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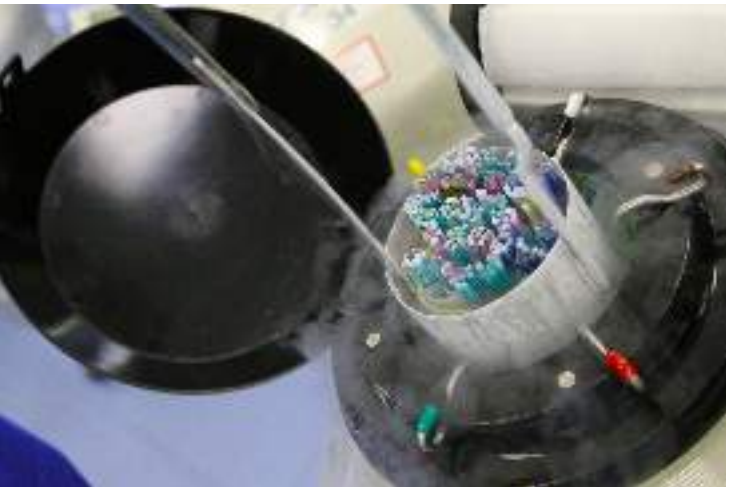

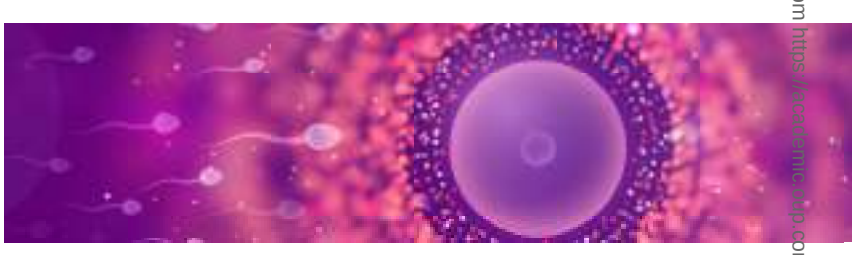

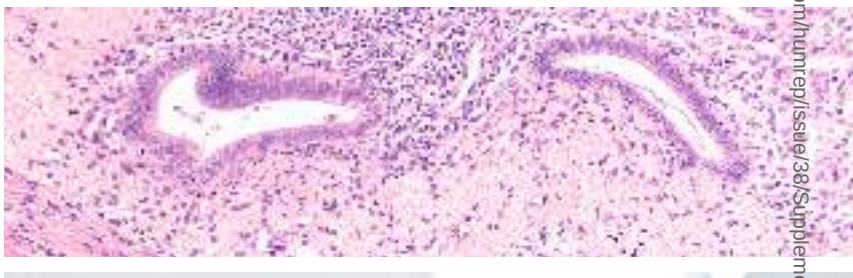

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Abstracts
39th Hybrid Annual Meeting of the European Society of Human Reproduction and Embryology

25 June to 28 June 2023

Abstracts

39th Hybrid Annual Meeting of the
European Society of
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ORAL PRESENTATIONS

Monday 26 June 2023

(*continued overleaf*)

Tuesday 27 June 2023

Wednesday 28 June 2023

- **INVITED SESSIONS**
- **SELECTED ORAL COMMUNICATION SESSIONS**
- **POSTER DISCUSSION SESSIONS**

POSTER VIEWING

Human Reproduction, Vol.38, Suppl 1, pp. i1–i598, 2023

human reproduction

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INVITED SESSION

SESSION 01: KEYNOTE SESSION

Monday 26 June 2023 Hall A 08:30 - 09:30

Abstract citation ID: dead093.001

O-001 Human Reproduction keynote lecture: Influence of diet and exercise on sperm and its epigenome

E. Willerslev[1]

[1]*University of Copenhagen, Lundbeck Foundation GeoGenetics Centre, Copenhagen, Denmark*

Abstract citation ID: dead093.002

O-002 Human genes: A journey through prehistory

R. Barrés[1]

[1]*University of Copenhagen, Novo Nordisk Foundation center of Basic Metabollic Research, Copenhagen, Denmark*

SELECTED ORAL COMMUNICATIONS

SESSION 02: NEW INSIGHTS INTO EMBRYO DEVELOPMENT

Monday 26 June 2023 Hall A 10:00 - 11:30

Abstract citation ID: dead093.003

O-003 Mechanics of human embryo compaction

J. Firmin[1], **N. Ecker**[2], **D. Rivet-Danon**[3], **O. Ozguc**[4], **V. Barraud-Lange**[3], **H. Turlier**[5], **C. Patrat**[6], **J.L. Maitre**[4]

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Study question: How the human embryo builds the blastocyst is crucial to improve ART, as morphology of human embryo is a prime determinant to assess implantation potential.

Summary answer: An evolutionarily conserved increase in cell contractility is required to generate the forces driving the compaction, which is the first morphogenetic event shaping the body.

What is known already: The shaping of the human embryo begins with compaction, during which cells come into close contact and form a tighter structure. ART studies suggest that human embryos fail compaction primarily because of defective adhesion. Based on our current understanding of animal morphogenesis, other morphogenetic engines, such as cell contractility, could be involved in shaping the human embryo. However, the molecular, cellular and physical mechanisms driving human embryo morphogenesis remain uncharacterized.

Study design, size, duration: A total of 54 frozen embryos have been used for this work. The use of human embryos donated for research was allowed by the Agence de la Biomédecine (approval number RE 17-011R) in compliance with the International Society for Stem Cell Research guidelines. Donated embryos were cryopreserved and stored at three different centers in Paris. Embryos were then transferred to the Institut Curie where they were immediately thawed and used for the research project.

Participants/materials, setting, methods: Using micropipette aspiration on human embryos, we mapped cell surface tensions during compaction. Drug inhibition and immunostaining of cell contractility and cell-cell adhesion in human embryos reveal what drives the surface tension responsible for compaction. To evaluate cell contractility we focused on F-actin and myosin motor proteins, for adhesion we focused on E-cadherin.

Main results and the role of chance: Mapping cell surface tensions during human compaction reveals a 4-fold increase of tension at the cell-medium interface, from 0.62 ± 0.04 to 2.35 ± 0.08 nN/µm (mean ± SEM of 147 measurements on 10 embryos, Student's t test $p < 10^{-5}$), while cell-cell contacts keep a steady tension ~0.6 nN/µm. Therefore, increased tension at the cell-medium interface drives human embryo compaction, which is qualitatively similar to compaction in mouse embryos (from ~0.2 to 0.4 nN/µm). Further comparison between human and mouse reveals qualitatively similar but quantitavely different mechanical strategies, with human embryos being mechanically least efficient. Inhibition of cell contractility and cell-cell adhesion in human embryos reveals that, while both cellular processes are required for compaction, adhesion involvement plays a permissive role. Only contractility controls the surface tension responsible for compaction. Interestingly, cell contractility and cell-cell adhesion exhibit distinct mechanical signatures when faulty. Analyzing the mechanical signature of naturally failing embryos, we find evidence that non-compacting embryos or partially compacting embryos with excluded cells have defective contractility.

Limitations, reasons for caution: Given the need to use embryos donated to research, which are valuable samples, our sample size is small but sufficient to show statistically significant differences. Moreover, these embryos come from infertile patients undergoing ART.

Wider implications of the findings: Excluded cells from compaction could be aneuploid to protect the embryonic tissue from chromosomal abnormalities. Is there a correlation between their mechanical signature and the fact that they may be aneuploid remains an open question.

How physical laws are used to produce the breathtaking diversity of the shapes of life?

Trial registration number: 'not applicable'

Abstract citation ID: dead093.004

O-004 Male and female blastocysts display differences in development such that embryos assigned an identical morphological grade may have differing viability dependent on their sex

M. Kilbee[1], A. Del Canto Cano[1], G. Clark[1,2], C. Coudereau[1], E. Fernandez Marcos[3], D. Babariya[1], D. Wells[1,2]

[1]*Juno Genetics, Clinical Genetics, Oxford, United Kingdom*
[2]*University of Oxford, Nuffield Department of Women's and Reproductive Health, Oxford, United Kingdom*
[3]*Juno Genetics, Clinical Genetics, Valencia, Spain*

Study question: Embryo selection methods based upon morphological or morphokinetic evaluation assume that male and female embryos have identical rates of preimplantation development, but is this true?

Summary answer: The distribution of trophectoderm grades differs for male and female blastocysts. Furthermore, male and female embryos of identical grade may have different probabilities of viability.

What is known already: Previous studies have suggested that male and female embryos may have subtle differences in their rates of preimplantation development. However, this possibility remains controversial. Apart from being of scientific interest, the question of whether the sex of an embryo can affect its growth trajectory is of clinical importance. Morphological grading, the primary method used by most IVF clinics when deciding which embryo to prioritise for transfer, assumes that developmental rates are independent of sex. Similarly, morphokinetic strategies for embryo evaluation, using data gathered from time-lapse incubators, are likely to be compromised if male and female embryos have differing developmental behaviour.

Study design, size, duration: 1,241 blastocysts underwent PGT-A and were shown to be euploid. Chromosome analysis also revealed the sex of the embryos, although this was not disclosed to patients. Standard morphological grading was carried out blindly with respect to the sex of the embryo. Information on clinical outcomes following embryo transfer was available for a subset of 336 embryos. Data was evaluated using various statistical methods to reveal any associations between embryo sex, morphology and clinical outcome.

Participants/materials, setting, methods: Embryos included in this study were derived from patients undergoing routine IVF with PGT-A. The only embryos excluded were those derived from patients carrying a monogenic disease mutation or a chromosome rearrangement. Embryos underwent PGT-A at the blastocyst stage on either day-5 or day-6, using a highly validated method. The euploid embryos were divided into male and female groups and blastocyst morphological grades were considered with respect to rates of implantation, miscarriage and ongoing pregnancy/birth.

Main results and the role of chance: The proportion of embryos biopsied on day-5 versus day-6 was identical for males and females (68% day-5 for both). No difference was observed in blastocyst expansion or inner cell mass grading on day-5. However, a highly significant difference was noted in the distribution of trophectoderm grades (A, B, C, D according to the system of Gardner and Schoolcraft, 1999) ($P < 0.0001$). This was characterised by a disproportionate representation of the highest grade amongst male embryos. 21.6% of male blastocysts had trophectoderm graded 'A', compared to 14.9% of females. Interestingly, euploid male embryos with a 'B' grade trophectoderm were associated with significantly higher implantation rates than females of the same morphological grade (82.1% vs. 58.7%; $P = 0.0002$), but the males of this grade also experienced a higher incidence of biochemical losses (16.7% vs. 4.2%; $P = 0.017$). As a group, male embryos do not have greater viability than female embryos, which implies that the greater proportion of high trophectoderm grades amongst these embryos is not an indicator of superior potential. In turn, this suggests that different criteria should be used for grading trophectoderm in males and females.

Limitations, reasons for caution: It may be desirable to introduce parallel morphological grading systems, one for male embryos and another for female. However, this would only be applicable in cycles involving PGT-A. The observation that embryos of equal grade can have different outcomes depending on their sex should be confirmed in a prospective study.

Wider implications of the findings: These results suggest the development of sex-specific morphological grading strategies might provide more reliable insights into embryonic potential, increasing the likelihood of selecting a viable embryo for transfer. Consideration of differences in the development of male and female embryos will also be important when developing morphokinetic algorithms for embryo selection.

Trial registration number: Not applicable

Abstract citation ID: dead093.005

O-005 The ToF-study - comparing the cumulative live birth rate of blastocyst-stage versus cleavage-stage embryo transfers in good prognosis IVF patients: a multicenter randomized controlled trial.

S. Cornelisse[1], K. Fleischer[2], B. Arends[3], J.J. Brink-van der Vlugt[4], J.P. De Bruin[5], M.H.J.M. Curfs[6], J.G. Derhaag[7], K.L. Deurloo[8], A.J.C.M. Van Dongen[9], J. Van Echten-Arends[10], M. De Hundt[11], C.A.M. Koks[12], Q. Pieterse[13], E. Slappendel[2], M.A.F. Traas[14], J. Visser[15], C.G. Vergouw[16], H.R. Verhoeve[17], L.A.J. Van der Westerlaken[18], Y.A. Wurth[19], M. Van der Zanden[20], D.D.M. Braat[1], M. Van Wely[21], L. Ramos[1], S. Mastenbroek[21]

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Abstract under embargo

Abstract citation ID: dead093.006

O-006 Novel and scalable non-invasive metabolic imaging of early embryos using a lab-on-a-chip approach

E. Vargas-Ordaz[1], H. Newman[2], S. Catt[2], R. Nosrati[1], V. Cadarso[1], A. Neild[1], F. Horta[2]

[1]*Monash University, Mechanical and Aerospace Engineering, Melbourne, Australia*
[2]*Monash University, Obstetrics and Gynaecology, Melbourne, Australia*

Study question: Is it feasible to safely determine metabolic imaging signatures to measure nicotinamide adenine dinucleotide (NADH) associated auto-fluorescence in early embryos using a scalable lab-on-a-chip approach?

Summary answer: We developed an optofluidic device capable of non-invasively obtain high-resolution 3D images of the metabolic activity of live mouse embryos using a lab-on-a-chip approach.

What is known already: Selecting the most suitable embryos for implantation and subsequent healthy live birth is crucial to the success rate of assisted reproduction and offspring health. Thus, developing non-invasive methods that are reliable to assess oocyte and embryo quality has been a significant aim for assisted reproduction. Besides morphological evaluation using optical microscopy, a promising alternative is the non-invasive imaging of live embryos to establish metabolic activity performance. However, metabolic imaging has been only achieved using highly complex advanced microscopy such as Fluorescence-lifetime imaging (FLIM) and hyperspectral microscopy, methods that are costly and challenging, limiting the potential for deployment in fertility clinics.

Study design, size, duration: The non-invasive nature of the system was investigated by assessing the development and viability of live embryos after embryo culture for 67hrs post metabolic imaging at the 2-cell embryo stage ($n = 115$), including a control for culture conditions and sham controls (system no-illuminated). Embryo quality of developed blastocysts was assessed by immunocytochemistry to quantify trophectoderm and inner mass cells ($n = 75$). Furthermore, inhibition of metabolic activity (FK866 inhibitor) during embryo culture was also assessed ($n = 18$).

Participants/materials, setting, methods: Optofluidic devices were manufactured by cast-moulding using a negative photoresist (SU8-2075; MicroChemicals GmbH, Ulm, Germany) by a standard UV-photolithography process. The microstructures fabricated of polydimethylsiloxane (PDSM) integrated Light Sheet Fluorescence Microscopy into a microfluidic system, including on-chip micro-lenses to generate a light sheet at the center of a microchannel. Super-ovulated F1 (CBA/C57Bl6) mice were used to produce 2-cell embryos and embryo culture experiments. Blastocyst formation rates and embryo quality (immunocytochemistry) were compared between study groups.

Main results and the role of chance: The optofluidic device was capable of non-invasively obtaining high-resolution 3D images of the metabolic activity in live mouse embryos. The system's design allowed continuous tracking of the embryo location, including high control displacement through the light-sheet, fast imaging of the embryos (<2 second) and keeping a low dose of light exposure (16 J cm^{-2} and 8 J cm^{-2}). Optimum settings for keeping sample viability showed that a modest light dosage was capable to obtain 30 times higher signal-noise-ratio images than images obtained with a confocal system ($p < 0.00001$; t-test). The results showed no significant differences between the control, illuminated and non-illuminated embryos (sham control) for embryo development as well as embryo quality at the blastocyst stage ($p > 0.05$; Yate's chi squared test). Additionally, embryos with inhibited metabolic activity showed decreased blastocyst formation rates as well as 47% reduction in metabolic activity measured by non-invasive metabolic imaging ($p < 0.0001$; t-test). This study reports an optofluidic device capable of non-invasive metabolic imaging of live embryos using a similar concept as previously reported using FLIM technology and hyperspectral microscopy, but allowing a novel, scalable and affordable system with implementation potential in standard IVF laboratory equipment.

Limitations, reasons for caution: The study was conducted using a mouse model focused on early embryo development. Further safety studies are required to assess embryonic health by investigating the impact of the use of light during embryo development, live birth, embryo gene expression and epigenome stability.

Wider implications of the findings: This lab-on-a-chip is novel, scalable and has the potential to be used in fertility clinics as a technology platform to enable real-time monitoring of the metabolic function of embryos prior embryo transfer. Future applications include potential integration into existing IVF laboratory equipment such as bench and time-lapse incubators.

Trial registration number: N/A

Abstract citation ID: dead093.007

O-007 Association of successful embryo development and pregnancy outcome with redox state as measured by human non-mercaptalbumin in embryo culture medium

Y. Kusumoto[1,2,3], M. Sato[3,4], K. Yasukawa[3], B. Uranbileg[3], H. Ikeda[3], Y. Yatomi[3]

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[2]*Akihabara ART Clinic, Culture section, Tokyo, Japan*
[3]*The University of Tokyo, Department of Clinical Laboratory Medicine- Graduate School of Medicine, Tokyo, Japan*
[4]*The University of Tokyo, Department of Gastroenterology- Graduate School of Medicine, Tokyo, Japan*

Study question: Is the redox state in the embryo culture medium, as measured by oxidized albumin, associated with successful embryo development and pregnancy outcome?

Summary answer: The level of oxidized albumin in the culture medium was the most important variable in predicting blastocyst formation andwas associated with miscarriage after transfer.

What is known already: Human serum albumin (HSA), the most abundant protein in the plasma, exerts important antioxidant activities against oxidative damage. HSA exists as oxidized human non-mercaptalbumin (HNA) and reduced human mercaptalbumin. HNA is attracting attention because of its

novel role as a marker reflecting oxidative state, and HNA levels are known to increase in oxidative stress diseases such as kidney disease, diabetes, liver disease or Parkinson's disease. On the other hand, in human IVF, most culture medium are supplemented with HSA as a protein source, but no previous studies have examined the relationship between HNA and IVF outcomes.

Study design, size, duration: We followed the pregnancy outcomes for 40 cycles of single blastocyst transfer out of the retrospective study, which enrolled a total of 173 embryos cultured to the blastocyst stage from a total of 91 patients who underwent IVF/ICSI cycle between February 2018 and July 2018.

Participants/materials, setting, methods: Prior to using the medium for embryo culture, the redox state was assessed with HNA level. HNA level, patient age, IVF/ICSI and oocyte maturity were analyzed as factors associated with blastocyst formation on day 5 and 6 after fertilization, and a machine learning model was developed by using the Random Forest (RF) algorithm to predict blastocyst formation. We also examined whether the factors made a difference in pregnancy outcomes.

Main results and the role of chance: The median %HNA in the culture medium was 89.36% (range, 80.12% to 94.84%), which was much higher than the value for blood in healthy humans, (approximately 25%). Blastocyst formation was observed in 41.04% (71/173) embryos. In both univariate and multivariate analyses, successful blastocyst development was associated with a lower %HNA in the culture medium ($p = 0.001$), a younger patient age ($p < 0.001$), and the use of standard IVF ($p = 0.007$). A prediction model for successful blastocyst formation was developed using a RF algorithm with four factors (%HNA, patient age, fertilizationmethod, and oocyte maturation stage). The RF model developed using 70% of samples (training set, n = 121) was validated in the remaining testing set (n = 52) and produced an area under the curve of 0.761, where %HNA in the culture medium was the most important variable for the prediction of blastocyst formation, followed by patient age. The HNA levels were significantly higher in the group of embryos that resulted in miscarriage after blastocyst transfer (93.15% vs. 89.28%, $P = 0.0498$).

Limitations, reasons for caution: This study was conducted in G-TL medium only. Further studies are needed to elucidate the association of %HNA and embryo development, and to predict the transfer success rate by using other commonly used media.

Wider implications of the findings: In the IVF medium, which is supposed to mimic the body environment, the %HNA showed a high oxidized state compared to the body itself; it is suggesting it may cause unsuccessful IVF results and lower live birth rate. Controlling the oxidation level may help to create a more appropriate environment.

Trial registration number: not applicable

Abstract citation ID: dead093.008
O-008 The composition of commercially available human preimplantation embryo culture media

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Study question: What is the composition of currently available commercial human preimplantation embryo culture media from eleven different brands for each stage of preimplantation embryo development?

Summary answer: Besides similarities in embryo culture medium composition between brands, there were also differences in composition for which there does not seem to be a rationale.

What is known already: Although a lot remains uncertain, there seems to be some consensus in the scientific literature on the environmental and nutritional needs of preimplantation embryos in *in vitro* culture, at least for certain components. Since suppliers do not disclose the exact formulations of their media, it is unclear whether the embryo culture media currently used in IVF laboratories meet these suggested conditions. We previously published composition analyses of a limited number of culture media, but have now repeated this analysis and have included a larger number of media from eleven brands of human preimplantation embryo culture media.

Study design, size, duration: Forty-seven human embryo culture media and protein supplements were purchased between December 2019 and June 2020. Upon arrival, each complete medium (n = 23), unsupplemented medium (n = 14), and supplement (n = 10) was aliquotted in Eppendorf tubes, snap frozen in liquid nitrogen and stored in -80°C until composition analysis in November 2021. Additionally, unsupplemented media were supplemented with each available supplement from the same brand (n = 33 combinations) and then also aliquotted, snap frozen and stored in -80°C until analysis.

Participants/materials, setting, methods: The concentration of forty components was determined in all collected samples (n = 80). Seven ions, glucose, three immunoglobulins, uric acid, alanine aminotransferase (ASAT), aspartate aminotransferase (ALAT), albumin, and the total amount of proteins were determined in each sample using a Cobas 8000 Analyser (Roche Diagnostics). Analysis of pyruvate, lactate, carnitine and twenty-one amino acids was achieved with Ultra-high Performance Liquid Chromatography-Mass Spectometry (UPLC-MS/MS).

Main results and the role of chance: Our analysis of the concentration of 40 components in ready to use embryo culture media of most available brands on the market showed that in general the culture media follow generally accepted assumptions on the changing needs of an embryo during early development. However, there were clear differences between brands, and no two embryo culture media were the same. For example, pyruvate concentrations were higher in fertilization and cleavage stage media and lower in blastocyst stage media, whereas glucose was relatively high in fertilization media, lower in cleavage stage media, and higher again in blastocyst media. This follows previous findings that demonstrate that pyruvate is the main energy source until blastocyst stage and that glucose becomes the main energy source for blastocysts. Other components, like lactate for example, followed different patterns in the sequential media of different brands, and concentrations also differed between brands. The composition of continuous media resembled the composition of the other media of the same brand, but differed between brands. Interestingly, some of the culture media brands belong to the same parent company, but differ in their composition. The rationale of such differences is not clear. Research providing such rationale seems warranted.

Limitations, reasons for caution: The analysed embryo culture media may contain other components that were not investigated in this study.

Wider implications of the findings: Detailed information on human embryo culture medium composition, including the exact concentrations of each ingredient, is much needed to in the end be able to understand its effect on IVF outcomes, and to facilitate the scientific improvement of media for human preimplantation embryo culture.

Trial registration number: not applicable

SELECTED ORAL COMMUNICATIONS
SESSION 03: NEW CONCEPTS: PCOS
Monday 26 June 2023 Hall D3 10:00 - 11:30

Abstract citation ID: dead093.009
O-009 The Role of Ethnicity and Polycystic Ovary Syndrome on Pregnancy Complications. An Analysis of a Population Database

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Study question: The goal of this study was to determine the independent effect of ethnicity on adverse obstetrical outcomes in women with Polycystic Ovary Syndrome (PCOS).

Summary answer: Among women with PCOS, African Americans, Hispanic and Asians are at an increased risk of certain adverse obstetrics outcomes, whereas Caucasians have fewer pregnancy complications.

What is known already: Polycystic ovarian syndrome is associated with an increased risk of pregnancy complications possibly due to the insulin resistance inherent in this syndrome. Variable ethnicities demonstrate different magnitudes of insulin resistance and in non-PCOS populations are known to be at modified pregnancy complication risks. Different ethnicities may have different phenotypes of PCOS, possibly further modifying outcomes. The role of ethnicity in women with PCOS and its effect on pregnancy complications has not been well studied.

Study design, size, duration: This retrospective population-based study utilized data from the Healthcare Cost and Utilization Project Nationwide Inpatient Sample (HCUP-NIS), over 11 years from 2004 to 2014. The data are representative of 20% of admissions to US hospitals and geographically represent over 96% of the American population.

Clinical modification ICD9-CM codes were used to identify women with PCOS and group them according to maternal ethnicity. In 2015 codes were changed to ICD-10 which are not comparable.

Participants/materials, setting, methods: All women with PCOS($n = 12782$) were grouped according to maternal ethnicity: Caucasian(9107), African American(1098), Hispanic(1288), Asian(741), and other. Pregnancy, delivery, and neonatal outcomes were collected per group and compared to the rest.

Chi-square tests were used to compare the baseline characteristics between the cohorts. Logistic regression analyses were conducted to explore associations between ethnicity and pregnancy outcomes through the estimation of odds ratio (OR) and 95% confidence intervals (CI) while controlling for confounding effects.

Main results and the role of chance: PCOS was found in 12,782 patients with included ethnicity results. Asian women had a higher prevalence of gestational diabetes(GDM) (aOR1.96, 95%CI 1.49-2.58, $p < 0.001$), chorioamnionitis (aOR3.41, 95%CI 2.12-5.47, $p < 0.0001$), operative vaginal delivery (aOR2.42, 95%CI 1.65-3.56, $p < 0.001$), postpartum hemorrhage(PPH) (aOR2.07, 95%CI 1.25-3.43, $p = 0.004$) and maternal infection (aOR2.84, 95%CI 1.80-4.49, $p < 0.001$). African Americans had a higher risk of pregnancy-induced hypertension (aOR1.38, 95% CI 1.06-1.80, $p = 0.02$), preeclampsia (aOR1.68, 95% CI 1.15-2.45, $p = 0.007$), preterm premature rupture of membrane (aOR2.75, 95%CI 1.58-4.78, $p < 0.001$), chorioamnionitis (aOR1.83, 95%CI 1.12-2.98, $p = 0.016$) and cesarean sections (aOR1.69, 95%CI 1.32-2.15, $p < 0.001$) and lower risk of operative vaginal delivery (aOR0.53, 95%CI 0.31-0.93, $p = 0.03$), spontaneous vaginal delivery (aOR0.67, 95%CI 0.52-0.85, $p < 0.001$), and maternal infection (aOR1.91, 95%CI 1.21-3.00, $p = 0.005$). The risk of GDM (aOR1.36, 95%CI 1.06-1.73, $p < 0.014$) and PPH (aOR1.58, 95%CI 1.01-2.47, $p = 0.045$) was increased among Hispanic patients. Caucasian patients were at lower risk of GDM(aOR0.67, 95%CI 0.57-0.79, $p < 0.0001$), chorioamnionitis (aOR0.39, 95%CI 0.28-0.55, $p < 0.0001$), casarean section (aOR0.83, 95%CI 0.73-0.95, $p < 0.008$), spontaneous vaginal deliveries (aOR1.25, 95% CI1.10-1.43, $p < 0.001$), PPH (aOR0.70, 95% CI0.50-0.98, $p < 0.035$), blood transfusion (aOR0.49, 95%CI 0.29-0.83, $p < 0.007$), maternal infection (aOR0.34, 95% CI 0.27-0.51, $p < 0.0001$) and small for gestational age infants (aOR0.64, 95%CI 0.44-0.93, $p < 0.018$). Rates of other pregnancy outcomes were similar for the groups.

Limitations, reasons for caution: This is a retrospective analysis utilizing an administrative database that relied on the accuracy and consistency of the individuals coding the data. There are known limitations to how accurately hospital coding is able to capture perinatal conditions and complications.

Wider implications of the findings: Our study demonstrated that among women with PCOS, specific ethnicities are at an increased risk of adverse obstetric outcomes. This underlines the importance of screening for certain pregnancy complications in PCOS women of African American, Hispanic, and Asian ethnicity. Studies should evaluate the role of insulin resistance in these outcomes.

Trial registration number: not applicable - This study used publicly accessible, anonymized data; therefore, according to articles 2.2, 2.4 of Tri-Council Policy Statement (2010), institutional review board approval was not required.

Abstract citation ID: dead093.010

O-010 The ULTRA trial: transvaginal ULTRAsound-guided ovarian ablation using the novel May Health device in women with PCOS-related infertility: first–in-human feasibility clinical trial

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Study question: Is transvaginal ULTRAsound (TVUS)-guided ovarian ablation using May Health device feasible, safe, and effective in inducing ovulation in Clomiphene citrate and/or Letrozole (CC/LTZ)-resistant PCOS women?

Summary answer: TVUS-guided ovarian ablation using the May Health device seems feasible, safe and effective in inducing ovulation in PCOS-related anovulatory infertility, with additional, larger studies needed.

What is known already: Laparoscopic ovarian drilling (LOD) is widely accepted and recommended as an effective second line treatment in PCOS-related infertility. However, it is an invasive procedure that requires general anaesthesia and carries significant risks. Furthermore, the off-the-shelf devices used for LOD are not specifically designed to precisely deliver the desired and effective amount of ablation. In contrast, the May Health device has been designed to induce a precisely calculated amount of ovarian ablation via a much less invasive route without general anaesthesia. May Health therefore transforms an invasive surgical procedure to an office-based technique similar in access to the well-established oocyte retrieval.

Study design, size, duration: This study included two phase-1 feasibility, single-arm clinical trials running in parallel in the US and EU assessing the May Health Device in performing TVUS-guided ovarian ablation in anovulatory PCOS women resistant to first-line ovulation induction drugs. Sample size was 35 participants with post-procedure follow-up (FU) of 24 months in EU and 12 months in US. Endpoints included procedure feasibility (successful ablation of at least one ovary), safety, and effectiveness (ovulation and pregnancy rates).

Participants/materials, setting, methods: Seven fertility centres (UK, France, Belgium, US) participated in the trials. Participants were CC/LTZ-resistant PCOS women aged 18-40 years. PCOS was diagnosed according to Rotterdam criteria. Participants underwent TVUS-guided ovarian ablation using May Health device. The initial five participants underwent laparoscopy concurrent with TVUS-ablation. Post-procedure, serum progesterone was measured weekly until confirmation of ovulation or up to 12 weeks. Women were evaluated at 3 and 6 months, then telephoned at 9, 12 and 24 months.

Main results and the role of chance: Twenty-three participants (mean ± sd age, 31.8 ± 3.1years; BMI, 29.8 ± 5.0kg/m2) underwent May Health TVUS-guided ovarian ablation and completed at least three-months FU. Of those, 10 (43.5%) ovulated spontaneously during the first three months. Six more women ovulated between three- and nine-months FU, some with CC/LTZ, giving a total ovulation rate of 69.6% (16/23). Of the 11 participants who completed nine-months FU, five conceived. Two more participants conceived before 9-months giving a total pregnancy rate of 53.8% (7/13).

In 19 cases (82.6%), ablation was achieved successfully in both ovaries, while in four cases (17.4%) one of the ovaries was not ablated either because it was too small (as per protocol) (n = 3) or inaccessible (n = 1).

Overall, 14 participants experienced 29 adverse events (AEs), of which 25 were mild, two moderate and two severe. Nine mild/moderate AEs were deemed related to the device/ablation procedure. Examples of mild/moderate AEs included mild self-limiting vaginal bleeding (n = 5), pain (n = 5) and headache (n = 2). None of the severe AEs were deemed related to the device/ablation procedure. One participant in phase1a who underwent laparoscopy concurrent with the ablation, sustained a bowel injury involving the ilium near the ileocecal junction. Independent expert investigation concluded that the injury was likely caused by laparoscopic veress needle.

Limitations, reasons for caution: One possible limitation of this study is the lack of a comparator treatment / placebo arm. However, this was not necessary for this feasibility phase 1 trial. Furthermore, given the nature of TVUS-ovarian ablation, it would be difficult to compare it with other ovulation induction drugs or to a placebo.

Wider implications of the findings: These preliminary data suggest that the novel May Health device offers a promising office-based second line ovulation inducing procedure for CC/Letrozole-resistant anovulatory PCOS women.

Trial registration number: NCT03760926

Abstract citation ID: dead093.011
O-011 Cardiovascular morbidity and mortality in women with PCOS: a cohort study of 75142 participants from the UK biobank

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Study question: Are women with PCOS at an increased risk of cardiovascular mortality and morbidity?

Summary answer: Although PCOS is strongly associated with cardiovascular risk factors, it does not increase the risk of cardiovascular morbidity and mortality in women aged 40-60 years.

What is known already: PCOS is one the commonest endocrinopathies in women of reproductive age characterised by anovulation, hyperandrogenism and polycystic ovaries in ultrasound surveillance. The prevalence of PCOS has been quoted 8 to13% of women in the UK, however, up to 70% of the cases may be undiagnosed. Women with PCOS are at increased risk of developing adverse cardiometabolic outcomes including insulin resistance, type 2 diabetes (T2DM), metabolic syndrome, hypertension and dyslipidaemia. Those unfavourable risk factors would place women with PCOS at an increased risk for cardiovascular mortality and morbidity, however, the evidence from epidemiological studies are so far heterogenous with conflicting results.

Study design, size, duration: We conducted a cohort study including a total of 75142 participants from the UK biobank, of whom 15747 had PCOS. Women were followed up for 11.1 years in average.

Participants/materials, setting, methods: The primary outcome was morbidity and mortality from ischemic heart disease and stroke. We constructed Cox regression analysis adjusted for confounders and risk factors to quantify the risk of cardiovascular morbidity and mortality in women with PCOS.

Main results and the role of chance: The incidence rate of cardiovascular events was 1.92/1000 person years in the PCOS population and 1.90/1000 person years in women without PCOS. PCOS was associated with a higher risk of obesity (OR 1.63, 95% CI 1.56-1.70), hypertension (OR 1.18, 95% CI 1.13-1.23) and type 2 diabetes (OR 1.44, 95% CI 1.31-1.58). In the adjusted cox regression model, PCOS doesn't increase the risk of CVD morbidity and mortality (HR 0.89, 95% CI 0.78-1.01), however, the cumulative hazard risk in women over 60 years old with a history of PCOS was greater compared to women without PCOS.

Limitations, reasons for caution: The low response rate (5.5%) to the UK biobank recruitment introduces a healthy responder bias which may limit the representation of the population. PCOS was both self-reported and assessed by clinical and biochemical features and other variables were also self-reported, hence reporting bias cannot be excluded.

Wider implications of the findings: The strong association of PCOS with cardiometabolic risk factors and that the CVD morbidity/mortality risk becomes comparable with that of women without PCOS after correcting for these factors, highlights the need to strengthen public health strategies for surveillance, lifestyle interventions and prompt treatment of those co-morbidities in women with PCOS.

Trial registration number: Not applicable

Abstract citation ID: dead093.012
O-012 Risk of epithelial ovarian tumors among women with polycystic ovary syndrome (PCOS): A nationwide population-based cohort study

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Abstract under embargo

Abstract citation ID: dead093.013
O-013 Metabolic characteristics of offspring born to women with polycystic ovary syndrome in childhood

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Study question: Does polycystic ovary syndrome (PCOS) affect the metabolic characteristics of offspring in childhood?

Summary answer: Girls born to PCOS mothers had increased cholesterol (CHOL) and low-density lipoprotein cholesterol (LDL) levels, whereas boys had comparable CHOL and LDL levels to controls.

What is known already: Maternal PCOS status may negatively influence childhood growth, endocrine and metabolic function. The long-term impact of PCOS on children health is poorly understood. Current findings reported by previous studies are divergent. Propensity score matching can reduce bias where strong confounding by indication is expected.

Study design, size, duration: This was a cohort study including 223 singletons born to women with PCOS and 2326 singletons born to non-PCOS women. All offspring were conceived by in-vitro fertilization (IVF) or Intracytoplasmic sperm injection (ICSI) from January 2001 to January 2018 in reproductive center of Women's hospital, Zhejiang University, School of medicine. Growth and development parameters and metabolic parameters were collected at age 3-6 years in Follow-up center of Women's hospital, Zhejiang University, School of medicine.

Participants/materials, setting, methods: We used the propensity score (PS) to match PCOS women and non-PCOS women in a 1:4 ratio. The variables in the PSM included maternal age at conception, paternal age at conception, maternal height, maternal preconception BMI, IVF or ICSI, fresh-embryo transfer (ET) or frozen-embryo transfer (FET), gender of offspring, age at follow-up, year of follow-up. The PCOS group consisted of 217 patients and the non-PCOS group consisted of 787 patients after PS matching.

Main results and the role of chance: Maternal preconception weight (58.3 ± 0.6 vs. 56.0 ± 0.2, $P<0.001$) and body mass index (BMI) (23.0 ± 0.2 vs. 21.9 ± 0.1, $P<0.001$) were significantly higher in mothers with PCOS before PS matching. After PS matching, parental demographic characteristics were similar between groups including maternal preconception weight and BMI. In PS matching cohort, the height, weight, BMI of children at age 3-6 years were comparable between PCOS group and non-PCOS group. Singletons born to PCOS women had significantly higher CHOL levels (4.43 ± 0.05 vs. 4.29 ± 0.01, $P=0.013$) and LDL levels (2.45 ± 0.04 vs. 2.37 ± 0.01, $P=0.049$) compared with non-PCOS group. Even though the girls had similar BMI in childhood between the two groups(15.26 ± 0.15 vs.15.13 ± 0.80, $P=0.449$), the girls born to PCOS women still exhibited elevated CHOL levels (4.52 ± 0.08 vs. 4.33 ± 0.04, $P=0.024$) and LDL levels (2.55 ± 0.06 vs. 2.39 ± 0.03, $P=0.007$). These differences in CHOL (4.34 ± 0.07 vs. 4.26 ± 0.04, $P=0.196$) and LDL levels (2.36 ± 0.05 vs. 2.36 ± 0.03, $P=0.866$) were not observed in male offspring between the two groups.

Limitations, reasons for caution: Mothers' weight gain during pregnancy, offspring lifestyle were not available for this study. This was a study carried out in a single center and was therefore susceptible to bias. Further studies remain necessary to fully confirm these results.

Wider implications of the findings: Our results support CHOL and LDL lipid metabolism as two of the earliest metabolic abnormalities features of the female offspring born to PCOS women. These findings suggest that further studies are needed to investigate the potential mechanisms and long-term metabolic changes associated with these differences.

Trial registration number: not applicable

Abstract citation ID: dead093.014
O-014 Clustering identifies distinct subtypes of PCOS – Towards a Rationale Approach to PCOS classification

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[4]Northwestern University Feinberg School of Medicine, Division of Endocrinology-Metabolism- and molecular Medicine, Chicago, U.S.A.

Study question: Does unsupervised clustering identify biologically distinct subtypes in a cohort of women with polycystic ovary syndrome (PCOS) diagnosed by Rotterdam criteria?

Summary answer: This study demonstrates that unsupervised hierarchical clustering of eight pre-defined quantitative reproductive and metabolic traits identifies biologically distinct subtypes in women with PCOS.

What is known already: PCOS is a common, heterogeneous, endocrine disorder in women of reproductive-age. PCOS diagnosed by NIH or non-NIH Rotterdam criteria or by self-report is generally genetically similar. Using Hierarchical Clustering (HC), we have previously identified discrete, stable PCOS subtypes, which we designated reproductive (higher LH, FSH, SHBG) and metabolic (higher BMI, insulin, glucose), in a United States cohort of ~900 PCOS women diagnosed by NIH criteria (Dapas *et al. PLoS Med*, 2020). The cases that did not cluster were designated "background subtype". The subtypes appeared to capture biologically meaningful differences because they were associated with distinct and novel genome-wide significant loci.

Study design, size, duration: In the current study, we applied HC to the same traits (BMI, LH, FSH, DHEAS, SHBG, testosterone, fasting insulin and fasting glucose). We then assessed whether additional traits differed between the subtypes thus identified: anti-Müllerian hormone (AMH), total follicle count, modified Ferriman-Gallwey score, estrogen, TSH, DHEA, cortisol, androstenedione, prolactin, LDL, HDL, triglycerides and cholesterol.

Participants/materials, setting, methods: Women of European ancestry, aged 13-45 years, n = 2502, with PCOS according to the Rotterdam criteria were included; n = 1067 also fulfilled NIH criteria. All quantitative traits were log-transformed to approximate a normal distribution. Z-scores were used to compare the differences between the three clusters using ANCOVA, corrected for age. Pair-wise comparison of the different clusters was performed using Fisher's least significance difference method and adjusted for multiple testing.

Main results and the role of chance: We replicated discrete subtypes in this large cohort of women with PCOS defined by the Rotterdam criteria. There were 1026 cases in the metabolic subtype, 450 cases in the reproductive subtype and 1026 in the background subtype. Cases in the reproductive subtype had significantly (all $P<0.001$) higher serum AMH levels, follicle

counts and HDL levels compared to the metabolic and background subtypes. These findings suggest that the reproductive subtype captures affected women with the alterations in folliculogenesis characteristic of PCOS, without using PCOM to define this subtype. In contrast, the cases in the metabolic subtype had significantly (all $P < 0.001$) higher triglyceride and LDL levels compared to the other subtypes providing further evidence that this subtype identifies cases with cardiometabolic risk. Androstenedione and TSH levels were significantly increased in the metabolic subtype compared to the background subtype ($P < 0.001$) and to both subtypes ($P = 0.004$), respectively. Cortisol and prolactin levels did not differ among the three subtypes. All results did not differ when the analysis was limited to NIH PCOS cases. Overall, our findings suggest that these PCOS subtypes have different etiologies and clinical outcomes. Subtyping may enable precision medicine approaches to the management of what is currently classified as PCOS.

Limitations, reasons for caution: A limitation of the study is that we have not replicated these findings in an independent cohort. We have not used an orthogonal method, such as genome-wide association, to confirm that the subtypes capture biologically distinct groups.

Wider implications of the findings: Taken together with our previous studies suggesting that the genetic architecture of these subtypes differs, the current study implies that PCOS consists of several etiologically distinct disorders. Our findings provide an example of the power of modern disease classification based on objective biologic differences rather than expert opinion.

Trial registration number: Not applicable

SELECTED ORAL COMMUNICATIONS

SESSION 04: FACTORS INFLUENCING SPERM (DYS)FUNCTION

Monday 26 June 2023 Hall D1 10:00 - 11:30

Abstract citation ID: dead093.015

O-015 Spontaneous alterations in semen parameters are associated with age, accessory gland function and the FSHB c.-211G>T variant

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Study question: Which parameters are associated with spontaneous alterations in semen analysis in infertile men?

Summary answer: Changes in semen parameters are modulated by abstinence time, accessory gland function and fluctuations in FSH serum concentration, which is bound to FSHB c.-211G>T variant.

What is known already: There is a strong within-subject alteration of semen parameters in men with infertility. The WHO therefore recommends two ejaculate analyses. However, it remains unknown in which subgroup variations are likely to occur and which semen parameters are affected.

We assume that in some men with idiopathic infertility, ejaculate parameters may spontaneously change without interventional medication apart from regression to the mean effects. The study aims to identify factors in conventional semen analysis based on the patients' clinical data, including reproductive hormones like follicle stimulating hormone (FSH), and factors impacting endocrine activity, like the FSHB-211 promoter gene.

Study design, size, duration: In this retrospective longitudinal study we selected 3456 deeply phenotyped men with idiopathic infertility who had visited our centre for fertility workup from 2010-2020. The patients were selected from our well curated database Androbase© for analysis of repeated ejaculate samples.

Participants/materials, setting, methods: Men with idiopathic infertility and at least two ejaculate analyses (70-90 days distance) were included. Exclusion criteria comprised: sperm concentration <1 million/ml, abnormal FSH or low testosterone, bitesticular volume (bTV) <10ml, orchiectomy, cryptorchidism, varicocele testis, oncological disease, genetic findings (i.e. abnormal karyotype, AZF deletions), medication affecting spermatogenesis.

Grouped linear 2-level nested mixed-effect models were applied. Analyzed parameters included: somatic, hormonal (including FSH and FSHB c.-211 variants), and semen parameters (including abstinence time, accessory gland markers).

Main results and the role of chance: Groups were built according to sperm concentration: A ($n = 397$): ≥1.0 to <5.0 mill/ml, B ($n = 708$): ≥5.0 to <15.0 mill/ml, C ($n = 2351$): ≥15.0 mill/ml.

A, B and C: changes in ejaculate volume were associated with alterations in total sperm count and motility ($p < 0.003$). Changes were, controlled for abstinence time ($p < 0.001$), related to alpha-glucosidase, fructose or zinc ($p = 0.005$-0.02).

A+B: fluctuations in FSH concentration influenced sperm concentration/count ($p = 0.004$-0.02), albeit only in men with FSHB c.-211 GG ($p = 0.007$-0.02). T-allele carriers did not show changes in FSH concentration ($p > 0.1$).

B: age <50 years ($p = 0.007$-0.01) and normal bTV ($p = 0.008$-0.02) were associated with spontaneous increases of sperm concentration, count and motility.

Semen parameters exhibit intra-individual alterations associated with organic, hormonal and genetic variables. Changes are pronounced in younger men with normal bTV and oligo- to almost normozoospermia. The effect is modulated by abstinence time, accessory gland function and fluctuations in FSH concentrations which, in turn, are bound to the FSHB-211 promotor gene polymorphism.

Limitations, reasons for caution: A limitation of our approach is the retrospective nature of the study, which cannot exclude the possibility of bias within the data set.

Wider implications of the findings: Judgement of semen analysis should be based on two semen samples, with abstinence time between 4-5 days. In future clinical trials, abstinence time should be kept constant. It might be investigated if younger men with normal bTV and FSHB c.-211 GT/TT benefit from improving accessory gland function and increasing FSH.

Trial registration number: not applicable

Abstract citation ID: dead093.016

O-016 Sperm motility has declined between 2017 and 2022 among candidate sperm donors in Denmark

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Study question: Has sperm quality among candidate sperm donors in Denmark changed in recent years (2017-2022)?

Summary answer: Although sperm concentration of candidate donors did not change, sperm quality (motility) declined by ~35% after controlling for age and other potential confounds.

What is known already: Questions remain about whether human sperm quality has declined in recent decades. Whilst some studies support this trend, others dispute it due to potential biases in the populations studied or the different methodological approaches to measuring sperm quality. Resolution of this issue is important because of the implications for human fertility, as well as for those involved in the recruitment of donors for use in Medically Assisted Reproduction.

Study design, size, duration: We analyzed the semen quality of 6,774 candidate sperm donors attending for their first semen analysis at Cryos International from 2017 to 2022 at four cities in Denmark: Aarhus, Aalborg, Copenhagen, and Odense. We analyzed only the first sperm sample, whether or not the candidate was eventually accepted as a donor. All donor

candidates were between 18 and 46 years old and lived in or near these four cities.

Participants/materials, setting, methods: Ejaculates were examined within one hour of production. Semen volume (mL) was estimated by weight and both total sperm concentration (10^6/mL) and the concentration of grade A and B spermatozoa were measured using the same protocols and CASA-system across all years at each site. Analyses were controlled for age, site, ejaculate volume, and the average monthly temperature when the ejaculate was produced. We used longitudinal data from accepted donors to test for methodological biases.

Main results and the role of chance: From 2017 to 2022, there was no evidence of changes in either semen volume (median = 3.5 mL) or sperm concentration (median = 58 million/mL) in the ejaculates of candidate donors. There was, however, clear evidence of a decline in the concentration (and total number) of grade A and B motile sperm. For the average candidate sperm donor that we studied, the concentration of grade A sperm declined from 5.15 [95% CL: 4.18, 6.34] million/mL in 2018 to 3.33 [2.71, 4.08] million/mL in 2022. This corresponds, for example, to a predicted decline in sperm quality of ~35% for a 25-year-old candidate from Aarhus in a month when the average daily high temperature was 18.5 °C. The same pattern was evident from all four cities, but candidates at Aarhus had lower overall sperm quality (grade A sperm motility) than candidates at the other three cities. Analysis of the longitudinal data from repeated donations from all 'accepted' donors during this same period (2017 – 2022), allowed us to rule out methodological factors (sperm collection, CASA, statistical anomalies) that might have influenced these findings.

Limitations, reasons for caution: We cannot rule out the possibility that men with poor sperm quality were more likely to apply to be donors during the global pandemic, or that the lifestyles of candidate donors had changed during this period because of lockdowns or changes in work patterns.

Wider implications of the findings: Candidate sperm donors are a useful population in which to monitor changes in human semen quality over time. The results may have implications for human fertility and the recruitment of sperm donors, where motile sperm concentration is an essential selection criterion.

Trial registration number: not applicable

Abstract citation ID: dead093.017
O-017 A novel diagnostic test identifies patients suffering from loss of CatSper function

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Study question: Is a loss of CatSper function in sperm a common cause of unexplained male infertility?

Summary answer: Loss of CatSper function leads to similar infertility phenotypes in mice and men and represents one of the most common causes of unexplained male infertility.

What is known already: Male infertility is often due to low sperm counts, impaired motility, and/or abnormal morphology. However, for a large fraction of infertile men, semen parameters are normal, suggesting that the infertility is rather due to an unexplained dysfunction of the sperm. There has been a growing body of evidence that human sperm dysfunction might involve the sperm-specific Ca^{2+} channel CatSper (cation channel of sperm) that translates changes in the chemical microenvironment of the female reproductive tract into changes in swimming behavior.

Study design, size, duration: In an observational study over the course of three years, a prototype of the "CatSper-Activity-Test" (CAT) was used to assess the function of CatSper in sperm from the left-over semen samples of 2286 unselected patients undergoing a semen analysis.

Participants/materials, setting, methods: The CatSper-Activity-Test (CAT) was developed at the Centre of Reproductive Medicine and Andrology, University Hospital Münster and subsequently performed by medical technical assistants in the diagnostic facility of this institution. Requiring only 40 µl of ejaculate, a standard light microscope, and a hands-on-time of a few minutes, the CAT enables a read-out of the activity of CatSper through a motility response: in the CAT buffer, CatSper-intact, but not CatSper-deficient sperm become immotile.

Main results and the role of chance: Using this CatSper-Activity-Test, we identified seven patients suffering from a loss of CatSper function in a cohort of men seeking medical advice for suspected male infertility. Standard semen analysis revealed that the CatSper-deficient patients are (with one exception) normozoospermic and were diagnosed with unexplained infertility. Notably, their sperm not only failed to fertilize the egg naturally but also upon intrauterine insemination (IUI) and in vitro fertilization (IVF), whereas intracytoplasmic sperm injection (ICSI) was successful. Two additional CatSper-deficient patients were identified among patients visiting our clinics for other reasons. We show that the loss of CatSper function is predominantly caused by a homozygous deletion of the *CATSPER2* gene; one patient featured compound heterozygous variants of the *CATSPERE* gene. According to the study results, CatSper dysfunction underlies 1.7% of cases of unexplained male infertility, for which a female factor can be excluded.

Limitations, reasons for caution: Results from this observational study were obtained from a single institute. A multi-center study involving various countries would enable a more precise determination of the prevalence of CatSper-related male infertility.

Wider implications of the findings: The CatSper-Activity-Test reliably identifies CatSper-deficient patients with a hands-on-time of a few minutes and requires neither special equipment nor specific training. We envisage the CatSper-Activity-Test as a novel tool for the early diagnosis of male infertility, thus, contributing to evidence-based treatment decisions and sparing patients unnecessary medical and financial risks.

Trial registration number: not applicable

Abstract citation ID: dead093.018
O-018 Impact of atypical bacterial semen infections on spermocytogram parameters and Spermatozoa DNA fragmentation and denaturation

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Study question: Does Spermocytogram parameters and Spermatozoa-DNA-Fragmentation (SDF) / Denaturation (SDD) levels are affected by atypical-bacterial semen infections in North-African men during the period between 2013-2022?

Summary answer: SDD presented positive-correlations with the presence of 3/5 atypical bacteria in the semen. Various Spermocytogram-parameters were also found to be negatively associated with atypical-bacterial-semen-infection.

What is known already: Atypical-Bacterial semen infections such as infections by Chlamydia-trachomatis (CT), Mycoplasma-hominis (MH) and genitalium (MG), Ureaplasma urealyticum (UU) and parvum (UP), were shown to be associated with poor sperm function (Prabha.et.al.,2009; Agarwal.et.al.,2012). In infections caused by CT, SDF was observed to be increased significantly and associated with male infertility (Karinen.*et.al.*,2004; Gallegos.et.*al.*,2008). In addition, MH and UU appeared to cause induction of nuclear decondensation and SDD, which damages sperm and has effects on sperm parameters. However, other studies failed to show any effect of MH, UU and CT on sperm parameters and SDD (Lee.et.al.,2013; Gdoura.et.al.,2008; Huang.et.al.,2015; Farsimadan and Motamedifar,2020).

Study design, size, duration: Retrospective study, including Sperm-culture-analyzes, Spermocytograms and SDF/SDD tests performed between

January,2013 and December,2022 of 10.386 patients from North-Africa aged between 19 and 73 years-old (y.o) (average-age: 43.2 y.o ± 7.16). Patients were classified in 2 main groups according to the presence (Group-1: n = 613) or not (Group-2: n = 9773) of atypical-bacteria in the semen and 5 subgroups of monobacterial-infected-semen which were extracted from Group-1 as: CT (n = 14), MH (n = 61), MG (n = 10), UU (n = 147) and UP (n = 73) subgroups.

Participants/materials, setting, methods: 8201-Tunisian-patients(79%), 1142-Algerians(11%) and 1038-Libyans(10%) presented for semen-analyzes. Samples were analyzed according to World-Health-Organization (WHO) guidelines. Bacterial-semen-infection was detected by real-time-polymerase-chain-reaction(RT-PCR). Spermocytogram analyzes were performed according to WHO2010-recommendations using Sperm-class-analyzer-software (SCA5/6(CASA-system)) and SCA-scope (Microptic®). SDF analyzed with terminal-uridine-nucleotide-end-labeling(TUNEL)/sperm chromatin dispersion(SCD) techniques. SDD analyzed with the Aniline-Blue-staining-method. Statistical-analyzes were performed using SPSS22.0 for Windows-software. Kolmogorov–Smirnov-test for normality-analysis and comparisons by Student-t-test/Mann–Whitney U-test, as appropriate. Pearson/Spearman' tests for correlations were used as appropriate, *P-value*<0.05 was considered as significant.

Main results and the role of chance: Sperm concentration was significantly lower in atypical-bacterial-semen-infected-group (Group-1) compared to controls (Group-2) with 11.39[0-311] vs 20.06[0-694]x10^6spz/mL, (p = 0.014); respectively, as well as progressive motility (14.6%[0%-64.47%] vs 21.89%[0%-81.35%], (p = 0.002); respectively) and typical morphology (5.34%[0%-18%] vs 9.29[0%-21%] (p < 0.001); respectively). However, Leucocytes concentration and SDD presented significant higher levels in Group-1 compared to Group-2 with 1.8[0.1-8.5] vs 0.5[0.1-2.45]x10^6 leucocytes/mL, (p < 0.001) and 21%[4%-60%] vs 15%[2%-75%], (p = 0.04); respectively. Similar significant differences were observed with 3/5 of the monobacterial-semen-infected-subgroups in comparison with Group-2 (MH, UU and UP-subgroups). No significant differences were observed in MG and CT-subgroups in except of SDD which presented significant higher levels in CT-subgroup in comparison with Group-2 (52%[4%-85%] vs 15%[2%-75%], (p = 0.002);respectively). Correlation-analyzes showed that SDD was positively correlated to MH (r = 0.069; p = 0.022) and UU (r = 0.084; p = 0.005) which is in accordance with the study of .Lee.et.al.(2013). Leucocytes concentration was also correlated positively to MH, CT, UU and UP (r = 0.047; p<0.001; r = 0.035; p=0.005; r = 0.074; p<0.001; r = 0.045; p=0.05; respectively). Sperm concentration, Progressive motility and Typical morphology presented Negative correlations with MH (r=-0.038; p=0.034; r=-0.048; p=0.05; r=-0.047; p=0.05; respectively), UU (r=-0.034;p=0.003; r=-0.04; p<0.001; r=-0.067; p=0.007; respectively) and UP (r=-0.072; p<0.001; r=-0.078; p=0.018; r=-0.057; p<0.05; respectively). Our results are in accordance with other studies such as of Prabha.et.al.(2009) and Agarwal. et al. (2012). However, SDF did not show any significant variation between our studied groups.

Limitations, reasons for caution: Our study is a retrospective-statistical survey that included patients presenting to the laboratory for fertility diagnosis or for a diagnosis due to a pathology and/or inflammation of the genital tract. Meta-analysis studies in addition to more prospective-randomized-controlled-trials in collaboration with IVF or other medical-analyzes-laboratories are necessary to confirm/refute our results.

Wider implications of the findings: These results should interest urologists, microbiologists, gynecologists, reproductive science fundamentalists, epidemiologists and embryologists who want to improve the investigations on the relationship between bacteria and semen parameters to the reasons of infertility, recurrent miscarriages and poor quality embryos in IVF.

Trial registration number: Not applicable

Abstract citation ID: dead093.019

O-019 Decreased Sperm Motility Associated With Bacterial Vaginosis Modeled in a Human Cervix Chip

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Study question: What is the underlying cause of infertility associated with bacterial vaginosis (BV)?

Summary answer: We provide the first evidence to suggest that BV-associated infertility could be the result of sperm dysfunction induced by exposure to poor cervical microbiome.

What is known already: Infertility is a global health concern, impacting 186 million individuals worldwide. In 25% of infertile couples the underlying cause remains unexplained. The complex interactions between the host mucosal epithelium, microbiome, and other microenvironmental factors play a significant role in health and disease of the reproductive tract. BV affects 30% of reproductive-aged women and is linked to overgrowth of pathogenic *Gardnerella vaginalis* bacteria. BV has a strong association with infertility, but it is not clear whether the resulting changes in the vaginal microbiome play a causal role, and as such BV screening is not currently included in infertility evaluation or treatment

Study design, size, duration: Female reproductive tract protective factors such as cervical mucus helps sperm retain their ability to fertilize for days compared to hours in suspension. We previously leveraged human organ-on-a-chip microfluidic culture technology to develop a physiological in vitro model of human cervix (Cervix Chip) that is lined with primary cervical epithelium interfaced with stromal fibroblasts. The epithelium forms a functional tissue barrier and produces mucus with compositional, biophysical, and hormone-responsive properties similar to the living cervix.

Participants/materials, setting, methods: We used 2D culture of sperm and bacteria as a screening and validated the effect of the identified bacteria on physiological in vitro Cervix Chip. A BV consortium containing E2 and E4 *Gardnerella vaginalis* bacteria was co-cultured with the Cervix Chip for 48 hours before adding human sperm samples. The motility of fluorescently labeled sperm was non-invasively evaluated in live chips using time-lapse imaging and quantitatively analyzed to obtain the percentage of motile sperm.

Main results and the role of chance: We initially analyzed the effects of bacterial co-cultur *L. crispatus* (optimal) or *G. vaginalis* (suboptimal) bacteria on sperm motility using a conventional 2D culture system. We observed a significant drop in sperm motility within 2 hours when exposed to *G. vaginalis* compared to *L. crispatus* or the control condition (no bacteria). We have established sperm staining and live tracking on Cervix Chip. Motile sperm was tracked on Cervix Chip for more than 7 days compared to 12 hours in suspension. We then tested this effect on-chip and observed that co-culture of live sperm with dysbiotic Cervix Chip (infected with *G. vaginalis*) resulted in a significant reduction in the sperm motility within 24 hours after exposure compared to the sperm exposed to the chips without bacteria. The dysbiotic Cervix Chip also exhibited a disease phenotype with elevated levels of proinflammatory cytokines including IL-1α, IL-1β, IL-6, IL-8, and TNF-α in the effluents of the chip's epithelial channel as well as significantly reduced thickness of the cervical mucus layer that correlated with reduced sperm motility. This finding is consistent with the observation that successful fertility is often associated with the quality of cervical mucus, which can be compromised in the BV dysbiotic condition.

Limitations, reasons for caution: Primary cervical epithelium cells used in the study were sourced from a single donor. Frozen sperm samples prepared for intrauterine insemination were used in the study

Wider implications of the findings: Our study suggests that infertility related to BV may be caused by sperm dysfunction, and BV screening should be considered in patients with unexplained infertility. We propose a model that can be used to identify dysbiotic conditions in the female reproductive tract that are unfavorable to sperm motility.

Trial registration number: N/A

Abstract citation ID: dead093.020

O-020 What is the recovery time for sperm parameters in men who have suffered a mild Covid-19 infection?

R. Nunez - Calonge[1], J.A. Guijarro[2], T. Rubio[3], P. Alberola[4], G. Fernández[5], N. Santamaria[6], S. Alonso[7], I. Barros[8]

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Abstract under embargo

SELECTED ORAL COMMUNICATIONS
SESSION 05: TRUST THE MENSTRUAL CYCLE - PROGESTERONE OR NOT?
Monday 26 June 2023 Hall D4 10:00 - 11:30

Abstract citation ID: dead093.021

O-021 The dose of micronised vaginal progesterone prior to frozen embryo transfer in artificially prepared cycles: the more the better?

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Study question: Does a higher daily dose of micronized vaginal progesterone (MVP) increase live birth rates (LBR) or diminishes risk of early pregnancy loss (EPL)?

Summary answer: An increased dose of MVP does not affect reproductive outcomes.

What is known already: Endometrial preparation in frozen embryo transfer (FET) cycles can be achieved either in a natural cycle (NC), a stimulated cycle or an artificial cycle (AC). Despite growing advocacy for utilization of NC for FET mainly because of pregnancy complications, there are still numerous indications and strengths of AC, such as convenient monitoring and planning. In AC-FET cycles exogenous estradiol (E2) and progesterone (P) are administered consecutively in order to mimic a natural cycle. Although MVP still being the most widely used medication for LPS in AC-FET cycles, dose finding studies are lacking.

Study design, size, duration: This is a retrospective study performed at a university-affiliated centre including patients performing a single blastocyst transfer from January 2016 till June 2021. Pregnancy outcomes were compared between patients receiving 600mg (3x200mg) or 800mg (2x200mg) of MPV in the second phase of an AC-FET cycle. The time period was chosen to incorporate the empiric change made in the inner guidelines regarding artificial cycles LPS in autumn 2018.

Participants/materials, setting, methods: Patients aged 19-39, who underwent the first frozen single blastocyst transfer were included. Only HRT cycles, where after exogenous estradiol endometrium preparation LPS was started with MVP 600mg or 800mg were included. Patients lost to follow-up, with missing data, with known uterine pathology, recurrent miscarriages, with switch to MNC, with more than one progesterone route planned or modified LPS were excluded.

Main results and the role of chance: 1825 artificial cycles for FET were included. 827 supplemented with 600mg of MVP -MVP600 group and 998 with 800mg of MVP – MVP800 group. The MVP600 and MVP800 groups did not differ in BMI 24.02 (SD 4.73) vs 23.81 (SD 4.6) ($p = 0.37$), rank of embryo transfer 1.93 (SD 1.41) vs 1.77 (SD1.19) ($p = 0.11$), or the thickness of the endometrium at planning 8.67mm (SD1.79) vs 8.51mm (SD 1.7) ($p = 0.06$). Yet, MVP 800mg group was slightly older than MVP600 group 31.99 (SD 3.72) vs 30.84 (SD 3.9) ($p < 0.001$). Positive hCG was 58.65% (485 out of 827) for MVP600 and in 62.12% (620 out of 998) for MVP800 group ($p = 0.13$). LBR per positive hCG 64.18% (292 out of 455) in MVP600 and 67.17% (397 out of 591) in MVP800 group ($p = 0.31$). Early pregnancy loss per positive hCG did not differ between the groups, and including biochemical losses, was 32.75% in MVP600 (149 out of 455) vs 30.8% in MVP800 (182 out of 591) ($p = 0.5$). Multivariable regression analysis adjusting for relevant confounders (eg. BMI, endometrium thickness) revealed that the dose of

MVP 600mg vs 800mg is not significantly associated with EPL OR 0.92 (95% CI 0.71-1.22) p > 0.592.

Limitations, reasons for caution: The main limitation is the retrospective design of our study, with an inherent risk of bias. Furthermore, we could not compare the blood progesterone values at the day of the transfer as in MVP600 they were not routinely performed, so the need for additional LPS could not be compared.

Wider implications of the findings: This is the largest study dose finding study comparing the two progesterone administration doses. The higher dose of MVP does not improve reproductive outcomes, but might be more convenient due to a twice daily application.

Trial registration number: BUN: 1432022000074 EC number: EC-2022-102

Abstract citation ID: dead093.022

O-022 Shall we go "natural" for endometrial preparation in egg donation programs? Live birth rates and obstetrical outcome from 797 frozen single-blastocyst transfers cycles

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Study question: Frozen single blastocyst transfer in modified natural cycle (mNC) increases success rates reducing obstetric risks in egg recipients, compared to Hormone Replacement Therapy (HRT) protocols?

Summary answer: In egg recipients, single-blastocyst transfer in mNC showed higher pregnancy rates, lower miscarriage rates, higher new-born rates compared to HRT, being preterm birth rate comparable.

What is known already: When choosing endometrial preparation protocols for egg recipients, we must consider medication safety and costs, cycle's cancellation rate, clinical outcomes as maternal and fetal risks. The superiority of mNC over HRT for frozen embryo transfers (FET) has been reported in terms of delivery and miscarriage rates as well as obstetrical complications, but few data are available on egg recipients, being HRT protocols the routine treatment in most cases.

In donor programs, when a blastocyst freeze-all strategy or egg banking is applied, there is no longer need for donor-recipient synchronization and many FET could be performed in mNC cycles.

Study design, size, duration: Retrospective single-centre comparative clinical study, including 797 frozen single-blastocyst transfer (FSBT) initiated cycles between January 2020 and December 2021: 590 (74%) in HRT protocol and 207 (26%) in mNC protocol.

The two groups were homogeneous for donor's age (25,4 yo HRT Vs 25,8 yo mNC), recipient's age (42,1 yo HRT Vs 42,4 yo mNC), severe male factor (6% HRT Vs 4% mNC), and the need for a sperm donor (28% HRT Vs 22% mNC).

Participants/materials, setting, methods: Among 797 started cycles, we compared cancellation rates between HRT and mNC groups due to either medical reasons (inadequate endometrium, bleeding, ovulatory leak) or other issues (no embryo survival, flight cancellations, COVID-19 restrictions).

We studied the clinical outcomes of the 683 FSBT performed, comparing HRT and mNC cycles for pregnancy rate, clinical pregnancy rate, miscarriage rate, live birth rate, and preterm birth rate <35 weeks. We used Chi-square test to compare groups (p < 0,05).

Main results and the role of chance: CANCELLATION RATES: Out of the 797 endometrial preparation cycles started, the overall cancellation rate was 14% from which 72,8% due to medical reasons (inadequate endometrium, bleeding, ovulatory leak) and 27,2% due to other issues (no embryo survival, SARS-Cov-2 related issues, flight cancellations, etc.); when evaluating cancellation for medical reasons, we didn't find significant differences between HRT protocol cycles (9%) and mNC protocol cycles (7%), p = 0,33.

CLINICAL OUTCOMES: Out of the 683 frozen single blastocyst transfers performed, no twin pregnancies occurred as expected.

There were no statistically significant differences between HRT Vs mNC endometrial preparation protocol when comparing pregnancy rates (59% Vs 63%, p = 0,35), clinical pregnancy rates (49% Vs 57%, p = 0,10) and miscarriage rates (19% Vs 16%, p = 0,48). Live birth rate was significantly higher in the mNC (47%) compared to HRT (38%), p = 0,046.

Overall preterm birth rate <35 weeks was 3,9%, including <32 weeks 1,1% and <28 weeks 0,3%. There were no significant differences between HRT and mNC endometrial preparation protocol for preterm birth rate <35 weeks (3,6% Vs 4,6%, p = 0,75).

Limitations, reasons for caution: Single-centre retrospective comparative trial. Homogeneity studies included several confounding variables, but other bias could have been studied such as uterine factor, PGT-A rate or previous fertility treatments.

Wider implications of the findings: In donor programs, the use of modified natural cycle should be encouraged as first option irrespectively from recipient's age for FET in normo-ovulatory patients, considering the increased live birth rates when compared to HRT-FET cycles. Moreover, mNC cycles are more patient's friendly and cost-effective.

Trial registration number: not applicable

Abstract citation ID: dead093.023

O-023 'The PiNC Trial': Progesterone in Natural Cycles for the treatment of unexplained infertility

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Abstract under embargo

definitive evidence exists regarding the optimal timing of transferring D6 blastocysts. Interestingly, a few studies have reported better reproductive outcomes if D6 blastocysts are transferred on the 5th day of progesterone administration instead of the 6th day of progesterone administration.

Study design, size, duration: This cohort study included a total of 718 vitrified D6 single blastocyst HRT-FET cycles performed between 2013 and 2022. 576 blastocysts were transferred on the 6th day of progesterone administration (2013-2021), and 142 blastocysts transferred on the 5th day of progesterone administration (2021-2022). Main outcomes were biochemical pregnancy rate (PR), implantation rate (IR), clinical pregnancy rate (CPR), and early pregnancy loss rate (EPLR).

Participants/materials, setting, methods: Patients undergoing HRT-FET in a public Fertility Clinic. Endometrial preparation included oral oestradiol (6mg/24hours), followed by vaginal micronized progesterone. As mentioned above, single blastocyst transfer was performed on either the 5th or 6th day of progesterone administration. Serum β-hCG level was measured 9-11 days after embryo transfer and biochemical pregnancy was defined as an hCG level >10IU/L. Clinical pregnancy was defined as visualization of a gestational sac and fetal heartbeat in gestational week 7.

Main results and the role of chance: In this cohort of a total of 718 HRT-FET cycles transferred on either the 5th or 6th day of progesterone administration the mean age of the patients at embryo transfer was 34.5 ± 4.5 years vs. 33.0 ± 4.9 years, respectively, p = 0.30. There was no significant difference between numbers of top-quality blastocyst transfers within the two groups 63% (89/142) vs. 64% (369/576), p = 0.76.

The overall, biochemical pregnancy rate (PR), implantation rate (IR), clinical pregnancy rate (CPR) and early pregnancy loss (EPLR) in the group of patients transferred on the 5th day of progesterone administration were 38% (54/142), 29% (41/142), 25% (36/142) and 33% (18/54). In the group of patients transferred on the 6th day of progesterone administration 41% (235/576), 33% (191/576), 30% (170/576) and 28% (65/235).

There were no significant differences between PR, IR, CPR and EPLR when transferring D6 vitrified blastocysts on either the 5th or the 6th day of progesterone administration (38% vs. 41%, p = 0.53, 29% vs. 33%, p = 0.33, 25% vs. 30%, p = 0.33 and 33% vs. 28%, p = 0.41).

Limitations, reasons for caution: Limitations of the study are the retrospective design and the limited number of D6 vitrified blastocysts transferred on the 5th day of progesterone administration. Furthermore, the two groups represented two different time periods.

Wider implications of the findings: Growing evidence shows that D5 vitrified blastocysts have better reproductive outcomes than slower developing embryos which are vitrified on day 6. Whether this is caused by intrinsic embryonic factors or poor timing of transfer in a subsequent FET cycle still needs to be unraveled.

Trial registration number: not applicable

Abstract citation ID: dead093.024

O-024 Exploring the optimal transfer day of day 6 (D6) vitrified blastocysts in Hormone Replacement Therapy Frozen Embryo Transfer (HRT-FET) cycles - a cohort study

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Study question: Should blastocysts cryopreserved D6 be transferred on the 5th or 6th day of progesterone administration in HRT-FET cycles?

Summary answer: No significant differences in outcome parameters were found when D6 vitrified blastocysts were transferred on either day 5 or day 6 of progesterone in HRT-FET.

What is known already: Several studies have shown that D6 blastocyst transfer should be avoided in fresh transfer IVF cycles; consequently, these more slowly developing embryos are transferred in a HRT-FET cycle on the 6th day of progesterone administration in line with day 5 (D5) blastocysts. For successful implantation, a receptive endometrium and optimal synchronization between the endometrium and embryo are important. However, no

Abstract citation ID: dead093.025

O-025 Livebirth delivery rates after natural versus artificial cycle in women receiving donated oocytes and the impact of female age.

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Study question: Is female age associated with worse outcomes following embryo transfer in either a natural cycle (NC) or artificial cycle (AC) in women receiving donated oocytes?

Summary answer: NC embryo transfers resulted in both lower miscarriage and higher livebirth rates, and the age of the female recipient did not modify this effect.

What is known already: Previous studies have demonstrated that NC may result in better outcomes than AC embryo transfer. When feasible, avoiding AC in advanced maternal age and/or couples using donated oocytes could be considered, given the potential compounding effect for certain negative maternal and perinatal outcomes such as gestational hypertension. However, there is limited research on the use of NCs in women of advanced maternal age and research thus far has shown that the luteal phase of the menstrual cycle in older women may be altered, thus potentially limiting the effectiveness of NC.

Study design, size, duration: This retrospective, multicentre, cohort study included all single blastocyst embryo transfers following oocyte donation performed between January 2010 and December 2019, subdivided according to the type of endometrial preparation performed (NC or AC). The oocyte donation model was preferred to minimize the potential confounding effect related to both poor embryo quality in older women and the influence that ovarian stimulation performed during autologous IVF may have on endometrial receptivity prior to a fresh embryo transfer.

Participants/materials, setting, methods: The main objective of the study was to compare livebirth delivery rates (LBR). The secondary outcomes included hCG positive pregnancy rate, clinical pregnancy rate (CPR) and miscarriage rate. Confounder-adjustment was performed using multivariable generalized estimating equations model regression analysis, adjusting for multiple confounders. Additionally, an interaction variable was added to the final multivariable model to assess whether female recipient age may modify the effect of each type of endometrial preparation on LBRs.

Main results and the role of chance: In total, 38259 embryo transfers were analysed, including AC (n = 34850) and NC (n = 3409). Mean recipient age was 42 years, with a range of 20-50 years. The results showed that despite being associated with a higher hCG positive pregnancy (57.1% vs 53.5%; OR 0.91 CI 0.84-0.98), AC were also associated with a higher miscarriage rate per hCG positive pregnancy (33.5% vs 27.9%; OR 0.70 CI 0.62-0.78) when compared to NC, resulting in slightly lower LBRs per transfer with AC (38.0% vs 38.6%; OR 1.13 CI 1.05-1.21). All primary and secondary outcomes were still significant following confounder adjustment. Confounder variables included were female donor and recipient age, recipient BMI, female factor infertility, male factor infertility, number of mature oocytes donated, oocyte status (fresh versus vitrified), sperm source (partner or donor), embryo status (fresh versus vitrified), embryo quality, endometrial thickness, and year of transfer. The interaction of endometrial preparation method with female recipient age was not statistically significant (aOR 1.02, 95% CI 0.99-1.05).

Limitations, reasons for caution: The retrospective nature of the study and the inherent risk of bias related to confounding may have impacted the results. Another limitation is the low numbers of natural cycles (9% of all cycles), which could be related to the low uptake to the treatment and/or to selection bias.

Wider implications of the findings: NC seems slightly better than AC and the differences between the two are unlikely to be modified by female age. Therefore, it seems reasonable to suggest NC for older women, as they would benefit from the decreased risk of miscarriage and hypertension during pregnancy.

Trial registration number: not applicable

Abstract citation ID: dead093.026
O-026 A new mNC protocol that allows a 7-day window for FET planning

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Study question: Is triggering necessary at 17 mm follicle diameter in a modified natural cycle (mNC), or does it allow flexible planning?

Summary answer: Embryo transfer can be scheduled when follicles measure 13 to 20 mm if endometrium is ready without impacting clinical outcome.

What is known already: Current practice is shifting, moving frozen embryo transfers (FET) from artificial cycles to natural cycles, which may complicate planning. The standard mean diameter to trigger in a mNC has classically been stablished in 17 mm, mimicking the size needed to obtain mature oocytes. Interestingly, research on triggering at different follicle sizes in a mNC has been limited. A previous study initiated progesterone based only on endometrial ultrasonographic characteristics and the presence of a dominant follicle of at least 12 mm and showed good results in ongoing pregnancy rates. However, it had a small sample size and rhCG was not administered.

Study design, size, duration: This is a multicenter, retrospective, observational study of 3,087 single frozen blastocyst transfers in mNCs carried out in 2,764 patients at our centers from January 2020 to September 2022.

Participants/materials, setting, methods: Selection criteria were the following: blastocyst on day 5/6 (minimum quality 3BB attending Gardner classification), regular menstrual cycles (26-35 days), normal uterine cavity assessed by ultrasound, serum progesterone <1.5 ng/mL and endometrial thickness ≥7 mm on the day of administration of rhCG, and absence of fluid in endometrial cavity. Triggering was done with a single dose of 250 µg sc rhCG, natural micronized progesterone 200mg bid was started two days later, then SET was performed.

Main results and the role of chance: Follicle size at time of triggering was stratified into three groups (13.0–15.9 mm; 16.0–18.9 mm; and ≥19.0 mm). No differences were seen regarding age, body mass index (BMI) and years of infertility, however, there were differences regarding egg donation (39.5%; 27.9%; and 27.4% respectively; p = 0.02) and the use of Preimplantational Genetic Testing for Aneuploidies (PGT-A) (19.4%; 34.01%; and 37.3%; p < 0.01). We found no differences in pregnancy rate (64.5%; 60.2%; and 57.4%; p = 0.19), clinical pregnancy rate (60.5%; 52.8%; and 50.6%; p = 0.10), implantation rate (62.10%; 52.9%; and 51.0%; p = 0.05) and miscarriage rate (15.0%; 22.2%; and 25.0%; p = 0.11), but differences were found in the ongoing pregnancy rate (OPR) (54.9%; 46.8%; and 43.1%; p = 0.02). however, those differences were not seen after adjusting for the use of PGT-A and egg donation: OPR at 16.0–18.9 mm vs 13.0–15.9 mm (aOR 2.37; 95% CI: 0.73–7.60; p = 0.15) and at 16.0–18.9 mm vs > 19mm (aOR 0.75; 95% CI: 0.54–1.05; p = 0.10).

Finally, OPR was assessed by follicle size by each millimeter from 13 mm (80,0%; 95% CI 29.9—99.0%) to 22 mm (54.6%; 95% CI 39.0—69.3%).

Limitations, reasons for caution: Follicle size at time of triggering 15 to 19 mm accounted for 84.7% of the mNCs included in this study, which leaves only a minority of cases in which triggering was done at "non conventional" follicle sizes. This results need to be confirmed by future prospective studies.

Wider implications of the findings: Our findings show that rhCG could be administrated from a follicle size of 13 to 22mm. Considering a follicular growth rate of 1-1.5mm per day, this approach could allow a flexibility of five to seven days, facilitating the planning of mNC FET in clinical practice.

Trial registration number: Not applicable

SELECTED ORAL COMMUNICATIONS

SESSION 06: ENDOMETRIOSIS: FROM PATHOGENESIS TO TREATMENT STRATEGIES

Monday 26 June 2023 Auditorium 10-12 10:00 - 11:30

Abstract citation ID: dead093.027
O-027 Efficacy results from a phase 3, randomized, placebo-controlled trial testing two doses of linzagolix in women with endometriosis-associated pain

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Study question: Does once-daily linzagolix for up to 6 months reduce the pain symptoms in women with endometriosis?

Summary answer: Once-daily oral use of the GnRH agonist linzagolix alone or in combination with combined hormonal add-back therapy (ABT) for 24 weeks improved endometriosis-associated pain symptoms.

What is known already: Linzagolix is an oral GnRH antagonist under development to treat endometriosis-associated pain (EAP). In the EDELWEISS 3 study linzagolix 200mg+ABT met its co-primary endpoints at 3 months by reducing dysmenorrhea (DYS) and non-menstrual pelvic pain (NMPP) in a clinically meaningful fashion along with a stable or decreased use of analgesics. Linzagolix 75mg significantly reduced DYS but not NMPP. Secondary endpoints were DYS, NMPP, overall pelvic pain, dyschezia, and dyspareunia at 6 months assessed with a numeric and/or verbal rating scale (NRS and/or VRS), and interference of pain with daily activities assessed with the Endometriosis-Health-Profile-30 (EHP-30) pain domain.

Study design, size, duration: EDELWEISS 3 was a randomized, double-blind, placebo-controlled Phase 3 study, investigating efficacy and safety of linzagolix 75mg and linzagolix 200mg with ABT (1mg estradiol/0.5mg norethindrone acetate) for up to 24 weeks in women with moderate to severe EAP. To qualify, subject had to demonstrate over 2 menstrual cycles at least moderate DYS and NMPP for $\geq$2 days each and at least moderate overall pelvic pain for $\geq$5 days.

Participants/materials, setting, methods: Eligible women with moderate to severe EAP were equally randomized to once daily oral: placebo, linzagolix 75mg or linzagolix 200mg+ABT. Efficacy assessments included DYS, NMPP, dyspareunia (assessed with a 4-point VRS), overall pelvic pain and dyschezia (assessed with an 11-point NRS), and interference of pain with daily activities (assessed with the EHP-30 pain domain). The co-primary endpoint was a responder analysis at Month 3. We present secondary endpoints at Month 6.

Main results and the role of chance: Participants (n = 484) had a mean age of 35 years, a BMI of 24kg/m^2 with 99% of subjects being White.

At Month 6, compared to placebo significant improvements in DYS and NMPP (VRS) were observed in the 200mg+ABT group, with an estimated mean change from baseline (CfB) of -1.83 (95%CI: 1.96, 1.70; difference with placebo $p < 0.001$) and 0.92 (95%CI: 1.03, 0.82; $p = 0.002$), respectively. For the 75mg group, the mean CfB was 1.10 (95%CI: 1.23; 0.97; $p < 0.001$) and 0.84 (95%CI: 0.95; 0.73; $p = 0.048$), respectively.

For overall pelvic pain (NRS), marked improvements were observed with an estimated mean CfB of 3.39 (95%CI: 3.74, 3.03; $p < 0.001$) for 200mg+ABT and 2.84 (95%CI: 3.20, 2.48; $p = 0.024$) for 75mg.

Similarly, dyschezia (NRS) was significantly improved for both groups with a mean CfB of 1.99 (95%CI: 2.29, 1.70; $p = 0.012$) for 200mg+ABT and -1.98 (95%CI: 2.28, 1.69; $p = 0.015$) for 75mg.

Both doses offered a marked, statistically significant improvement in the interference of pain with daily activities as measured on the EHP-30 Pain Dimension, with an estimated mean CfB of 35.60 (95%CI: 38.73, 32.48; $p < 0.001$) and -27.37 (95%CI: 30.50, 24.25; $p = 0.001$) for the 200mg+ABT and 75mg group, respectively.

Both doses did not provide significant improvements in dyspareunia.

Limitations, reasons for caution: We report data of the EDELWEISS 3 study for up to Month 6. An extension study of this trial will provide more information on the sustained effect and potential symptom recurrence after stopping treatment.

Wider implications of the findings: The linzagolix 200mg+ABT group provided substantial improvement in endometriosis-associated pain symptoms. The 75mg dose was less effective but demonstrated still marked improvements. There exists an substantial need for different treatment regimens in women suffering from endometriosis.

Trial registration number: NCT03992846

Abstract citation ID: dead093.028

O-028 Genital tract infection and pelvic surgery contribute to the development of endometriosis

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Study question: Was the incidence of endometriosis in women with a recent history of genital infection, pelvic surgery, or both increased?

Summary answer: The pelvic inflammation resulting from genital infection and pelvic surgical injury may play a role in developing endometriosis.

What is known already: Endometriosis is a multifactorial disease, and inflammation is considered a core pathology. Inflammation occurs due to pathogenic infection and tissue injury, and genital tract infection and surgical injury can be inducers for pelvic inflammation in endometriosis.

Study design, size, duration: A retrospective cohort study using the Korean National Health Insurance Service -National Sample Cohort 1 (KNHIS-SC I) from 2002-2013 (N = 1,025,340) was performed.

Participants/materials, setting, methods: 20- to 49-year-old women diagnosed with genital tract infections (GTI) or who underwent pelvic surgeries between 2002 and 2008 were collected and followed up for five years. A total of 30,336 women were diagnosed with GTI, 2,894 women underwent pelvic surgery, and 788 women with GTI and pelvic surgery were enrolled in each study. The comparison groups matched sociodemographic factors for each group were collected. The incidence of endometriosis in each group was analyzed.

Main results and the role of chance: The incidence of endometriosis per 1,000 person-year was 5.37, 5.17, and 20.81 in each case group and was significantly higher than in each comparison group. A recent history of GTI increased an adjusted hazard ratio (aHR) of 2.29 (1.99-2.63, 95% confidence interval) for the development of endometriosis. The aHRs of pelvic surgery history and the history of both GTI and pelvic surgery were 2.10 and 7.82, respectively.

Limitations, reasons for caution: The diagnosis and treatment codes registered on the NHIS data are used to claim HIRA NHI benefits, which may differ from the diagnosis or treatment performed in practice and it does not contain the used diagnostic method, disease severity, and the disease led to pelvic surgery were not specified.

Wider implications of the findings: Active treatment of genital infections and careful surgical procedures to minimize tissue injury may reduce the incidence of pelvic endometriosis.

Trial registration number: Not Applicable

Abstract citation ID: dead093.029

O-029 The role of non-coding RNAs in endometriosis diagnosis: A systematic review and meta-analysis

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Study question: Can non-coding RNAs (ncRNAs) serve as sensitive biomarkers to differentially diagnose endometriosis accurately and precisely?

Summary answer: Non-coding RNAs appear to be promising biomarkers for endometriosis diagnosis, providing adequate diagnostic efficiency, especially when co-evaluated as a model and not as single markers.

What is known already: Endometriosis is a chronic, inflammatory gynecological disease characterized by endometrial-like tissue development outside the uterus. This benign, estrogen-dependent inflammatory disorder affects 6–10% of women of reproductive age and over 40% of patients with endometriosis present with infertility. Despite advances, endometriosis diagnosis remains challenging, and it is mainly relied upon surgical assessment, commonly via laparoscopy. The invasive nature of endometriosis diagnosis could be avoided if sensitive biomarkers for endometriosis deferential diagnosis were available. In the context of developing non-invasive diagnostic methods, the role of ncRNAs has been investigated and this study aims to collectively synthesize and critically analyze existing evidence.

Study design, size, duration: A systematic review was performed in PubMed/Medline and Embase up to April 2022 followed by a meta-analysis. Original, full-text articles in English comparing the diagnostic validity of ncRNAs versus conventional methods for endometriosis diagnosis were included. Study inclusion requirements were the following: presenting detailed information regarding the tissue where ncRNAs were isolated, methods employed for ncRNA detection, and finally inclusion of data on ncRNA diagnostic efficiency, namely sensitivity, specificity, and area under the curve (AUC).

Participants/materials, setting, methods: The studied population consisted of women with endometriosis confirmed via conventional diagnostic methods, including laparoscopy and histology. The control group consisted of women without endometriosis. Data indicating ncRNA diagnostic efficiency, including sensitivity, specificity, and AUC were extracted. Following extraction, data were meta-analyzed employing univariate analysis with Clopper-Pearson. Confidence intervals were calculated employing the random-effects model. Bivariate analysis to plot the summary receiver operating characteristic (SROC) curve was also employed. R Programming Language was used.

Main results and the role of chance: Twenty-six studies were included and 64 ncRNAs were analyzed. Included studies were categorized into two groups. In the first group the diagnostic efficiency of single ncRNAs was evaluated. In the second group the diagnostic efficiency of models comprising of two or more ncRNAs was assessed. Considering the first group, two ncRNAs, namely miR-200c and miR-199a, differentially diagnosed endometriosis with an AUC of 1.000. Meta-analysis revealed that the total diagnostic efficiency of single ncRNAs is characterized by an AUC of 0.783, sensitivity 78.0% (95%CI:72.3-82.8%), specificity 73.8% (95%CI:67.8-79.1%), Positive Predictive Value (PPV) 81.1% (95%CI:77.1–84.5%), and Negative Predictive Value (NPV) 69.5% (95%CI:62.4–75.9%). Sensitivity analysis presenting the total diagnostic efficiency of single ncRNAs evaluated from two or more individual studies was performed. The total AUC was reported to be 0.824, sensitivity 81.8% (95%CI:72.0–88.7%), specificity 80.0% (95%CI:68.8-87.9%), PPV 87.6% (95%CI:80.3–92.4%), and NPV 72.2% (95%CI:57.4–83.3%). Regarding the second group, the ncRNA model comprised of miR-125b-5p, miR-451a and miR-3613-5p reporting the highest diagnostic efficiency, with an AUC of 1.000. Meta-analysis revealed that the total diagnostic efficiency of ncRNA models is characterized by an AUC of 0.896, sensitivity 86.8% (95%CI:81.9–90.5%), specificity 80.3% (95%CI:68.9-88.2%), PPV 88.3% (95%CI:82.2–92.5%), and NPV 78.0% (95%CI:68.2–85.4%).

Limitations, reasons for caution: The main limitation is the high heterogeneity observed among most of the studied outcomes. This is mainly attributed to the fact that the number of the participants significantly ranged between the studies. Another reason for caution is the high heterogeneity observed regarding the methods employed for ncRNA profiling and validation.

Wider implications of the findings: Data presented herein indicate that ncRNAs appear as highly promising non-invasive biomarkers for endometriosis diagnosis, especially when two or more ncRNAs are co-evaluated as a model. Larger scale studies should focus on developing artificial intelligence based diagnostic algorithms incorporating ncRNAs profiling coupled by molecular, biochemical, and imaging diagnostic data.

Trial registration number: Not applicable

Abstract citation ID: dead093.030

O-030 Gut microbiome in endometriosis: a cohort study on 1000 individuals

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Study question: Do gut microbial composition and functionality differ between women with and without endometriosis?

Summary answer: Gut microbiome diversity and composition (species and microbial pathways) were not significantly different between women with and without endometriosis.

What is known already: Endometriosis, defined as the presence of endometrial-like tissue outside of the uterus, is one of the most common female reproductive disorders. Although different theories of the possible causes of endometriosis have been proposed, its pathogenesis is not clear. Novel studies indicate that the gut microbiome may be involved in the etiology of endometriosis, nevertheless, the connection between microbes, its dysbiosis and the development of endometriosis remains unexplored. This study aims to analyze and compare the gut microbiome profile in women with and without endometriosis in a large cohort to identify microbial targets potentially involved in the development of the disease.

Study design, size, duration: This case-control study included a subsample of 1000 women (age = 45.61 ± 10.36 years; BMI = 25.67 ± 5.59) of the Estonian Microbiome (EstMB) cohort, a volunteer-based sub-cohort of the Estonian Biobank created in 2017. 136 women with endometriosis and 864 control women who have not been diagnosed with endometriosis or any of its most prevalent comorbidities (systemic lupus erythematosus, rheumatoid arthritis, autoimmune thyroiditis, celiac disease, multiple sclerosis and irritable bowel syndrome) were included in this study.

Participants/materials, setting, methods: Microbial DNA from fecal samples was extracted and sequenced by paired-end metagenomic shotgun sequencing (Illumina Novaseq 6000 platform). Microbial functional pathways were annotated using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (https://www.genome.jp/kegg/). Partitioning around medoids (PAM) algorithm was performed to cluster the microbial profile of the Estonian population. The alpha- and beta-diversity and differential abundance analyses were performed to assess the gut microbiome (species and KEGG orthologies [KO]) in both groups.

Main results and the role of chance: After metagenomics analysis, 17180 microbes and 7869 KO were detected. Those bacteria and KO with a relative abundance > 1 % were used for the diversity and differential abundance analyses, resulting in 2442 species and 1974 KO. PAM clustering analysis stratified the study population into two enterotypes: one characterized by a high abundance of *Prevotella copri* and the second presented a high abundance of *Bacteroides* genus (PERMANOVA, p = 0.001). However, the enterotypes were not associated with the presence/absence of endometriosis. Microbial alpha-diversity (observed richness and Shannon's index) was not significantly different between women with and without endometriosis (all p-value > 0.05). Beta-diversity analyses on the microbial and functional profile (species and KO profile) indicated no significant dissimilarity between groups

(PERMANOVA, all $p > 0.05$). No differential species nor KO were detected after multiple testing adjustment (all FDR $p > 0.05$).

Limitations, reasons for caution: This case-control study did not identify a distinct gut microbial profile in women with endometriosis. A deeper analysis considering potential confounders (specifically hormonal treatment in patients) is needed to further confirm our results.

Wider implications of the findings: Endometriosis is a widespread disorder affecting ~10% of reproductive-age women. To the best of our knowledge, this is the biggest metagenome study performed in women with endometriosis. Our findings do not find enough evidence to support the existence of a gut microbiome-dependent mechanism implicated in the pathogenesis of endometriosis.

Trial registration number: Not applicable

Abstract citation ID: dead093.031

O-031 The natural history of endometriosis in pregnancy: An ultrasound study of the morphological features of deep endometriosis (DE) and ovarian endometrioma

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Study question: What is the impact of pregnancy on the morphological features and behaviour of ovarian endometrioma and deep endometriotic nodules?

Summary answer: For the majority of women, despite features of decidualization being common in the first and second trimesters, endometrioma and deep nodules will regress during pregnancy.

What is known already: Deep endometriosis and endometrioma subtypes are thought to affect approximately 5% of women in pregnancy, with about 50% being unaware of their condition. Pregnancy has a major effect on the size and morphological features of endometrioma, with published studies reporting a tendency for cyst regression. Decidualization, a hormonally induced pregnancy-related phenomenon, effects endometriomas and may raise suspicion of an ovarian malignancy. The behaviour of deep endometriosis in pregnancy is poorly understood and there is limited available literature on the subject.

Study design, size, duration: This was a prospective observational cohort study conducted over three years at a single centre. We included 65 women with a viable eutopic pregnancy and concomitant ultrasound features of deep and/or ovarian endometriosis. The study was conducted at University College London Hospital, which is a tertiary level referral unit for early pregnancy complications and an accredited Endometriosis Centre.

Participants/materials, setting, methods: All women who participated provided written consent and were invited for surveillance ultrasound examinations at the time of their routine scans in pregnancy. All scans were performed by a single operator to minimise interobserver error. The change in size of endometrioma and nodules were reported as change in their mean diameter. Endometrioma with irregular thick inner walls, hyperechoic papillary projections and/or high vascularity and hyperechoic nodules with moderate to high vascularity were reported as decidualized.

Main results and the role of chance: Sixty five women were included in the study. Their median age was 34 years (23-44), and the gestation at presentation was 7 + 6 weeks (3 + 6 to 18 + 0). 47/65(72%) were nulliparous, 48/65(74%) had a background of endometriosis and 19/65(29%) conceived following IVF. There were 10/65(15%, 95%CI 7-24) women with endometrioma alone, 28/65(43%, 95%CI 31-55) with nodules alone and the remaining 27/65(42%, 95%CI 30-54) had both.

29/34(85%, 95%CI 73-97) women with endometrioma experienced cyst regression, 2/34(6%, 95%CI 0-14) experienced cyst growth and in 10/34(29%, 95%CI 14-45) there was complete resolution of all cysts. 43/51(84%, 95%CI 74-94) women with nodules experienced nodule regression, 2/51(4%, 95%CI 0-9) experienced nodule growth and in 4/51(8%, 95%CI 0-15) there was complete resolution of all nodules. 5/37(14%, 95%CI 3-25) women who attended postnatal follow-up, experienced complete resolution of all endometriotic lesions during pregnancy .

In 10/34(29%, 95%CI 14-45) women with endometrioma and 27/51(53%, 95%CI 39-67) with nodules, a pattern of growth was observed in the first and second trimesters, which preceded regression in later pregnancy.

Features of decidualization were observed in 17/34(50%, 95%CI 33-67) women with endometrioma, most commonly in the 1st trimester, and 25/51(49%, 95%CI 35-63) women with nodules, most commonly observed in the 2nd trimester.

Limitations, reasons for caution: The lack of extended follow-up fails to establish the long-term impact of pregnancy, lactation and postnatal contraception on the behaviour of endometriosis. This study relies on ultrasound alone for the detection of moderate/severe disease with no correlation with laparoscopy.

Wider implications of the findings: Sonographic changes of endometriosis in pregnancy are difficult to differentiate from characteristics of malignant lesions. Better understanding of the appearance of endometriosis in pregnancy is vital to reduce unnecessary surgical procedures, associated morbidity to mothers and babies and will help clinicians to counsel women regarding the significance of their condition.

Trial registration number: The study was registered on Research Registry (Unique identifying number: researchregistry4569).

Abstract citation ID: dead093.032

O-032 Effect of relugolix combination therapy in women with endometriosis-associated pain who received prior first-line hormonal treatment: SPIRIT 1 and 2

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Study question: To assess the effect of oral relugolix combination therapy (Relugolix-CT) in the subgroup of women with endometriosis-associated pain who received prior first-line hormonal treatment.

Summary answer: Relugolix-CT significantly reduced dysmenorrhoea and non-menstrual pelvic pain (NMPP) through 24 weeks in women with endometriosis-associated pain who received prior first-line hormonal treatment.

What is known already: ESHRE recommends hormonal contraceptives or progestogens as first-line therapy for endometriosis; however, these treatments may not be efficacious for all women. SPIRIT 1&2 were Phase 3, replicate, randomised studies of oral Relugolix-CT (relugolix 40 mg, estradiol 1 mg, norethisterone acetate 0.5 mg) in premenopausal women (aged 18–50 years) with moderate-to-severe endometriosis-associated pain. In SPIRIT 1&2, Relugolix-CT significantly improved dysmenorrhoea, NMPP and daily functioning (measured by the Endometriosis Health Profile-30 pain domain score) compared with placebo over 24 weeks. Relugolix-CT was well-tolerated and bone mineral density showed a minimal decline (<1%) from baseline to Week 24.

Study design, size, duration: At screening, women were required to have at least moderate menstrual and non-menstrual pain during their last cycle. In total, 1261 women with diagnosed endometriosis and moderate-to-severe dysmenorrhoea and NMPP at baseline were randomised 1:1:1 to receive once-daily Relugolix-CT or placebo for 24 weeks, or delayed Relugolix-CT (relugolix 40 mg then Relugolix-CT; 12 weeks each). Study candidates who received hormonal treatment for endometriosis completed a washout period prior to the screening and run-in period.

Participants/materials, setting, methods: Analyses were performed using pooled SPIRIT 1&2 data in women previously receiving first-line hormonal treatment (e.g. oral contraceptives and/or progestogens; N = 392). Co-primary endpoints: proportion of dysmenorrhoea and NMPP responders at Week 24. Responders were women achieving a predefined, clinically meaningful reduction from baseline in daily Numerical Rating Scale (NRS [0=no pain, 10=worst pain imaginable]) score and no increase in analgesic use. Least squares (LS) mean changes in NRS scores were assessed by a mixed-effects model.

Main results and the role of chance: In total, 121 women in the Relugolix-CT group and 134 in the placebo group had received prior first-line hormonal treatment, of which combined oral contraceptives and/or dienogest were the most common medications. Data for the delayed Relugolix-CT group were used for safety assessment only and are not reported here. Baseline demographics and clinical characteristics were comparable between the subgroup of women who received prior first-line hormonal treatment and the overall study population.

In this subgroup, the proportion of dysmenorrhoea responders with Relugolix-CT vs placebo at Week 24 was 70.2% vs 27.6%, respectively ($p < 0.0001$); the proportion of NMPP responders was 60.3% vs 40.3%, respectively ($p = 0.0013$).

In the Relugolix-CT group, LS mean NRS scores for dysmenorrhoea decreased from 7.2 (severe) at baseline to 1.7 (mild) at Week 24, with a significant change from baseline vs placebo ($p < 0.0001$). LS mean NRS scores for NMPP decreased from 5.6 (moderate) at baseline to 2.8 (mild) at Week 24 with Relugolix-CT, with a significant change from baseline vs placebo ($p = 0.0097$).

The proportion of women who were analgesic-free at baseline was 7.4% with Relugolix-CT and 3.7% with placebo; this increased to 48.8% with Relugolix-CT and 18.7% with placebo at Week 24 ($p < 0.0001$).

Limitations, reasons for caution: Assessment of endometriosis-associated pain at screening visit was done after the wash-out period; therefore, data for endometriosis symptoms experienced by women while receiving hormonal treatment prior to study entry, as well as the duration of the previous treatments, were not available.

Wider implications of the findings: Relugolix-CT significantly reduced dysmenorrhoea and NMPP, vs placebo, through 24 weeks in women with endometriosis-associated pain who received prior first-line hormonal treatment. The proportion of women in this subgroup who were analgesic-free increased through 24 weeks with Relugolix-CT. Results were comparable to the overall SPIRIT 1&2 population.

Trial registration number: NCT03204318; NCT03204331

SELECTED ORAL COMMUNICATIONS

SESSION 07: CHALLENGES IN COMPLEX DECISIONS: INFORMATION PROVISION AND PSYCHOSOCIAL CARE

Monday 26 June 2023 Hall D5 10:00 - 11:30

Abstract citation ID: dead093.033

O-033 A randomised control trial of an online decision-aid for women considering elective egg-freezing

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[8]University of Edinburgh, MRC Centre for Reproductive Health- Queens Medical Research Institute, Edinburgh, United Kingdom

Study question: Does a Decision-Aid for those considering elective egg-freezing impact decisional conflict, the decision-making process, and quality of the decision made, compared to existing information?

Summary answer: The Decision-Aid significantly reduced decisional conflict and improved preparedness for decision-making.

What is known already: Elective egg-freezing offers women the potential to extend their reproductive years. The decision to freeze eggs is complex, and our previous data shows that 78% of women had high decisional conflict (uncertainty) when they considered elective egg-freezing. Decision-Aids are the gold-standard for supporting complex health-related decisions. They have been shown to reduce decisional conflict and improve knowledge, risk perception, and decision alignment with personal values, when compared to standard care alone across a range of health conditions. We developed an online Decision-Aid to support women considering elective egg-freezing.

Study design, size, duration: A single-blinded, two-arm parallel group Randomised Control Trial. Participants were recruited September 2020-March 2021 (target n = 286). Randomisation was 1:1 to the intervention (Decision-Aid plus existing information) or control (existing information only) group, stratified by Australian state/territory and prior consultation with an IVF specialist about elective egg-freezing. Existing information was the Victorian Assisted Reproductive Treatment Authority website. Online surveys were completed at recruitment (baseline), 6, and 12-months.

Participants/materials, setting, methods: Australian women aged ≥18 years, considering elective egg-freezing, proficient in English, and with internet access were recruited using various methods including social media, Google advertising, newsletters, and clinic referrals. Participants completing the baseline survey were randomised (intervention n = 150, control n = 156) and emailed their allocated resources. Subsequent surveys were emailed 6 and 12-months post-randomisation. Surveys covered decisional conflict (primary outcome), distress, egg-freezing and fertility knowledge, decision made/not made, preparedness for decision-making, informed choice, and decisional regret.

Main results and the role of chance: Of 306 participants (mean age=30 years, SD: 5.2), 50% were single and 65% worked in professional occupations. At 6-months, the intervention group was substantially more prepared for decision-making than the control (Preparation for Decision-Making Scale; mean score difference: 9.22 [95% CI: 2.35, 16.08], $p = 0.009$). Overall, 77% of participants completed the primary outcome at 12-months (intervention n = 113, control n = 124). Between baseline and 12-months, the mean reduction in Decisional Conflict Scale (DCS) scores was greater for the intervention group than the control (mean score difference: -6.99 [95% CI: -12.96, -1.02], $p = 0.022$), whilst there were no group differences in distress (Depression Anxiety and Stress Scale; mean score difference: 0.61 [95% CI: -3.72, 4.93], $p = 0.783$), knowledge (study-specific scale; mean score difference: 0.23 [95% CI: -0.21, 0.66], $p = 0.309$) and whether a decision had been made about egg-freezing (odds ratio: 1.95 (95% CI: 0.67, 5.69), $p = 0.221$). No differences were observed between groups in informed choice (Multi-Dimensional Measure of Informed Choice; relative risk: 1.00 [95% CI: 0.81, 1.25]) or decision regret (Decisional Regret Scale; median score difference: -5.00 [95% CI: -15.30, 5.30]) for participants who had made their decision about egg-freezing at 12-months (intervention n = 48, control n = 45).

Limitations, reasons for caution: The total eligible population reached cannot be calculated due to the broad recruitment methods used. Adaption of some scales to improve relevancy may impact validity. Biases from self-selection and the use of self-reported data are possible, and some results had small sample sizes limiting the inferences made.

Wider implications of the findings: This is the first study to evaluate the effectiveness of a Decision-Aid for women considering elective egg-freezing. It demonstrates the benefit of a Decision-Aid in supporting women's decision-

making. Given these results, the Decision-Aid will be made publicly available. There is also potential to adapt the tool for international relevancy.

Trial registration number: ACTRN12620001032943

Abstract citation ID: dead093.034

O-034 Staff and patient evaluation of fertility staff performance during sharing bad news conversations.

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[2]*Cardiff University, School of Medicine, Cardiff, United Kingdom*

Study question: What are fertility staff and patients' evaluation of staff performance during sharing bad news (SBN) conversations?

Summary answer: In positive SBN conversations, staff and patient ratings of staff performance are similarly good, but in negative SBN conversations patients' ratings are lower than staff's.

What is known already: Bad news, defined as news that negatively alters patients' view of their future, happens at all stages of fertility care. Fertility staff perceive SBN among the biggest challenges of their job. 47% of patients express staff could improve bad news delivery. SPIKES is an evidence-based protocol that has shown promise in guiding SBN in fertility care via breaking the task in six steps: Setting the scene, evaluating patients' Perception, getting patients Invitation, sharing Knowledge, addressing Emotions, and Summarizing and planning future care. Knowledge about how staff perform during SBN conversations and which factors are associated with performance is lacking.

Study design, size, duration: Two cross-sectional mixed-methods online surveys were distributed to staff (via ESHRE) and patients (via charities, social media). Inclusion criteria for staff were working at a fertility clinic and SBN at least once a month and for patients being 18 years of age and having had a SBN conversation with staff in past two months. 334 staff and 345 patients clicked survey links, of which 217 staff and 222 patients completed it (65% and 64% completion).

Participants/materials, setting, methods: Dependent variable was staff SBN performance across SPIKES steps, measured with CARE (empathy) and the Breaking Bad News Assessment Schedule (other steps). Independent variables were actor (staff, patient) and overall evaluation of the SBN conversation (positive, negative). Factors assessed related to staff and patients' background (e.g., gender, age), fertility care context (e.g., patients: parental status, treatment stage, staff: role, burnout, time pressure, SBN training), and SBN conversation (e.g., patients: person vs remotely, alone vs accompanied).

Main results and the role of chance: Crosstabs of actor and evaluation showed a higher proportion of staff (85%) than patients (67%) evaluated the SBN conversation as positive ($X^2(1) = 38.863, P<.001$).

MANOVA investigating differences in staff's SBN performance according to actor and overall evaluation revealed significant effects of actor (Hotelling's Trace $T = .236, F(388,6) = 15.279, p<.001, \eta^2_p = .191$), overall evaluation ($T = .344, F(388,6) = 22.233, p<.001, \eta^2_p = .256$), and their interaction ($T = .127, F(388,6) = 8.243, p<.001, \eta^2_p = .113$). Follow-up ANOVAs showed the same pattern for all SPIKES steps: in positive SBN staff and patient ratings of staff performance were similar (~4 from 1 = poor/not at all to 5 = excellent/completely), but in negative SBN patients' ratings were lower than staff's (~2.5 vs 4, η^2_p ranged .160[Setting] to .289[Perception]).

Among staff, physicians and nurses tended to rate their SBN performance better than embryologists across all SPIKES steps except Knowledge and Emotions (β ranged .166[Invitation] to .312[Perception]). Reporting ≥1 burnout symptom was associated with worse performance in Emotions ($\beta = -.168, P = .049$), time pressure was associated with worse performance in Perception ($\beta = -.185, P = .019$), and having attended SBN training with better performance in Setting ($\beta = .295, P<.001$).

Patients who were alone during the SBN conversation rated staff SBN performance worse than patients who were accompanied in all SPIKES steps (β from -.154[Summary] to -.403[Setting]). Patients with children rated staff performance in Setting ($\beta = .156, P = .015$), Emotions ($\beta = .158, p = .031$), and Summary ($\beta = .142, p = .048$) better than childless patients.

Limitations, reasons for caution: Online data collection attracted mostly UK patients while staff were from all over the world. SBN performance was reported and not based on behavioral observation, though based on sound questionnaires that evaluate different aspects of performance.

Wider implications of the findings: Staff and patients agree about what good SBN conversations are, but patients are more critical of bad SBN conversations. Staff SBN performance seems more a function of the context in which news are shared than of actors' background. SBN training can support staff recognizing bad SBN conversations and improving performance.

Trial registration number: not applicable

Abstract citation ID: dead093.035

O-035 Research-informed educational materials to promote the routine implementation of psychosocial care for unsuccessful fertility treatment (PCUFT) at clinics: healthcare professionals' and patients' views

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Study question: Are educational materials to promote the routine implementation of PCUFT at clinics acceptable and feasible for healthcare professionals (HCPs) and patients?

Summary answer: HCPs and patients expressed high demand for PCUFT and welcomed educational materials to support this endeavour but expressed different views about how to offer it.

What is known already: Nine in ten patients want to discuss the possibility of fertility treatment being unsuccessful as part of routine care offered at clinics, but only 35% of patients report having this opportunity. Offering PCUFT, defined as assistance and guidance on the implications of treatment being unsuccessful, could promote patients' positive adjustment to this loss. However, it is unknown if and how PCUFT varies across countries and HCPs' and patients' perceived barriers towards its implementation. The present international qualitative study investigated HCPs' and patients' willingness and preferences about using research-informed educational materials to support the implementation of PCUFT at fertility clinics.

Study design, size, duration: Seven Focus Groups were conducted with HCPs (March 2022) from Europe (Belgium/Finland/Germany/Italy/Portugal/Spain/UK) and South America (Argentina/Brazil/Chile), and patients and patient advocates (March-December 2022) also from Europe (UK/Portugal) and South America (Argentina/Chile). Participants were invited to participate through fertility charities/associations and social media. Eligibility criteria were being aged 18 or older, working at a fertility clinic (HCPs) or charity (advocates), or waiting to initiate or undergoing fertility treatment or having completed treatment within six months (patients).

Participants/materials, setting, methods: Semi-structured script following Bowen's (2009) framework. Section one evaluated demand and acceptability of implementing PCUFT. Section two introduced a proposal of research-informed educational materials to promote PCUFT (*MyJourney* webpages - for HCPs: practical advice on introducing PCUFT and addressing patients' FAQs; for patients: information and support for unsuccessful treatment in video, text, FAQs). Questions elicited views on the materials' acceptability, practicality and adaptation. Focus groups were recorded, transcribed verbatim, and data analysed with Framework Analysis.

Main results and the role of chance: Thirty-four patients, seven advocates, 15 HCPs participated. Patients were 38 years old and trying to conceive for around three years, most were female(91.18%) and childless(73.53%). HCPs were mostly psychologists(40.00%) or physicians(33.33%) in the field for around 22 years. Framework analysis generated four themes and one meta-theme, reflecting a need for a normative shift towards having PCUFT as part of routine care. Themes were: (1) need for better collaboration and support, strongly endorsed by patients, who perceived PCUFT

would enable them to better cope with treatment, make more long-term informed-decisions, and feel supported, particularly after treatment; (2) current PCUFT approaches are almost non-existent/non-optimal. Patients and HCPs agreed PCUFT is not offered but expressed different views about its appropriateness. HCPs considered PCUFT more appropriate at later treatment stages and expressed lack resources and know-how to implement it; (3) PCUFT requires an empathic, hopeful, multidisciplinary approach. While patients want to receive in-depth medical advice about their full treatment options, potential outcomes and support to prepare for (unsuccessful) treatment, most HCPs envisioned present-focused information-sharing and support tailored to patients' treatment stage; and (4) high demand for educational materials to promote PUCFT. *MyJourney* package prototype is beneficial but needs improvements to be acceptable and feasible.

Limitations, reasons for caution: Non-probability sample. Although the patients' sample was heterogeneous (including heterosexual and homosexual couples; from private and public clinics), patients were primarily white, well-educated, employed, and childless women, limiting the generalisation of results and comparisons across gender.

Wider implications of the findings: HCPs and patients agree PCUFT is needed and beneficial, but HCPs' will need reassurance about the right timing and support on appropriate ways to implement it given its sensitivity and perceived potential adverse effects. Educational materials, including the *MyJourney* package, are seen as added value to promote a cultural shift.

Trial registration number: Not applicable

Abstract citation ID: dead093.036

O-036 Are male infertility patients and male partners to infertility patients impacted differently by fertility challenges?

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Study question: How do male infertility patients and male partners to infertility patients differ on the impact of diagnosis on their daily lives, relationships and mental health?

Summary answer: Significantly more male patients than male partners agreed that diagnosis impacted their activities of daily living, partner relationships and mental health.

What is known already: Globally, male factor infertility is causal (solely or with other factors) in approximately 50% of infertility cases[1]. The male patient perspective on infertility remains under-researched as is that of the male partner to the infertile patient. A recent James Lind Alliance priority setting study, including health professionals, patients and health care providers, identified psychological aspects of male infertility as one of the top 10 research priorities within male infertility[2]. Previous literature has documented that men with male factor infertility can experience significant mental distress compared to other diagnoses. However, there is a paucity of evidence about other aspects of life.

Study design, size, duration: This is a secondary analysis of the male subpopulation of the international, online, 30-minute, quantitative '1000 dreams' survey, which collected data from March to May 2019 among 1944 respondents. The male subpopulation consisted of 847 respondents from nine countries (United States [US], United Kingdom [UK], Germany, Spain, Italy, France, China, Australia, and Canada). Male respondents had either been diagnosed with infertility (patients) or had a partner who had received a diagnosis of infertility (partners).

Participants/materials, setting, methods: Infertile patients (n = 351) and partners (n = 496) were at different stages of the treatment journey (diagnosis, treatment). Average age at time of questionnaire completion was 36.33 years (SD = 10.06). Survey topics covered impact on mental health, relationships and daily activities. Respondents indicated their extent of agreement with each statement on a scale from 1 to 7 (do not agree at all–completely agree). Response categories 5-7 (somewhat to fully agree) were combined. Chi-square comparison tests were used.

Main results and the role of chance: Significantly more male patients (57%, n = 351) than male partners (40%, n = 496) agreed with some impact of their diagnosis on their activities of daily living ($p < 0.001$). Of those reporting *any* impact, male patients were significantly more likely to agree that they withdraw from social engagements than male partners (55% vs 38%, $p < 0.001$). A significantly greater proportion of male patients agreed that the diagnosis had a negative impact on their work-life balance compared with male partners (58% vs 42%, $p < 0.001$) and a negative impact on their career progression (51% vs 36%, $p < 0.001$). Similarly, 60% of male patients and 52% of male partners agreed that there were some impacts on their relationship with their partners ($p = 0.03$). Of these, more male patients than male partners somewhat or fully agreed that the financial commitment began to put a strain on their partner relationship (54% vs 43%, $p < 0.01$). Among male patients (n = 351), 62% felt that their diagnosis had an impact on their mental health, and of these, 30% sought professional support services. Among male partners (n = 496) 47% reported an impact of their partners diagnosis on their mental health and of these 23% sought professional support services (all differences were NS).

Limitations, reasons for caution: This survey is based on an anonymous, self-reported online survey with no opportunities to validate data by health care professionals and respondents were not able to ask clarifying questions. There is a risk that the data is not fully representative of the male patient/partner population.

Wider implications of the findings: The presence of a male factor diagnosis impacts the emotional experience of infertility on males. It highlights the importance of recognizing that while infertility is a couple diagnosis, men may have varying needs for support depending on whether the source of fertility problems is male.

Trial registration number: Not applicable

Abstract citation ID: dead093.037

O-037 Sex and reproductive health education in the UK: What are we teaching teenagers?

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Study question: Are we giving teenagers in the UK a comprehensive sex and reproductive health education?

Summary answer: Teenagers report learning about how not to get pregnant but have little education in other reproductive health topics.

What is known already: Both the World Health Organization (WHO) and the United Nations Educational, Scientific and Cultural Organization (UNESCO) agree that sex education is a necessary component of education. But globally most countries teach limited information on sex education, concentrating on how not to get pregnant. They may cover puberty, contraception and sexually transmitted infections (STIs) but rarely teach how to get pregnant, PCOS, endometriosis or menopause. We have analysed the UK curriculum at the two exam stages (age 16 and 18), which has shown that the majority of topics that come under reproductive health education are not covered.

Study design, size, duration: We conducted a mixed-methods study, distributing an anonymous, online survey using multiple choice and open-ended questions on Qualtrics software to schools for students aged 16-18 years. The schools distributed the survey either in the classroom or the link was given to the students. The survey was live for 62 weeks (10th May 2021 – 18th July 2022). The final sample size was n = 1224.

Participants/materials, setting, methods: The study had ethical approval from UCL research Ethics Committee ID no: 9831/006. A total of 1244 students completed or partially completed the survey: 754 girls, 344 boys, 38 others, 17 prefer not to say. The qualitative data was analysed thematically.

Main results and the role of chance: Over half of the teenagers' rated the sex education they received in their schools' to be adequate or below (55%, 708/1080). The top three most popular topics taught in schools was contraception (90%, 968/1080), STI's (85%, 914/1080) and puberty (83%, 899/1080). Outside of schools the most popular topics learnt by teenagers were abortion (63%, 675/1080), puberty (60%, 648/1080) and the menstrual cycle (60%, 650/1080). The majority learnt about reproductive health topics through the internet (69%, 745/1080) and social media (60%, 646/1080) and over half (74%, 800/1076) of these teenagers do not or only sometimes, talk to their parents/guardian about reproductive health topics. Common reason for not talking to their parents/guardian was because it was uncomfortable or embarrassing (46%, 386/842).

Half of the teenagers (49%, 502/1024) did not know when a woman is most fertility during their menstrual cycle. Teenagers estimated the oldest age women can naturally have children as: 40 years old (10%, 104/1024), 45 years old (23%, 263/1024), 50 years old (21%, 214/1024), and the oldest age a man can naturally have children: 60 years old (12%, 122/1024), 70 years old (12%, 122/1024), 80+ years old (36%, 366/1024). Teenagers showed better knowledge of female fertility than of male fertility.

Limitations, reasons for caution: Only 20 schools participated so the study results are not representative of all UK schools. Personal contacts and a teacher forum were used for survey promotion. Schools in more deprived areas, faith schools and teens without internet access and a digital device did not participate in the survey.

Wider implications of the findings: Schools should be teaching all aspects of reproductive health. The data from this study, and school talks given by the second author has helped the development of a free teachers' reproductive health education PowerPoints and guide which is being promoted by the ESHRE International Fertility Education.

Trial registration number: Not applicable

Abstract citation ID: dead093.038
O-038 Is a 10-year retention period sufficient after elective oocyte vitrification (EOV)?

J. Nekkebroeck[1], L. Leunens[1], E. Buyse[1], R. Cooreman[1], H. Tournaye[1], M. De Vos[1]

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Study question: In Belgium, by law, a retention period of 10-years is standard. Is this period sufficient for women to return to the clinic and use their vitrified oocytes?

Summary answer: A standard cryopreservation period of 10-years seems sufficient since half of the women returned to the fertility clinic and 96% used the vitrified oocytes.

What is known already: The main driver for elective oocyte cryopreservation is to buy more time to find a suitable partner. The question remains whether 10 years of cryopreservation is sufficient for women to accomplish their goal of finding a partner and procreate with or without using their vitrified oocytes. The return rates and the utilisation rates of the vitrified oocytes reported in the literature are low. Most women do not use their vitrified oocytes. Little is known about women's reproductive pathways after EOV and the destination of the expired surplus vitrified oocytes.

Study design, size, duration: Computerized clinical data were retrieved from a pioneer cohort of women who underwent EOV in our centre for age-related reasons more than 10 years ago (between 2009 and 2012). Hence, the legal cryopreservation period of their oocytes expired between 2019 and 2022. We documented reproductive choices for those who returned to the centre and we investigated the intended destination of the expired vitrified oocytes for those who did not return.

Participants/materials, setting, methods: Data were collected from clinical charts of women who vitrified oocytes. According to Belgian law, unused vitrified oocytes expire when reaching the age limit of 48 years for use or by reaching the standard cryopreservation period of 10 years. We evaluated whether women eventually started treatment, assessed their relational status, the utilisation rate of the vitrified oocytes and the intended destination of the vitrified oocytes as stated in their informed consents.

Main results and the role of chance: 117 women vitrified their oocytes at least 10 years ago. Women's oocyte cryopreservation period expired because they were either beyond the age limit for use (n = 82) or because the legal oocyte cryopreservation period of 10 years was reached (n = 35). Five women requested transport of their oocytes for treatment in a centre abroad.

Fifty-two out of 117 women (44.4%) returned for assisted reproduction. Eventually, 96% (50/52) of them used their vitrified oocytes for treatment and 21/50 had a child or ongoing pregnancy. Upon return to the clinic, 22 women were single and 27 had found a partner, two women returned with a co-parent and one woman had a female partner. Furthermore, 7/117 women returned to the clinic but eventually refrained from treatment.

Fifty-one women never returned to the centre. Only five women asked to prolong the cryopreservation period for medical reasons. The others not using their vitrified oocytes, did not initiate further action concerning the cryopreservation period, use or destination of their stored oocytes. Hence, the destination of the cryopreserved oocytes was according to the women's choice stated in the informed consent. The majority donated their oocytes for research (60%), others (31%) opted to destroy the oocytes after 10 years of cryopreservation.

Limitations, reasons for caution: It was not possible to map out the reproductive pathway of women who did not return to the centre and whose vitrified oocytes expired for use. Follow-up of reproductive choices and outcomes in women who did not return for treatment is required for a comprehensive appraisal of EOV.

Wider implications of the findings: The vast majority of the women who returned for treatment used their vitrified oocytes. Those not returning did not initiate further action concerning the use or destination of their vitrified oocytes. These results add to our knowledge on the utilisation rate after EOV, essential to improve counselling of future EOV-candidates.

Trial registration number: not applicable

POSTER DISCUSSION SESSION
SESSION 08: REPRODUCTIVE ENDOCRINOLOGY
Monday 26 June 2023 Hall D2 10:00 - 11:30

Abstract citation ID: dead093.039
P-589 How Does Hypothyroidism Alter Perinatal Outcomes in Patients with Polycystic Ovary Syndrome? - An Evaluation of a Population Database

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Study question: Does concomitant hypothyroidism in polycystic ovary syndrome (PCOS) patients have an additive effect on the risk for adverse pregnancy, delivery and neonatal outcomes?

Summary answer: In patients with PCOS, hypothyroidism significantly increases the risk for preeclampsia, however risks of other adverse perinatal outcomes are unaltered.

What is known already: PCOS and hypothyroidism are common endocrinopathies during reproductive age, and each has been shown to be associated with pregnancy complications. While PCOS has been associated with gestational diabetes, gestational hypertension, and preeclampsia, overt hypothyroidism has been associated with pregnancy loss, intrauterine growth restriction, preeclampsia, and preterm birth. Furthermore, PCOS and thyroid abnormalities are known to be linked, with a higher prevalence of thyroid dysfunction in pregnant PCOS patients compared to controls. However,

information on obstetric and neonatal outcomes in women with both PCOS and hypothyroidism is lacking.

Study design, size, duration: A retrospective population-based study utilizing data from the Healthcare Cost and Utilization Project—Nationwide Inpatient Sample (HCUP-NIS), was performed. A dataset of all deliveries between 2004 and 2014 inclusively, was created. This population-based cohort study included 14,882 women with an International Classification of Diseases (ICD)-9 diagnosis of PCOS in the United States. IN 2015 data was converted to ICD-10 codes which are not comparable to ICD-9 codes.

Participants/materials, setting, methods: All pregnancy admissions that resulted in delivery or maternal death were included so that patients were counted only once per pregnancy. We compared PCOS women with a concurrent diagnosis of hypothyroidism to those without. Women with hyperthyroidism were excluded. Pregnancy, delivery, and neonatal outcomes were compared between the two groups. Multivariate logistic regression analysis was used to adjust for confounding effects.

Main results and the role of chance: Overall, 14,882 women met the inclusion criteria. Amongst them, 1,882 (12.65%) had a concomitant diagnosis of hypothyroidism, and 13,000 (87.35%) did not. Women with concomitant hypothyroidism, compared to those without, were characterized by increased maternal age (25.5% $\geq$35 years vs. 18%, $p < 0.001$, respectively), and had a higher rate of multiple gestations (7.1% vs. 5.7%, $p = 0.023$). Interestingly, pregnancy, delivery, and neonatal outcomes were comparable between the groups, except for a higher rate of small-for-gestational-age (SGA) neonates in the group with hypothyroidism (4.1% vs. 3.2%, $p = 0.033$), when not accounting for confounding effects. In a multivariate logistic regression adjusting for potential confounders, hypothyroidism was no longer found to be associated with SGA (adjusted odds ratio [aOR] 1.32, 95% confidence interval [CI] 0.99-1.75, $p = 0.057$), but was found to increase the odds for preeclampsia (aOR 1.30, 95% CI 1.06-1.59, $p = 0.012$). Of note when controlling for confounding effects, all other pregnancy, delivery, and neonatal outcomes were comparable between the groups, including pregnancy-induced hypertension; eclampsia; gestational diabetes mellitus; preterm premature rupture of membranes; preterm delivery; placental abruption; cesarean delivery; hysterectomy; post-partum hemorrhage; wound complications; maternal death; blood transfusions; maternal infection; venous thromboembolism; disseminated intravascular coagulation; SGA; intrauterine fetal death; and congenital anomalies.

Limitations, reasons for caution: Due to our study's retrospective nature, some data is missing such as TSH levels, TPO antibody status, the time period in which hypothyroidism was diagnosed (pre-gestationally or during the 1st, 2nd or 3rd trimesters), and Levothyroxine treatment dose and compliance.

Wider implications of the findings: Patients with PCOS and hypothyroidism during pregnancy should be managed with consideration of instituting both preventive measures for preeclampsia development and heightened blood pressure, proteinuria, and symptom monitoring. Interestingly, hypothyroidism did little to affect the already increased rates of pregnancy complications in PCOS. This should be further studied.

Trial registration number: not applicable

Abstract citation ID: dead093.040

P-596 Rectal progesterone administration secures high ongoing pregnancy rate in a personalized Hormone Replacement Therapy Frozen Embryo Transfer (HRT-FET) protocol - a prospective interventional study

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Study question: Can supplementation with additional rectal administration of progesterone secure high ongoing pregnancy rates (OPR) in patients with low serum progesterone (P4) at blastocyst transfer (ET)?

Summary answer: Rectally administered progesterone starting on the blastocyst transfer day secures high OPR in patients with serum P4 levels lower than 35 nmol/l (11ng/ml).

What is known already: Low serum P4 levels at peri-implantation in HRT-FET cycles impact reproductive outcomes negatively. However, studies have shown that patients with low P4 after a standard vaginal progesterone treatment can obtain live birth rates (LBR) comparable to patients with optimal P4 levels if they receive additional subcutaneous progesterone, starting close to the day of blastocyst transfer. In contrast, increasing vaginal progesterone supplementation in low serum P4 patients does not increase LBR. Another route of administration rarely used in ART is the rectal route, although progesterone is well absorbed and serum P4 levels reach a maximum level after approximately two hours.

Study design, size, duration: This prospective interventional study included a cohort of 488 patients treated with single blastocyst HRT-FET, in which a total of 374 patients had a serum P4 level $\geq$35nmol/l (11ng/ml) at ET, and 114 patients had a serum P4 level <35nmol/l (11ng/ml). The study was conducted from January 2020 to November 2022.

Participants/materials, setting, methods: Patients undergoing HRT-FET in a public Fertility Clinic. Endometrial preparation included oral oestradiol (6mg/24hours), followed by vaginal micronized progesterone, 400mg/12hours. Single blastocyst transfer and P4 measurements were performed on the 6th day of progesterone administration and patients with serum P4 <35nmol/l (11ng/ml) additional rectal administration of progesterone (400mg/12hour) was started the same day. In pregnant patients, vaginal and rectal administration continued until week 8, and oestradiol and vaginal progesterone treatment continued until week 10.

Main results and the role of chance: Among 488 HRT-FET single blastocyst transfers, the mean age of the patients at oocyte retrieval (OR) was 30.9 $\pm$4.6 years and the mean BMI at ET 25.1 $\pm$3.5 kg/m^2.

The mean serum P4 level after vaginal progesterone administration on the day of blastocyst transfer was 48.9 $\pm$21.0 nmol/l (15.4 $\pm$6.6ng/ml), and a total of 23% (114/488) of the patients had a serum P4 level lower than 35nmol/l (11ng/ml). The overall, positive hCG rate, clinical pregnancy rate, OPR week 12, and total pregnancy loss rate were 66% (320/488), 54% (265/488), 45% (221/488), and 31% (99/320), respectively. There was no significant difference in neither OPR week 12 nor total pregnancy loss rate between patients with P4 $\geq$35nmol/l (11ng/ml) and patients with P4 <35nmol/l, who received rescue in terms of rectally administered progesterone, 45% vs. 46%, $p = 0.77$ and 30% vs. 34%, $p = 0.53$, respectively.

OPR did not depend on whether patients had initially low P4 and rectal rescue or were above the P4 cut-off. Logistic regression analysis showed that only age at OR and blastocyst scoring correlated with OPR week 12, independently of other factors like BMI, and vitrification day of blastocysts (day 5 or 6).

Limitations, reasons for caution: In this study vaginal progesterone (Cyclogest®), a solid pessary with progesterone suspended in vegetable hard fat, was used vaginally as well as rectally. It is unknown whether other vaginal progesterone products could be used rectally as easily with the same rescue effect.

Wider implications of the findings: A substantial part of HRT-FET patients receiving vaginal progesterone treatment has low serum P4. Adding rectally administered progesterone in these patients increases the reproductive outcome. Importantly, rectal progesterone administration is considered convenient by the majority of patients; moreover, progesterone pessaries are easy to administer rectally and of low cost.

Trial registration number: EudraCT no.: 2019-001539-29

Abstract citation ID: dead093.041

P-660 No association between BMI, oocyte yield and maturation following different trigger strategies: a large observational study including 5000 cycles.

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Study question: Is patients' BMI associated with oocyte yield and maturation following different trigger strategies with r-hCG, GnRH agonist (GnRHa) or dual trigger?

Summary answer: Patients' BMI is not associated with oocyte yield and oocyte maturation following triggering with rhCG, GnRHa or dual trigger.

What is known already: Triggering final oocyte maturation is a key step of ovarian stimulation for IVF/ICSI treatment. Although hCG has traditionally been the gold standard for final oocyte maturation, GnRHa is increasingly used as optimal trigger in "freeze-all" era. Recent evidence has demonstrated excellent results with dual-trigger, a combination of hCG and GnRHa.

Despite this swift in clinical practice, early studies demonstrated that hCG plasma levels depend on hCG trigger dose and BMI. Furthermore, although dose-finding studies following for GnRHa trigger demonstrated equal efficacy of triptorelin doses ranging from 0.1-0.4mg, these studies pertained only to low BMI patients.

Study design, size, duration: This is a retrospective observational study including 5190 consecutive cycles ovarian stimulation cycles (OS) performed between January 2019 -September 2022 in a tertiary Fertility Unit within a University Affiliated Hospital. Overall 5190 ovarian stimulation cycles were analyzed: 2691 cycles triggered with subcutaneous administration of 0.2 mg of GnRHa (triptorelin), 1110 with 250 mcg of r-hCG and 1389 with a dual trigger (combination of both 0.2 mg triptorelin+ 250 mcg of r-hCG).

Participants/materials, setting, methods: Ovarian stimulation was performed with recombinant FSH or HMG at a starting dose 150-300IU depending on patients' ovarian reserve and BMI. Control of LH surge was accomplished by a flexible GnRH antagonist or PPOS protocol. The primary outcome measures were oocyte maturation rate (MII/oocytes) and FOI (oocytes/AFC); secondary outcomes were oocyte and MII yield. Multivariable regression models were performed to evaluate the interaction between BMI and type of trigger in relation to study's outcomes.

Main results and the role of chance: Overall, oocyte and MII yield was significantly different between different types of trigger mainly due the fact that GnRHa was primary used in freeze-all cases, with excellent ovarian reserve. This resulted in a higher number of oocytes (15.95 ± 8.88) and MIIs (12.31 ± 7.27) as compared with cycles triggered with in rhCG (7.83 ± 5.42 and 5.86 ± 4.22 respectively) or dual trigger (8.51 ± 5.70 and 6.44 ± 4.54 respectively) was utilized. Nonetheless, when oocyte maturation rate was comparable between trigger groups (% [95% CI]): GnRHa 77.2 [76.6; 77.8] vs rhCG 74.9 [73.3; 76] vs dual 75.6 [74.5; 76.7].

Multivariable regression analysis, adjusting for confounding factors, demonstrated that BMI was not associated with oocyte maturation rate (OR:1.00 [95% CI: 0.99;1.01]) and FOI (Beta 0.52 [95% CI: -0.49;1.54]). Similarly BMI was not associated number oocytes (Beta 0.02 [95% CI: -0.08; 0.13]) or on the number of MIIs (Beta 0.01 [95% CI: -0.08; 0.10]) retrieved. Interactions between BMI and trigger groups were considered in each model. All analyses were conducted considering patients' weight, but no association was revealed.

Limitations, reasons for caution: This is a retrospective study, and we cannot exclude the presence of residual bias despite the large series, the multiple regression models and the indexes like maturation rate and FOI we adopted to account for confounding factors.

Wider implications of the findings: Although previous small observational studies supported an association between BMI and hCG levels, our large analysis of > 5000 cycles clearly shows that the efficiency of r-hCG, triptorelin or dual trigger is not associated with the patient's BMI and weight, as no significant differences were found in maturation rate and FOI.

Trial registration number: Not applicable

Abstract citation ID: dead093.042

P-653 Natural proliferative phase frozen embryo transfers: a novel, safe and efficient transfer strategy

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Study question: How do natural proliferative phase for frozen embryo transfers (NPP-FET) compare to traditional artificial and natural cycle FETs in terms of pregnancy and neonatal outcomes?

Summary answer: NPP-FETs were associated with similar ongoing pregnancy rates after 22 weeks, but with less miscarriage rates comparing to artificial cycles.

What is known already: The best FET protocol for endometrial preparation is still controversial. A natural cycle (NC) FET may require more visits to the clinic and provides less flexibility. Conversely, artificial cycles (AC), although being more flexible in terms of scheduling, have been previously associated with higher rates of miscarriage and maternal morbidity. Here we describe an alternative FET strategy (NPP-FET) in which, during an unmediated ovulatory cycle, progesterone is initiated as soon as the endometrium proliferation reaches 7 mm of thickness, regardless of the size of the dominant follicle and without the administration of exogenous hCG or spontaneous LH peak.

Study design, size, duration: We performed a single center retrospective cohort study of FET cycles performed between January 2020 and June 2022 (n = 2158). Only single embryo blastocyst stage transfers were included. The main outcome was ongoing pregnancy rate after 22 weeks. Secondary outcomes included the number of visits to the clinic during monitoring, serum levels of progesterone on the day of FET, livebirth (LBR) and miscarriage rates, and maternal/perinatal outcomes.

Participants/materials, setting, methods: FETs cycles were divided in three subgroups: NC, NPP and AC. For the main outcome measures, a multivariable logistic regression was performed, to adjust for potential confounding (oocyte age/source, use of PGT-A and embryo quality), followed by pairwise comparisons whenever statistically significant.

Main results and the role of chance: In total, 2158 FET cycles were analysed (1219 NC, 277 NPP and 662 AC). The mean number of visits before FET planning were 2.14, 1.83 and 1.33 for NC, NPP and AC FETs, respectively ($p < 001$ for all pairwise comparisons). Mean progesterone levels on the day of transfer were significantly higher after NPP and NC (29.4 ng/mL and 40.2ng/mL, respectively), compared to AC (12.7 ng/mL). Progesterone rescue therapy (administered whenever serum progesterone <8.8 ng/mL) was also significantly more frequently needed following AC (24,9% for AC versus 0.6% and 2.6% in NC and NPP, respectively). The ongoing pregnancy rates after 22 weeks were 57.3% for NC, 55.6% for NPP and 54.5% for AC. LBRs following NPP were comparable with NC (38.9% and 42.7%, respectively), and significantly lower with AC (36.0%) compared to NC. The miscarriage rates were significantly lower after NPP and NC-FET when compared to AC (18.4%, 24.3% and 30.7% respectively). Considering maternal outcomes, NPP and NC were associated with a significantly lower risk of first trimester bleeding compared to AC (17.5%, 13.8% and 36.7%, respectively). The rate of gestational hypertensive disorders did not vary significantly (7.5% for NPP, 9.9% for NC and 12.6% for AC).

Limitations, reasons for caution: While serum progesterone and miscarriage rates comparable to NC-FET allude to the possibility that ovulation may still occur in NPP-FETs, this study cannot establish such causality due to its retrospective design. Moreover, the study may have been underpowered to detect clinically relevant subgroup differences such as for hypertensive disorders.

Wider implications of the findings: NPP-FETs seems to be an effective and safe alternative, potentially wielding both the advantages of the natural proliferative endometrium of the NC with the ease for scheduling of ACs.

Nonetheless, further investigation is warranted attempting also to confirm whether ovulation does indeed still occur despite the progesterone administration.

Trial registration number: not applicable

Abstract citation ID: dead093.043

P-673 Women exposed to higher levels of DEHP display altered microRNA expression profile in the pre-ovulatory follicular fluid

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Study question: Can higher concentrations of di-2-ethylhexyl phthalate (DEHP) in follicular fluid (FF) lead to an altered microRNA expression profile?

Summary answer: The present study indicates the upregulation of miR-203a-3p, miR-150-5p and miR-28-3p in the follicular fluid of women with higher DEHP concentrations (FDR<0.05).

What is known already: Women are exposed daily to endocrine-disrupting chemicals (EDCs) such as phthalates which can be found in hundreds of personal care products and plastic items. In studies of rodents exposed to certain phthalates, high doses have been shown to change hormone levels, disrupt folliculogenesis and cause birth defects. Recently, we reported a strong inverse association between DEHP metabolites and ovarian sensitivity index (OSI) in females undergoing ART treatment. Several mechanisms have been proposed to explain the association between DEHP and fertility in rodents, however not much is known about the mechanisms in humans.

Study design, size, duration: FF was collected from 96 women undergoing IVF treatment after controlled ovarian stimulation during ovarian puncture. 48 participants from Estonia were recruited at Nova Vita Clinic AS in Tallinn, Estonia in 2019 and 48 participants from Sweden were recruited at Carl von Linnekliniken in Uppsala, Sweden in 2016. In both cohorts, patients signed a written informed consent form. Both cohorts were matched for age, BMI, parity and the number of previous IVF treatments.

Participants/materials, setting, methods: DEHP metabolite concentrations in the FF samples were measured in our earlier study by LC-MS/MS. Cell free RNA was extracted from FF samples with Qiagen miRNeasy Micro kit, sequencing libraries were prepared with QIAseq miRNA library kit and sequenced on Illumina NextSeq 550 instrument. Differential miRNA expression analysis was performed with the DESeq2 package. Pathway enrichment analysis for differentially expressed miRNA targets was conducted with the miEAA tool integrated with WikiPathways.

Main results and the role of chance: In total, 465 miRNAs were observed at least twice in ≥ 24 samples. Differential expression analysis was conducted between DEHP-high and DEHP-low groups in combined cohorts using the cohort as a covariate. 16 miRNAs were found to be differentially expressed between DEHP groups with FDR<0.1. Three miRNAs: miR-203a-3p, miR-150-5p and miR-28-3p, were differentially expressed with FDR<0.05 and were more abundant in women with high DEHP-levels in both cohorts. Of these, miR-203a-3p was negatively correlated with OSI (Pearson R = -0.27, p = 0.0089). Six miRNAs (miR-10b-5p, miR-112-5p, miR-132-5p, miR-203-3p, miR-205-5p and miR-27-3p) were found to have predicted targets in the androgen receptor signalling pathway.

Limitations, reasons for caution: The miRNAs found in this study need to be validated in a larger set of patients as a cohort-dependent difference in the results was observed. There is a need for further functional studies to investigate miRNAs as possible mediators of the association between DEHP and ovarian sensitivity index.

Wider implications of the findings: The current study presents evidence that DEHP exposure alters the expression of FF miRNAs which are associated with androgen receptor signalling. These results provide novel evidence of the potential influence of endocrine-disrupting chemicals on female reproduction.

Trial registration number: NA

Abstract citation ID: dead093.044

P-676 Bone health in Australian women with early menopause: a 23-year longitudinal analysis

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Study question: What is the frequency of osteoporosis, fractures, and osteoporosis management in women with early menopause (menopause <45years; EM)? What factors influence osteoporosis, screening, and treatment?

Summary answer: Osteoporosis risk is 80% higher in women with early versus natural age menopause. Despite higher screening and treatment, gaps persist in managing EM bone health.

What is known already: A treatment gap in osteoporosis care exists, with low diagnosis (using dual X-ray absorptiometry, DXA), and primary or secondary fracture prevention. Women with EM have increased rates of osteoporosis. Clinical guidelines recommend screening with DXA and menopause hormone therapy (MHT) for most women with EM to reduce osteoporosis and fracture risk. However, studies suggest osteoporosis knowledge, guideline uptake and management adherence by clinicians and women is limited.

Study design, size, duration: The Australian Longitudinal Study on Women's Health is a prospective longitudinal study of Australian women. This study uses the cohort of women born between 1946 – 1951, surveyed nine times between 1996 – 2019. Data from Australian administrative health records including the Pharmaceutical Benefits Scheme (PBS) (MHT, Bone specific agents; BSA) and Medicare benefits schedule (DXA) was linked to survey data.

Participants/materials, setting, methods: Respondents with self-reported age of menopause were included. EM defined as above. T-test/chi-square used for comparisons at baseline ($p < 0.05$ indicates significance). Generalised estimating equations (GEE) for panel data explored longitudinal outcomes of osteoporosis, fractures, DXA rates, MHT use and BSA (in women with osteoporosis/fracture). Univariable regression was performed, and variables retained where $p < 0.2$, to form the multivariable model, and bootstrapping with 100 repetitions at 95% sampling of the original dataset to ensure robustness of results.

Main results and the role of chance: 8,603 women were included: 610 (7.1%) with EM. Mean (SD) baseline age was 47.6 (1.45) years in the entire cohort, and mean (SD) age of menopause was 38.2 (7.95) and 51.3 (3.04) years in women with EM and natural age menopause, respectively ($P < 0.001$). Overall, 421 (69.0%) women with EM had DXA screening, 305 (50%) had a fracture/osteoporosis, 474 ever used MHT (77.7%) and 142 (46.6%) used BSA.

Using the multivariable model, women with EM had increased risk of osteoporosis (Odds Ratio (OR) 1.80; 95%CI 1.43,2.26), fractures (OR 1.48; 1.18,1.85), MHT use (OR 6.98; 5.78,8.43) and BSA use (OR 1.69; 1.24,2.32).

In EM women, increasing age was associated with greater risk of osteoporosis / fracture (OR 1.09; 1.08,1.11), and MHT use did not significantly reduce this risk (OR 0.78; 0.55,1.11). In EM women, age (OR 1.32; 1.12,1.15), BMI (OR 0.94; 0.91,0.96), current smoking (OR 0.51; 0.35,0.74) and inner (OR 0.69; 0.52,0.91) or outer regional (OR 0.66; 0.46,0.95) residential location were associated with DXA screening. In EM women, increasing age (OR 1.15; 1.13,1.18) and lower BMI (OR 0.91; 0.86,0.95) were associated with BSA use.

Limitations, reasons for caution: Survey data were self-reported by participants, and fracture questions were not included in the 2001 survey. PBS data was only available from 2004, and hospital admissions data for all of Australia was only available from 2007.

Wider implications of the findings: Osteoporosis and fractures affect a significant number of women with EM. Focus must be made on improving screening and treatment of these women.

Trial registration number: N/A

INVITED SESSION

SESSION 09: THE TROUBLE TO ASSEMBLE TWO GENOMES

Monday 26 June 2023 Hall D3 11:45 - 12:45

Abstract citation ID: dead093.045
O-039 Fertilization: Mechanisms of pronuclear migration and genome unification

T. Cavazza[1]

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Most early human embryos contain aneuploid cells. Aneuploidy often arises during the early mitotic divisions of the embryo, but its origin remains elusive. Human zygotes that cluster their nucleoli at the pronuclear interface are thought to be more likely to develop into healthy euploid embryos.

I will present data showing how the parental genomes cluster with nucleoli in each pronucleus in human and bovine zygotes. Parental genome clustering is required for the reliable unification of the parental genomes after fertilization. During the migration of intact pronuclei, the parental genomes polarize toward each other in a process driven by centrosomes, dynein, microtubules, and nuclear pore complexes. The maternal and paternal chromosomes eventually cluster at the pronuclear interface, in close proximity to each other, yet separated. Parental genome clustering ensures the rapid unification of the parental genomes upon nuclear envelope breakdown. However, clustering often fails, leading to chromosome segregation errors and micronuclei that are incompatible with healthy embryonic development.

In summary, our data reveal why nucleolar clustering correlates with the formation of healthy euploid embryos. In addition, we used automated analysis of nucleolar trajectories and clustering. Our results support the use of nucleolar clustering as a parameter to identify zygotes with higher developmental potential.

Abstract citation ID: dead093.046
O-040 Clinical competence of oocytes with developmental abnormalities

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For decades we have looked for marker of embryo competence. And while we have come a long way in terms of identifying markers related to embryonic competence, a lot of research is still focused on finding this simple measurable indicator predicting a positive outcome of the individual treatment. Many potential indicators have been evaluated. This includes the cumulus oocyte complex, zona pellucida, perivitelline space, polar bodies, vacuoles, refractile bodies, SER clusters, cytoplasmic granularity, and many others. For some of these there are data suggesting potential prediction of negative outcome (for review see Bartolacci et al. 2022). Embryo autonomy may result in cleavage and development despite the lack of competence to establish a pregnancy. And pregnancies may occur without the competence to go all the way to a live birth. In a study by Lundin et al. we looked into chromosomal constitution after rescuing immature (developmentally abnormal) human oocytes. And while 15% of the oocytes developed into an embryo only very few appeared chromosomally normal after cytogenetic analysis. The study concluded that the majority of hCG exposed oocytes that have not reached the MII stage at time of pick-up are defect and consequently that GV or MI oocytes should not be used in ART programs.

As reproductive specialists our main task is to assist our patients in achieving their dream of having a child. We are supposed to use all our combined expertise, knowhow, craftmanship, routine and experience to optimize the chance for the patients to have a baby.

However, in our ambition to help our patients we sometimes lose touch of the underlying biology of human reproduction. Oocytes from a stimulated ovary are not like those we buy in the supermarket of equal quality. The depressing fact is that only 5.0 % of the oocytes in a first cycle is biologically competent and in around 2/3 of all cycles, none of the oocytes have the potential to result in the birth of a child (Lemmen et al 2016). We also have very limited ability to determine competence of individual sperm cells – the strongest prognosticator being if they look and move like a "normal" sperm sample.

Are we getting a bit desperate when we start to look for normality in the abnormal as suggested in the title of this talk? Have we reached a point were patient expectations overrides our biological training and education resulting in low willingness to accept a negative outcome dictated by our biology? And is this reinforced by patient perceptions like "*we did not pay a lot of money for the oocytes not being fertilized/developed/implanted*".

Embryo competence evaluation is based on markers of what have happened rather than indicators of what will happen. At the end of the day, we as health care professionals as well as our patients needs to accept the fact that there is no such thing as "CLINICAL COMPETENCE OF OOCYTES WITH DEVELOPMENTAL ABNORMALITIES". And what will be the consequence of a marker indicating a lower implantation potential in an otherwise ok embryo? It might affect the ranking of the embryos from a particular cycle, but we would be willing transfer it anyway if needed. Embryo evaluation don't increase pregnancy chance per se. But it may decrease time to pregnancy by improved selection between the embryos available. Maybe it is time to re-focus our efforts and move from a binary normality approach identifying "developmental abnormalities" toward a more biological oriented approach mapping "developmental variation" in embryos and their impact on pregnancy potential and thus recognizing the multi-factorial nature of embryo maturation and ongoing developing competence.

INVITED SESSION

SESSION 10: DATA REPORTING SESSION

Monday 26 June 2023 Hall D1 11:45 - 12:15

Abstract citation ID: dead093.047
O-041 Data from the ESHRE PGT Consortium - year 2021

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Study question: Which are the trends shown in data collection XXIII of the European Society of Human Reproduction and Embryology (ESHRE) PGT Consortium compared with previous years?

Summary answer: Data collection XXIV, the year 2021, represents valuable data on PGT activity in (mainly) Europe and reports on the main trends observed, being the further expansion of blastocyst biopsy and NGS-based comprehensive testing technologies.

What is known already: The ESHRE PGT Consortium was set up in 1997 and from that time has been collecting data on PGT-M, PGT-SR and PGT-A. Since 1999, the PGT consortium has collected 105 000 embryo analyses and published 21 previous data collection sets in 17 reports. Since the year 2016 a prospective cycle-by-cycle data collection is in place.

Study design, size, duration: IVF cycle management and genetic analysis techniques are getting more complex and require more details to be reported. Therefore, ESHRE uses an online data collection system in which data are collected prospectively from oocyte retrieval to analysis, embryo transfer and pregnancy/live birth. Data are collected cycle by cycle on a voluntary basis.

Participants/materials, settings, method: For the 2021 data, individual centres (36) from 19 countries directly entered the analysis data (n = 3067) into the PGT database through software developed by ESHRE. Data were analysed at ESHRE Central Office and include all aspects of the PGT/PGT-A cycles.

Main results and the role of chance: The indications for PGT included inherited chromosomal abnormalities (n = 386 analyses), monogenic disorders (n = 1329 analyses), aneuploidy testing (n = 1147 analyses), HLA typing (alone or in combination with PGT-M (n = 4 analyses), mitochondrial disorders (5 analyses) or combinations of the above (n = 111 analyses). 85 analyses were reported without indication. In addition, 894 clinical pregnancies and 496 deliveries have been analysed in detail. The methods used for biopsy were polar body (1%), cleavage stage biopsy (16%) and blastocyst biopsy (83%), showing a continuous increase in blastocyst biopsy compared to 2020 and earlier years. The methodology used for diagnosis is what evolved most over the last years but seems to stay comparable with 2020. Data set XXIV (2021) shows around 4% of FISH, 31% of PCR and 62% of WGA. Within WGA 84% of the analyses were done using NGS, in 8% of the cases SNP arrays were used, PCR accounted for 2.5% of the analyses and in 1% array-CGH was used. In the remaining cases, combinations of the above were used. The overall clinical pregnancy rate of 29% per analysis shows an increase compared to 2020. The baby data show that it is difficult for most centres to have a detailed follow-up.

Limitations, reasons for caution: The findings apply to the 36 participating centres and may not represent worldwide trends in PGT. Data were collected prospectively, but details of the follow-up on PGT pregnancies and babies born were limited.

Wider implications of the findings: The ESHRE PGT Consortium continues its activities as an important forum for PGT practitioners to share data and exchange experiences. The information extracted from the data collection helps to monitor quality issues in PGT and survey the introduction and effectiveness of new PGT technologies and methods.

Trial registration number: XXXX

Abstract citation ID: dead093.048

O-307 Aneuploidy in oocytes of women of advanced maternal age: impact on embryo development and evidence for a novel mechanism of meiotic error

P. Verdyck[1, 2], G. Altarescu[3], S. Santos-Ribeiro[4, 5], C. Vrettou[6], U. Koehler[7], G. Griesinger[8], V. Goossens[9], C. Magli[10], C. Albanese[10], M. Parriego[11], L. Coll[11], R. Ron-El[3], K. Sermon[2], J. Traeger[6]

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Abstract: Study question: In oocytes of advanced maternal age (AMA) women, what is the association of aneuploidy with embryo development and what are the mechanisms leading to aneuploidy?

Summary answer: We confirmed precocious separation of sister chromatids as the main cause of aneuploidy. Known chromosome segregation errors explained 90.4% of the observed abnormal chromosome copy numbers in polar bodies.

What is known already: Meiotic chromosomal aneuploidies in oocytes correlate with AMA (>35 years), and can affect over half of oocytes in this age group. This underlies the rationale for polar body (PB) biopsy as a form of early PGT-A, as performed in the 'ESHRE STudy into the Evaluation of oocyte Euploidy by Microarray analysis' (ESTEEM) randomised controlled trial (RCT). So far, chromosome analysis of oocytes and PBs has shown that precocious separation of sister chromatids (PSSC), meiosis II (MII) nondisjunction (ND) and reverse segregation (RS) are the main mechanisms leading to aneuploidy in oocytes.

Study design, size, duration: Data was sourced from the ESTEEM study, a multicentre RCT from seven European centres to assess the clinical utility of PGT-A on PBs using array comparative genomic hybridisation (aCGH) in patients of AMA (36-40 years). This included data on the chromosome complement in PB pairs, and on embryo morphology in a subset of embryos, up to day 6 post insemination (dpi), from both the intervention (PB biopsy for PGT-A) and from control (no biopsy) arms.

Participants/materials, setting, methods: ESTEEM recruited 396 AMA patients: 205 in the intervention group and 191 in the control group. Complete genetic data from 693 PB pairs were analysed. Additionally, the morphology from 1034 embryos generated from fertilized oocytes (2 pronuclei) in the PB biopsy group and 1082 in the control group were compared using age-adjusted multilevel mixed-effect ordinal logistic regression for each day of embryo scoring and summarized with adjusted odds ratios (aOR) and 95% confidence intervals (CI).

Main results and the role of chance: Overall, 461/693 PB pairs showed abnormal segregation in 1162/10810 chromosomes. The main observed abnormal segregations were compatible with PSSC in meiosis I (MI) (n = 568/1162; 48,9%), ND of chromatids in MII or RS (n = 417/1162; 35.9%) and less frequently ND in MI (n = 65/1162; 5.6%). For 112 chromosomes (112/1162; 9,6%), we observed a chromosome copy number in PB1 and PB2 that is not explained by any of the known mechanisms causing aneuploidy in oocytes.

We observed that embryos in the PGT-A arm of the RCT did not have a significantly different morphology between 2 and 6 dpi compared to the control group, indicating that polar body biopsy did not affect embryo quality.

Following age-adjusted multilevel mixed-effect ordinal logistic regression models performed for each embryo evaluation day (using the before-mentioned embryo scoring subcategories), aneuploidy was associated with a decrease in embryo quality on day 3 (aOR 0.62, 95% CI 0.43-0.90), day 4 (aOR 0.15, 95% CI 0.06-0.39) and day 5 (aOR 0.28, 95% CI 0.14-0.58).

Limitations, reason for caution: RS cannot be distinguished from normal segregation or MII ND using aCGH. The observed segregations were based on the detected copy number of PB1 and PB2 only and were not confirmed by the analysis of embryos. The embryo morphology assessment was static and single observer.

Wider implications of the findings: Our finding of frequent unexplained chromosome copy numbers in polar bodies indicates that our knowledge of the mechanisms causing aneuploidy in oocytes is incomplete. It challenges the

dogma that aneuploidy in oocytes is exclusively caused by missegregation of chromosomes during meiosis I and II.

Study funding/competing interest(s): Data was mined from a study funded by ESHRE. Illumina provided microarrays and other consumables necessary for aCGH testing of polar bodies. None of the authors have competing interests.

This work is dedicated to the memory of Professor Joep Geraedts.

Trial registration number: NCT01532284

Study funding: Yes

Funding source: Funding by national/international organization(s)

INVITED SESSION

SESSION 11: ASRM EXCHANGE SESSION:THE FUTURE OF REPRODUCTIVE MEDICINE

Monday 26 June 2023 Hall D4 11:45 - 12:45

Abstract citation ID: dead093.049
O-042 The future of endometriosis

H. Taylor[1]

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Abstract citation ID: dead093.050
O-043 The future of ART

M. Cedars[1]

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Abstract citation ID: dead093.051
O-044 The future of contraception

M.A. Thomas[1]

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In 1960, the first hormonal contraceptive agent, Enovid approved by the Food and Drug Administration in the United States. The pill contained mestranol 150 mcg and norethynodrel 10 mg. High dose pills containing >50 mcg of estrogen were discontinued in 1988 due to a higher risk of myocardial infraction and stroke secondary to thrombosis. Until recently, the only estrogens used in combination OCPs were mestranol and ethinyl estradiol (EE). Estetrol (E4) and 17-beta estradiol have been added to newer pill and intravaginal ring (IVR) formulations in order to reduce the risk of thrombosis. Progestins confer the primary contraceptive effect in combination agents and can be used without estrogens. First generation progestins include norethynodrel, ethynodiol diacetate, norethindrone and norethindrone acetate (estranes). Second generation progestins include norgestrel and levonorgestrel (gonanes). Third generation include gestoden, desogrestrel and norgestimate, which are less androgen versions of the second generation progestins. Fourth generation progestins have no androgenicity include drospirenone. The fourth generation progestin is a 17 carbon compound which separates it from the other progestins that are primarily 19 carbon derivatives of androgens. Segesterone acetate (SA) is a newer progestin that is less potent and has no androgenic properties. SA, combined with EE, is currently being used in an IVR. However, clinical trials are ongoing using SA with 17-beta estradiol in an IVR and another with EE as a contraceptive gel. Other new methods of hormonal contraception include progestins in a biodegradable implant, longer acting injectables that can last 6-12 months, transdermal patches that are imbedded with an array of microneedles where either the contraceptive agent is in a reservoir or in the needles themselves. Microchip technology is being developed by the Gates Foundation that would allow levonorgestrel to be released systemically at will over a 16-year period using a remote device. Multipurpose technologies are being developed utilizing contraceptive pills, injectables and IVRs containing hormonal agents as well as medications to prevent STI/HIV acquisition.

INVITED SESSION

SESSION 12: IMPROVING SPERM QUALITY WITH THE RIGHT HABITS

Monday 26 June 2023 Auditorium 10-12 11:45 - 12:45

Abstract citation ID: dead093.052
O-045 Avoiding some things you're exposed to might improve your fertility

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Abstract citation ID: dead093.053
O-046 Nutritional habits might improve your fertility

C. Dupont[1], **N. Sermondade**[1], **G. Bachelot**[1], **R. Lévy**[1]

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Many lifestyle factors have been identified as risk factors for male infertility. For example, an unbalanced diet leading to overweight, obesity, metabolic disorders, and micronutrient deficiencies can have consequences for male reproductive functions. Obese men present more often altered semen parameters and a reduced chance of pregnancy naturally or after medically assisted procreation. In some cases, weight loss can reverse the adverse effects of obesity. However, few interventional studies have demonstrated the effectiveness of a preconceptional intervention in men.

In contrast, more and more studies are highlighting the importance of a balanced diet and physical activity to improve fertility. A qualitatively adequate and balanced diet and appropriate daily intake of micronutrients are fundamental for male reproductive functions. Some teams have highlighted the benefits of the Mediterranean diet on sperm production, whereas the Western diet, including reconstituted meats, fried foods, and sweet foods and drinks, was associated with altered semen parameters. However, few interventional studies have confirmed the impact of men's diets on the chances of natural or medically assisted pregnancy and live birth, and their results are controversial.

Therefore, robust randomised controlled studies are needed. Practical advice and education can be recommended to men who wish to conceive to help them maintain or achieve the public health recommendations that encourage a balanced diet, such as the Mediterranean diet.

POSTER DISCUSSION SESSION
SESSION 14: REPRODUCTIVE SURGERY
Monday 26 June 2023 Hall D2 11:45 - 12:45

Abstract citation ID: dead093.054
P-751 Comparing levonorgestrel intrauterine system versus hysteroscopic niche resection in women with postmenstrual spotting related to a niche in the uterine caesarean scar

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Study question: To compare the effectiveness of levonorgestrel-releasing intrauterine system (LNG-IUS 52mg) with hysteroscopic niche resection in the reduction of niche-related postmenstrual spotting.

Summary answer: In women with niche-related postmenstrual spotting, LNG-IUS did not more often reduce spotting days with 50% at 6th month than hysteroscopic niche resection.

What is known already: Both Levonorgestrel intrauterine system (LNG-IUS) and hysteroscopic niche resection are widely implemented to reduce niche-related postmenstrual spotting. The study aimed to compare the effectiveness of LNG-IUS 52mg with hysteroscopic niche resection in the reduction of niche-related postmenstrual spotting.

Study design, size, duration: A randomised, open-label, controlled trial conducted at one medical center in Shanghai, China. From September 2019 to January 2022, we randomised 208 women to LNG-IUS (N = 104) or hysteroscopic niche resection (N = 104).The primary outcome was reduction of postmenstrual spotting at 6th month after randomisation, defined as percentage of women with a reduction of at least 50% in spotting days from baseline. Efficacy and safety were assessed by an intention-to-treat analyses.

Participants/materials, setting, methods: Women with symptoms of postmenstrual spotting after CS, with a niche depth of at least 2 mm and residual myometrium of at least 2.2mm on Magnetic Resonance Imaging (MRI), and no intention to conceive within the next year were randomly assigned to treatment with LNG-IUS 52mg or a hysteroscopic niche resection.

Main results and the role of chance: At 6th month follow-up, a 50% reduction of spotting had occurred in 78.4% (80/102) women in the LNG-IUS group and 73.1% (76/104) women in the hysteroscopic niche resection group (RR = 1.07, [95%CI 0.92-1.25]; P = 0.370). Spotting reduced over time (P_{trend}=0.001), with a stronger reduction in the LNG-IUS group (P = 0.001), while there was also a significant interaction between time and treatment (P = 0.007). From nine months onwards, reduction of spotting occurred significantly more after LNG-IUS than hysteroscopic niche resection (9th month 89.2% vs. 72.1%, RR = 1.24, [95% CI 1.08-1.42]; 12th month 90.2% vs. 70.2%, RR = 1.29, [95% CI 1.12-1.48]).

Moreover, compared with the hysteroscopic niche resection group, the LNG-IUS group had significantly fewer postmenstrual spotting days and total bleeding days from six months onwards (all P < 0.001), and less pelvic pain from three months onwards (all P < 0.010).

Intervention-related complications were not reported in any of the groups. During follow-up, eleven (10.8%) women reported hormonal related side-effects and two (2.0%) women had spontaneous partial expulsion in the LNG-IUS group, while in the hysteroscopic niche resection group three unintended pregnancies were reported.

Limitations, reasons for caution: Due to the nature of the intervention, it was not possible to blind participants and gynaecologists for treatment allocation.The cut-off value chosen for our primary outcome and the moment of assessment for our primary endpoint can both be debated.

Wider implications of the findings: LNG-IUS provides an alternative treatment for women with niche-related gynaecological symptoms and no active desire to become pregnant.

Trial registration number: Chinese Clinical Research Center (ChiCTR1900025677)

Abstract citation ID: dead093.055
P-757 The classification and management of adnexal masses identified during pregnancy

J. Barcroft[1], M. Pandrich[2], C. Landolfo[1], S. Del Forno[3], N. Parker[1], N. Cooper[1], M. Pikovsky[1], S. Murugesu[1], A. Novak[1], C. Kyriacou[1], M. Al Memar[1], J. Yazbek[2], D. Timmerman[4], S. Saso[1], T. Bourne[1]

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[4]KU Leuven, Department of Development and Regeneration, Leuven, Belgium

Study question: How do we classify adnexal masses (ovarian cysts) identified during pregnancy and how should we manage them?

Summary answer: Adnexal masses (ovarian cysts) should be managed expectantly in pregnancy, given the low rate of complications and low prevalence of malignancy.

What is known already: Conservative management of adnexal masses (ovarian cysts) is preferable during pregnancy, due to the maternal and fetal risks associated with surgery. Accurate diagnosis is key to safe expectant management. Various ultrasound-based models exist to classify adnexal masses; however, none have been validated for use in pregnancy. Adnexal mass morphology can change during pregnancy due to decidualisation, which is often difficult to distinguish from underlying neoplastic processes. We aim to evaluate:(1) the performance of current methods of adnexal mass classification in pregnancy, (2) understand the natural course of adnexal masses in pregnancy, particularly the presence of decidualisation and incidence of complications.

Study design, size, duration: Retrospective analysis of prospectively collected data between January 2017- November 2022. To classify adnexal masses (ovarian cysts), we evaluated: Expert subjective assessment (SA), IOTA Simple Rules (SR) and the IOTA Assessment of Different Neoplasias of the Adnexa (ADNEX) model. The end point was either the histological examination of tissue removed at surgery or the subjective classification of adnexal masses at the postnatal scan in women managed conservatively.

Participants/materials, setting, methods: Women with an adnexal mass identified on gynaecological ultrasound in pregnancy at a tertiary London University Hospital were included. Relevant clinical data was extracted, including age, gestation and cyst-related complications. Adnexal masses were classified at the first antenatal and postnatal ultrasound examination according to SA, SR and the ADNEX model (10% risk of malignancy cut off) and correlated to histology for adnexal masses managed surgically. IOTA simple descriptors were used to classify benign adnexal masses.

Main results and the role of chance: 254 women (median age 33-years old, range:18-49) with an adnexal mass/cyst were included at a median gestation of 12 weeks, (range:4-36). 13 (5.1%) conceived through assisted reproductive techniques. Spontaneous resolution occurred in 24.5% of cases, and 21 were lost to follow up (8.3%). According to Simple Descriptors,

38.6% were simple, 22.0% were endometriomas and 19.7% were dermoid cysts.

Antenatally, SA outperformed ADNEX, based on specificity (94.6 vs.92.2%) and sensitivity (60 vs.55.6%) respectively. SA and ADNEX had a negative predictive value(NPV) of 96.1% and 98.3% respectively. Postnatally, SA had a higher sensitivity than ADNEX (75 vs.50%), but a lower specificity (94.1 vs.96.9%). SA and ADNEX had a NPV of 94.1% and 97.5% respectively.

Three (1.3%) underwent acute surgery during pregnancy: one ovarian torsion (9/40), and two cyst ruptures (11/40, 30/40). Presumed decidualisation occurred in 29.5% of endometriomas. Three (1.3%) underwent cyst removal during caesarean section, two for suspicion of BOTs (one had suspected decidualisation) and one for patient preference. On histology, the suspected decidualised mass was a serous BOT, and the suspected BOT was a struma ovarii.

In the postnatal period (12 weeks), four underwent surgery for suspicion of BOTs, two were confirmed BOTs and two were benign cystadenomas on histology.

Limitations, reasons for caution: The dataset as expected contains a relatively small number of malignancies (prevalence 3.9%) and so any analysis of test performance to discriminate between benign and malignant disease must be interpreted with caution. A larger prospective multicentre study is needed to do this.

Wider implications of the findings: Our data suggest that a number of cysts in pregnancy will resolve, a few will be associated with acute complications and the risk of malignancy is very low. Accordingly, the study supports an expectant management approach for adnexal masses (ovarian cysts) detected in pregnancy.

Trial registration number: Not applicable

Abstract citation ID: dead093.056

P-755 Timeless importance of the embryo transfer technique: a real-life retrospective study comparing direct versus afterload catheters

F. Cirillo[1], V. Immediata[1], T. Carletti[1], C. Ronchetti[1], E. Morenghi[2], E. Albani[1], A. Baggiani[1], P.E. Levi-Setti[1]

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[2]Humanitas Research Hospital- IRCCS, Department of Biomedical Sciences-Humanitas University, Rozzano Milan, Italy

Study question: Can the embryo transfer (ET) technique influence clinical pregnancy rate (CPR)?

Summary answer: Our study demonstrated that CPR is significantly affected by the ET technique. In particular, with the afterload technique both CPR and easy transfer rates increased.

What is known already: Despite it having widely demonstrated that the occurrence of difficult ETs reduces the success of assisted reproductive technology (ART) cycles and that afterload technique has showed a decrease in the number of difficult transfers compared to the direct one, few data are available assessing pregnancy outcomes in the two different techniques. The present study represents the progression of a published prospective randomized controlled trial (RCT) performed in the same setting on a smaller population comparing direct and afterload techniques on the difficult transfer rate, but not designed to demonstrate differences in terms of CPR.

Study design, size, duration: Single center retrospective cohort analysis of 8,189 fresh and frozen single blastocyst transfers performed between January 2016 and December 2021 in a tertiary University affiliated fertility center. Of the total, 2,000 ETs were performed with the direct technique and 6,189 with the afterload one. All fresh single day-5 and single day-5 and day-6 frozen blastocyst transfers performed during the study timeframe were included.

Participants/materials, setting, methods: Direct technique was the only one used from January 2016 to September 2017. In the clinical trial recruitment period, the choice was given by randomization. From April 2019, only the afterload technique was used: the operator never had chance to choose the technique. Preimplantation genetic testing cycles, gamete donation and day-7 blastocyst transfers were excluded. CPR was the primary outcome, while difficult transfer rate the secondary one. Univariate and multivariate logistic regression have been performed.

Main results and the role of chance: During the period, 8,189 single blastocyst transfers were performed. Afterload ET showed a percentage of difficult transfers which was almost one third compared to that of the direct ET, being respectively 9.06% and 26.85% (OR 0.27, 95% CI 0.24-0.31p <0.001). Overall, the CPR between the two groups was significantly different: 44.69% in afterload ET and 41.65% in direct ET (OR 1.13, 95% CI 1.02-1.25, $p = 0.017$). As for the interaction between the two techniques and difficult transfer on CPR, only the direct difficult ET resulted in a significantly lower CPR with an OR of 0.62 (95%CI 0.49-0.77, $p < 0.001$). Lastly, among the 15 operators that performed the ETs, a range of difficult transfers from 3.8% to 45.4 % in the direct group and 0.8% to 20.5% in the afterload group was found ($p < 0.001$).

Limitations, reasons for caution: The main limitations of the study are the difference in sample sizes between the two groups, the wide range of time considered, and the 15 operators included. A further possible reason is the high prevalence of difficult transfer, probably due to the broad definition we used.

Wider implications of the findings: The study points out how in embryo transfers the use of the afterload technique instead of the direct one significantly improves clinical pregnancy rate and easy transfer rate. Since there is a limited knowledge on the embryo transfer technique impact, our findings aim to increase the effectiveness of embryo transfers.

Trial registration number: NCT05364528.

Abstract citation ID: dead093.057

P-748 Fertility and anatomical outcome following hysteroscopic adhesiolysis of intrauterine adhesions classified according to symptoms, imaging findings and hysteroscopic appearance of the uterine cavity

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Study question: To classify patients with dense intrauterine adhesions based on clinical characteristics as well as, ultrasound, hysterosalpingography (HSG) and hysteroscopy findings into different prognostic groups.

Summary answer: We have defined a prognostic model to classify patients with dense intrauterine adhesions which could easily be implemented in patient counseling and management.

What is known already: Intrauterine adhesions are a major cause of hypoamenorrhea and failure to conceive. A universally agreed classification to categorize intrauterine adhesions is paramount to provide individualized counseling and care. Previous efforts for categorization suffer from limitations such as variable assessment of anatomical outcomes, application of different techniques, pooling of results of different operators and lack of long-term follow-up. Many experts have voiced a call for a prognosis-oriented classification system. An ideal classification model should consider clinical characteristics, findings from imaging techniques (including ultrasound and HSG) together with hysteroscopic appearance of the uterine cavity and demonstrate a high predictive value.

Study design, size, duration: This is a retrospective analysis of 281 patients treated for intrauterine adhesions by a single operator (B.U.) between 2010 and 2021. Lysis of adhesions was affected in 479 office hysteroscopy procedures using the Versapoint bipolar cutting electrode under transabdominal ultrasound guidance. 227 patients were followed for at least 15 months after the last surgical procedure. Patients were classified into five

categories (Class I to V) according to their symptoms, ultrasound, HSG and hysteroscopy findings.

Participants/materials, setting, methods: Clinical data and operative findings were reviewed from patient files and video recordings. The number of hysteroscopic interventions needed to restore the cavity and the reproductive outcome in women who were desirous for pregnancy were recorded. Predictive power of the model was assessed using the live birth rate as the primary and rate of cavity restoration and number of interventions as the second outcome parameters. Groups were compared using ANOVA, ROC and regression analyses.

Main results and the role of chance: Adhesions were classified as class I in 43 (15.3%), class II in 72 (25.6%), class III in 57 (20.3%), class IV in 82 (29.2%) and class V in 27 (9.6%) patients. They were due to previous curettages of pregnancies (79,7%) or retained products of gestation (3.9%), prior uterine surgery (6.8%), prior hysteroscopy of inappropriate technique (6.8%) and tuberculosis (2.8%). The cavity was septate in 12 and unicornuate in 2 patients.

The mean age of the study group was 29.8 ± 3.7 (20-40). Age was not related with the severity of adhesions ($p = 0.335$). While the majority of patients with curettage-related adhesions were classified as Class II, uterine surgery, iatrogenic and tuberculosis related adhesions were higher in severity. The number of hysteroscopic adhesiolysis procedures (from 1.0 ± 02. to 2.3 ± 0.5) needed for optimal restoration of the cavity was directly related and the rate of full restoration (from 100% - 18.5%) was indirectly related with the severity of adhesions according to the proposed classification ($p = 0.0001$ for both). The live birth rates were 54.3%, 45%, 31.7%, 21% and 12.5% for patients in Class I to V, respectively ($p = 0.0001$). The proposed classification was fairly predictive (AUC: 0.654, 95%CI: 0.582-0.727) for live birth.

Limitations, reasons for caution: This is a retrospective analysis of consecutive patients with intrauterine adhesions in routine practice. The follow-up for reproductive outcome is limited. The study is hospital-based and single-center. Thus, the predictive value of the proposed classification needs to be validated in an external data set preferably in a prospective series.

Wider implications of the findings: In patients with intrauterine adhesions, a classification system based on patient symptoms, imaging findings and hysteroscopic appearance of the uterine cavity reliably predicts the postoperative outcome in terms of the extent of anatomical restoration of the uterine cavity and pregnancy and live birth rates.

Trial registration number: Not applicable

INVITED SESSION

SESSION 15: NEW INSIGHTS INTO FERTILITY AND REPRODUCTION

Monday 26 June 2023 Hall D3 14:00 - 15:00

Abstract citation ID: dead093.058
O-048 Mechanisms of ovarian ageing

E. Hoffmann[1]

[1]*University of Copenhagen, Copenhagen, Denmark*

Abstract citation ID: dead093.059
O-049 Novel strategies to monitor spermatogenesis in vitro and their potential benefit for the clinics

J-B. Stukenborg[1]

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INVITED SESSION

SESSION 16: UPDATE ON EMBRYO BIOPSY AND REUTILISATION

Monday 26 June 2023 Hall D1 14:00 - 15:00

Abstract citation ID: dead093.060
O-050 The impact of embryo biopsy on obstetric and neonatal outcomes

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Background: Preimplantation genetic testing of embryos (PGT) was performed first in 1990 to prevent the transmission of X-linked disease. Since then, the technique has improved to include couples who are both carriers of the same autosomal recessive disorder, to those with balanced translocations having recurrent pregnancy loss, and to women with advanced maternal age who are at higher risk for having aneuploid embryos. Originally, PGT involved biopsy of the polar body or of one blastomere of a cleavage stage embryo for diagnosis. The contemporary practice of PGT involves the culture of embryos to the blastocyst stage (5-7 days after fertilization), with biopsy of several trophectoderm cells that would become the placenta, transport of biopsy tissue off-site for genetic analysis, and cryopreservation of blastocysts until biopsy results are available. A single euploid embryo is then transferred into the uterus as a frozen embryo transfer.

Objective: We sought to determine if removal of these cells for preimplantation genetic testing was associated with adverse obstetrical or neonatal outcomes after frozen-thawed single embryo transfer compared to frozen-thawed single embryo transfer without biopsy. Further, we review other studies of contemporary practice of PGT to draw conclusions about the safety of the practice.

Study design: We linked assisted reproductive technology surveillance data from the Society for Assisted Reproductive Technology Clinic Outcome Reporting System to birth certificates and maternal and neonatal hospitalization discharge diagnoses in Massachusetts, USA, from 2014-2017, considering singleton births after frozen-thawed single embryo transfer. We compared outcomes of cycles having embryo biopsy ($n = 585$) to those having no biopsy ($n = 2,191$), adjusting for mother's age, race, education, parity, body mass index, birth year, insurance, and all infertility diagnoses (Sites CK et al, Am J Obstet Gynecol 2021;225:285.e1-7).

Results: With no biopsy used as the reference, we found no differences between no biopsy and biopsy groups with respect to preeclampsia, pregnancy-induced hypertension, placental disorders (placental abruption, placenta previa, placenta accreta, placenta increta, and placenta percreta), preterm birth, low birthweight, cesarean delivery, gestational diabetes mellitus, or maternal or infant length of stay after delivery. Comparing our outcomes to 4 other studies of contemporary PGT practice of frozen-thawed embryo transfers (Zhang WY Fertil Steril 2019, Li M Am J Obstet Gynecol 2020, Makhijani R Hum Reprod 2021, He H Fertil Steril 2019), our data are consistent with no effect of biopsy on low birth weight, pregnancy-induced hypertension, gestational diabetes, placenta previa, and placenta accreta. A slight decrease in preterm delivery (RR 1.10, CI 1.02-1.18), slight reduction in cesarean section (RR 0.90, CI 0.82-0.99), and increase in intrauterine growth restriction (RR 1.21, CI 1.06-1.38) are reported with biopsy in a systematic review (Hou W, Fertil Steril 2021). The increase in risk for intrauterine growth restriction with biopsy was influenced by one study (Li M Am J Obstet Gynecol 2020).

Conclusion: Embryo biopsy of trophectoderm cells for PGT in contemporary practice appears to be safe generally with respect to maternal and neonatal outcomes. There is no clear increase in diagnoses related to placentation (preeclampsia, pregnancy-induced hypertension, placental disorders, preterm birth, low birthweight), cesarean delivery, gestational diabetes

mellitus, or maternal or infant length of stay after delivery. Further study of a possible increase in intrauterine growth restriction with biopsy is indicated.

Abstract citation ID: dead093.061
O-051 Refreezing and rebiopsy – are these worth it?

W. Verpoest[1]

[1]*UZ Brussel, Brussels IVF Fertility Centre, Jette- Brussels, Belgium*

Preimplantation genetic testing of embryos offers the opportunity to avoid the transmission of hereditary disorders to the offspring by selecting out embryos affected by a single gene mutation or mutations (PGT-M), structural rearrangements of chromosomes (PGT-SR), aneuploidy (PGT-A) as well as polygenic risk (PGT-P). Over the last two decades, laboratory techniques have seen tremendous developments including extended embryo culture to blastocyst stage, trophectoderm biopsy and vitrification. The latter technique is highly efficient and allows multiple freezing and warming cycles when performed in proficient laboratories. At the same time whole genome analysis techniques provide the opportunity to analyse embryonic DNA in great detail for multiple indications simultaneously.

The PGT process is sensitive to both biological and technical issues which sometimes lead to a failed PGT result. Failure of PGT diagnosis is reported to occur in between 2-12% of PGT cycles depending on the methodology used. The main causes of inconclusive diagnosis are DNA amplification failure and non-concurrent results. Independent variables that influence inconclusive results are day of embryo biopsy, number of cells taken at biopsy and the quality of the IVF laboratory. In order to seize every opportunity in reaching a diagnosis and optimize reproductive outcome for the patient, rebiopsy and retesting (double biopsy and double cryopreservation) can be performed. In situations where embryos need to be biopsied for PGT for reasons of newly found genetic indications or the clinical need for PGT-A, and after an initial cryopreservation, single biopsy and double cryopreservation may be performed with potential effect on subsequent reproductive outcome.

A limited number of studies have addressed both the feasibility as well as the success rate of rebiopsy and refreezing. Most studies conclude that the technique is efficient in terms of diagnostic efficiency and reproductive outcome, when performed in expert laboratories. A few studies have addressed the health of the children after multiple biopsy and multiple freezing and have not shown increased risks. Larger studies are needed to establish the true clinical and health-economic value of rebiopsy and refreezing. The speaker will give an overview of existing evidence in literature and assess whether it is safe and worthwile to go the extra mile and consider refreezing and rebiopsy for PGT.

Trial registration number: XXXX

INVITED SESSION
SESSION 17: FSANZ AND CFAS EXCHANGE SESSION
Monday 26 June 2023 Hall D4 14:00 - 15:00

Abstract citation ID: dead093.062
O-052 Morphometric and morphokinetic differences in the sperm- and oocyte- originated pronuclei of male and female human zygotes: a time-lapse study

L.S. Orevich[1], **Y. Liu**[1], **K. Watson**[1], **K. Ong**[2], **I. Korman**[2], **R. Turner**[3], **D. Shaker**[4]

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Study question: Do morphometric and morphokinetic profiles of pronuclei (PN) following intracytoplasmic sperm injection (ICSI) vary between male and female human zygotes?

Summary answer: Male and female zygotes displayed different PN morphometrics and morphokinetics. Additionally, variations were identified between sperm-originated (SPN) and oocyte-originated (OPN) pronuclei.

What is known already: Previous studies have investigated the use of PN-associated parameters via static observations as indicators of zygote viability, including size equality or juxtaposition. However, recent clinical application of time-lapse videography (TLV) provides a novel opportunity to assess these pronuclear events with greater accuracy and precision of morphometric and morphokinetic measurement. A number of recent TLV studies have also investigated potential live birth prediction by such PN associated measures, however whether or not there are sex associated differences in such measures which could in turn confound live birth prediction is unknown.

Study design, size, duration: This retrospective cohort study included 94 consecutive autologous single day 5 transfer cycles (either fresh or frozen) performed between January 2019 and March 2020. Only ICSI cycles (maternal age <40 years) leading to a singleton live birth (43 males and 51 females) were included for analysis. All oocytes were placed in the EmbryoScope incubator for culture immediately post sperm injection with all annotation performed retrospectively by one embryologist (L-SO).

Participants/materials, setting, methods: Timings included 2nd polar body extrusion (tPb2), SPN(tSPNa)/OPN(tOPNa) appearance (differentiated by proximity to Pb2) and PN fading (tPNF). Morphometrics were evaluated at 8 (stage 1), 4 (stage 2) and 0 hour before PNF (stage 3), measuring PN area (um^2), PN juxtaposition, and nucleolus precursor body (NPB) arrangement. Means ± standard deviation were compared using student t test or logistic regression as odds ratio (OR) and 95% confidence interval (CI), and proportional data by chi-squared analysis.

Main results and the role of chance: Logistic regression indicated that male zygotes had longer time intervals of tPb2_tSPNa than female zygotes (4.8 ± 1.5 vs 4.2 ± 1.0 h, OR = 1.442, 95% CI 1.009-2.061, p = 0.044), but not tPb2_tOPNa (4.7 ± 1.8 vs 4.5 ± 1.3 h, OR = 1.224, 95% CI 0.868-1.728, p = 0.250) and tPb2_tPNF (19.9 ± 2.8 vs 19.1 ± 2.3 h, OR = 1.136, 95% CI 0.957-1.347, p = 0.144). SPN increased in size from stage 1 through 2 to 3 (435.3 ± 70.2, 506.7 ± 77.3, and 556.3 ± 86.4 um^2, p = 0.000) and OPN did similarly (399.0 ± 59.4, 464.3 ± 65.2, and 513.8 ± 63.5 um^2, p = 0.000), with SPN being significantly larger than OPN at each stage (p < 0.05 respectively). However, relative size difference between SPN and OPN was similar between male and female zygotes at 3 stages (33.6 ± 61.7 vs 38.6 ± 50.8 um^2, p = 0.664; 38.5 ± 53.1 vs 45.7 ± 71.9 um^2, p = 0.585; 38.4 ± 77.4 vs 45.8 ± 63.9 um^2, p = 0.615; respectively). More male than female zygotes reached central PN juxtaposition at stage 1 (77% vs 51%, p = 0.010), stage 2 (98% vs 86%, p = 0.048) and stage 3 (98% vs 86%, p = 0.048). Furthermore, more OPN showed aligned NPBs than in SPN at stage 1 (45% vs 29%, p = 0.023), but similar proportions at stage 2 (64% vs 50%, p = 0.056) and stage 3 (76% vs 72%, p = 0.618). There were no sex associated differences detected in NPB alignment in either SPN or OPN (p > 0.05 respectively).

Limitations, reasons for caution: The retrospective design does not allow for control of unknown confounders. Sample size is considered relatively small. PN area measurement may not truly represent volume as PN may not be perfectly spherical. Findings were based on women <40 years old so may not apply to older population.

Wider implications of the finding: These findings augment and extend previous studies investigating PN parameters via static observations. The reported variations between male and female embryos may confound live birth prediction when using pronuclei morphometrics and morphokinetics. Larger scaled studies are warranted to verify these findings.

Study funding/competing interest(s): N/A.

Trial registration number: N/A.

Abstract citation ID: dead093.063

O-053 Link between cannabis use and fertility: effects on gamete competence and early embryonic development

L. Favetta[1]

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Worldwide human fertility has been facing a significant decline over recent years. Therefore, attention has been focused on identifying environmental and lifestyle risk factors that may be contributing to impact human reproductive function. The use of alcohol, tobacco and certain medications have undergone substantial assessment into their effects on fertility. As physicians aim to offer the most appropriate recommendations to their patients, it is of utmost importance to understand the side effects of readily available substances and their mechanisms of action. Cannabis falls into this group of substances used regularly by women and men of reproductive age, with its consumption steadily rising with the onset of legalization in Canada in 2018 also amongst pregnant women. At present, the Society of Obstetricians and Gynecologists of Canada recommends that women of reproductive age, especially if considering pregnancy, discontinue cannabis use. There is, however, inconclusive evidence that links cannabis and fertility.

This presentation will outline the most recent data from our laboratory on the effects of Delta-9 tetrahydrocannabinol (THC), the main psychoactive component of cannabis, on oocytes competency and sperm fertilization capability. In fact chronic exposure to cannabis also adversely affects the male reproductive system, inducing oligospermia, morphologically abnormal sperm, and impaired sperm motility in rodents. Using a bovine model as translational model for humans, we identified effects of THC on oocyte developmental parameters, gene expression as well on sperm parameters, such as motility, morphology, capacitation and mitochondrial potential. The general hypothesis at the basis of this research program aims to elucidate **the effects of THC at clinically relevant doses on gene and epigenetic modifications in sperm, oocytes and embryos, ultimately affecting fertility and pregnancy outcome.** We showed a significantly reduced ability of THC-treated oocytes to undergo nuclear maturation, ultimately leading to decreased oocyte fertilization capability and poor embryonic development. We showed that THC acts as a partial agonist at the Cannabinoid receptors 1 and 2 (CB1 and CB2), as THC effects were reverted by the addition of antagonists. The significant decrease in maturation and cleavage rate of oocytes exposed to the higher doses of THC, representative of high recreational use of Cannabis, allows us to speculate that THC might act as a selection factor for only the highest quality oocytes, that overcome the THC effects. The more immature oocytes may be negatively affected by THC by being pushed towards meiotic resumption, leading to early maturation, prior to gaining developmental competence. Levels of connexin gap-junction proteins were used as indicator of oocyte competency, as they highly correlate to oocyte fertilization potential and embryo quality. Our results demonstrated a significant decrease in *connexin 37* (CX37) and *connexin 43* (CX43) mRNA levels measured by digital droplet PCR (ddPCR), in oocytes only in the low dose THC group, representative of the therapeutic use of Cannabis. More recently we have also shown how THC affects methylation in granulosa cells, via disrupting methylation and de-methylation enzymes, ultimately determining oocyte competency and, therefore, fertility. THC exerts parallel effects on the male gametes as well, increasing capacitation of *in vitro* treated sperm, affecting their mitochondrial potential and disrupting the expression of key microRNAs in sperm that are linked to early embryonic development, therefore affecting pre-implantation development and, ultimately, pregnancy outcome.

Our results suggest an overall detrimental effect of THC on the competency and fertilization potential of both female and male gametes, negatively influencing fertility and the likelihood of a viable pregnancy, especially of importance when undergoing artificial reproductive technologies procedures.

Trial registration number: XXXX

SELECTED ORAL COMMUNICATIONS
SESSION 18: REPRODUCTIVE SURGERY
Monday 26 June 2023 Auditorium 10-12 14:00 - 15:00

Abstract citation ID: dead093.064

O-054 Live Births following Laparoscopic Caesarean Scar Niche Repair in patients with Secondary Infertility: A Self-Controlled Case Series and Literature Review.

M. Deivanayagam[1], **O. Tsonis**[1], **K. Kunde**[2], **Y. Kopeika**[1]

[1]*Guys' & St Thomas' Hospital, Assisted Conception Unit, London, United Kingdom*
[2]*Guys' & St Thomas' Hospital, Gynaecology, London, United Kingdom*

Study question: Does laparoscopic repair of caesarean scar niches as a treatment for secondary infertility improve live birth rates?

Summary answer: In our self-controlled case series, the laparoscopic repair of caesarean section niches in patients with secondary infertility has improved pregnancy and live birth rates.

What is known already: A niche is an iatrogenic myometrial defect due to abnormal tissue healing at a previous caesarean section scar site. Patients with a niche present with symptoms of vaginal discharge, abnormal uterine bleeding, chronic pelvic pain, dyspareunia and secondary infertility. As a niche is secondary to caesarean sections, rising caesarean section rates will lead to an increase in the rate of niche presentation and diagnosis.

Study design, size, duration: A retrospective review was performed of all women undergoing a hysteroscopy assisted laparoscopic niche repair between 2016 and 2023 at Guy's and St Thomas' Hospital in London. The primary outcome measure was live births. A literature review was performed to interrogate the currently available evidence on live birth rate following niche repair.

Ultrasound images of the niche prior to and post repair, as well as intraoperative laparoscopic and hysteroscopic images and videos, are also presented.

Participants/materials, setting, methods: The cases were identified from the Assisted Conception Unit database. Only women who presented with secondary infertility with a diagnosis and repair of a niche were reported.

Niche diagnosis was made in patients presenting with abnormal discharge, bleeding and fluid in the uterine cavity. Any incidental myometrial defect found on transvaginal ultrasound was also a diagnostic characteristic.

Main results and the role of chance: There are some reports in the literature on pregnancy outcome following niche repair. A scarcity of studies reporting specifically on live birth rates following repair, suggests this case series as unique.

Eleven patients underwent caesarean section niche repair by two surgeons at our unit. The mean age at repair of the patients was 36. All patients had two or more years of subfertility on presentation, with a proportion having several failed in-vitro fertilisation (IVF) cycles prior to repair.

The pregnancy rate, clinical pregnancy rate and live birth rate were 73% (8/11), 64% (7/11) and 45% (5/11) respectively. One patient had an early miscarriage at age 48 following repair and has not conceived again since. One patient underwent a second repair and is awaiting further investigations. Two patients have not yet conceived following repair, one of which had sickle cell disease, age factor and endometriosis.

Our findings suggest a positive correlation between surgical repair of the niche and live births. However, this could be due to a strict selection criteria in a small sample size. In the two patients that did not have live births following repair, other factors of subfertility such as age, male factor and comorbidities could also play a role.

Limitations, reasons for caution: The small sample size can lead to selection bias and impairs the external validity of our findings. The likelihood of random error is high. Lack of a control group prevents any comparisons.

Furthermore, all repairs were performed by the two surgeons which introduces operator bias. Outcome assessors were not blinded.

Wider implications of the findings: Subfertility secondary to niches is likely to be a growing challenge as caesarean section rates continue to increase. Our study suggests that surgical repair may improve live birth rates. We present our findings to augment the current international data on live birth rates following niche repair.

Trial registration number: not applicable

Abstract citation ID: dead093.065
O-055 Efficacy of Laparoscopic Abdominal Cerclage in Women With Prior 2nd Trimester Losses/Preterm Birth: Perinatal Outcomes of Case Series

E. Pabuccu[1], R. Pabuccu[2], G. Israfilova[3], A. Valadova[4]

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[4]*Private Cagsu Hospital, Obstetrics and Gynecology, Düzce, Turkey*

Study question: What is the efficacy of laparoscopic abdominal cerclage (LAC) that placed during periconceptional period in singletons with prior 2nd trimester pregnancy losses/preterm deliveries (PTDs)?

Summary answer: LAC seems to prolong pregnancy duration and enhance perinatal outcomes in singletons prior 2nd trimester losses/PTDs.

What is known already: Cervical insufficiency (CI) is defined as the inability of the uterine cervix to retain a pregnancy till term without the signs and symptoms of clinical contractions ,with an incidence up to 1% (Berghella, 2011). Cervical cerclage (CC) has been proposed as an effective treatment for CI in terms of successfully prolonging the pregnancy period and improving the perinatal prognosis. The common clinical approach is transvaginal cervical cerclage (TVC), which is a feasible and relatively easy technique. However, some group of women may experience late abortions and/or PTD and LAC has been proposed to enhance pregnancy outcomes.

Study design, size, duration: Case series consisting of singletons with at least 2 prior PTDs and managed with LAC was reviewed between January 2019-November 2022. After placement of LAC, reproductive and perinatal outcomes of women were reviewed for 2 years.

Participants/materials, setting, methods: All women with at least 2 prior 2nd trimester losses and/or PTDs were offered to LAC procedure during the period. After having informed consents, total of 28 LAC procedures were performed. All surgeries were performed by a single experienced laparoscopist in the same center. Non-absorbable mersilene tape was placed during preconceptional period. Reproductive outcomes including spontaneous and assisted conception cycles and perinatal outcomes were reported.

Main results and the role of chance: A total of 28 LAC proceudres were performed during the period. Mean age of women was 34 ± 2 and mean numbers of previous 2nd trimester losses/PTDs was 2.8 ± 0.8, of which all of them were <24 weeks. Total of 8 women have had gynecologic surgery in their history. Less than 12 months interval, 19 out of 28 women got pregnent; 13/28 conceived spontaneously and 6/28 conceived with after IVF treatment.

For the perinatal outcomes; there were no single case of 2nd trimester losses/PTDs <24 weeks in the entire cohort.

15 women delivered >34 weeks and remaining 4 delivered at term (>37 weeks).

During the gestation, no additional medication was provided to pregnants such as progesterone treatment.

The mean gestational age was 35 4/7 weeks.

There were no remarkable adverse events during the pregnancy. All newborns were healthy without remarkable issues, only 2 infants required intensive care for less than 2 weeks.

Limitations, reasons for caution: Small sample size, lack of a control group and randomization and heterogenious patient population.

Wider implications of the findings: LAC appears to have favorable pregnancy outcomes in singletons with prior pregnacy losses and/or PTDs. To precisely conclude, randomized controlled trials are needed with different intervention arms in the same population.

Trial registration number: non applicable

Abstract citation ID: dead093.066
O-056 Dysmorphic uterus: lateral angle might associate with euploid blastocyst implantation

A. Romito[1], D. Cimadomo[2], D. Golino[3], E. Pittana[4], C. Exacoustos[5], C. De Angelis[6], M. Guido[7], I. Romito[8], L. Rienzi[2,9], F.M. Ubaldi[2], A. Vaiarelli[2]

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[5]*University of Rome 'Tor Vergata', Department of Biomedicine and Prevention, Rome, Italy*
[6]*Sapienza University of Rome, Department of Maternal and Child Health and Urology, Rome, Italy*
[7]*University of L'Aquila, MeSVA Department, L'Aquila, Italy*
[8]*San Camillo-Forlanini Hospital, Department of Woman's and Child's Health, Rome, Italy*
[9]*University of Urbino "Carlo Bo", Department of Biomolecular Sciences, Urbino, Italy*

Study question: Are there a morphological features measured in coronal plane obtained by tridimensional transvaginal sonography (3D-TVS), associated with euploid blastocyst implantation?

Summary answer: Mean laeral angle (mLA) below 143 degrees was associated with lower clinical pregnancy rate among 47 euploid blastocyst transfers.

What is known already: The ESHRE/ESGE 2013 consensus on uterine malformation clustered in Class U1 (namely Dysmorphic uterus): T-shaped uterus (class U1a), uterus infantilis (Class U1b) and Class U1c including other minor abnormalities. Diagnostic criteria are still under major debate, however a few studies report an association between dysmorphic uterus and impaired fertility outcomes. In the literature, no evidence has been published on a selected population of euploid blastocyst transfers. Moreover, sonographic predictors of successful transfer in patients with dysmorphic uterus undergoing ART are not available.

Study design, size, duration: Cohort study involving 122 infertile couples undergoing ICSI with NGS-based PGT-A on trophectoderm biopsies between July-2022 and December-2023. A 3D-TVS was acquired in the luteal phase before starting ovarian stimulation. The primary outcome was to assess whether uterine morphological features associate with clinical pregnancy rate, defined as an ultrasonographic evidence of a gestational sac with fetal heartbeat per transfer. To date, 47 patients underwent vitrified-warmed single euploid blastocyst transfer.

Participants/materials, setting, methods: Uterine morphology was assessed in a coronal plane. Following measurements, we registered: (1) the distance between the two internal tubal ostia, (2) the width of the uterine cavity at corpus-isthmic level, (3) the lateral angle between the corpus-isthmic cavity, (4) the lateral indentation. The optimal cut-off values of mLA was evaluated with ROC curve; $p < 0.05$ was considered statistically significant. SPSS version 25.0 was used for statistics.

Main results and the role of chance: Median age was 38 (IR:26-43). The main cause of infertility was tubal/ovulatory in 22 cases (46%), severe male factor in 18 (39%) and idiopathic in 18 (39%). Thirteen women (28%) experienced a previous miscarriage, and half of them were submitted to a uterine cavity revision. The mLA ranged from 126 to 180 degrees (median:156 degrees), whereas mean indentation ranged from 0 to 7.2 mm (median: 3.0 mm). The patients were categorized in 2 groups by the optimal cutoff

value of mLA calculated based on ROC curve analysis. Dysmorphic uterus was defined in case of mLA (mean between right and left lateral angle) below 143 degrees (sensitivity 90%, specificity 54%; AUC=0.67). mLA levels were higher than this threshold in 37 women (80%). Relative risk to have a clinical pregnancy was 0.35 (95%CI 0.15-0.83) in dysmorphic uterus. Two women (25%) got pregnant in dysmorphic uterus group compared to 18 (72%) in normal uterus (p:0.026). After adjusting blastocyst quality and day of transfer, mLA association with a clinical pregnancy remained significant (RR 7.38, 95%CI 1.09-50.18 p = 0.04, Post hoc power: 65.3%).

Limitations, reasons for caution: Preliminary results. A larger sample is required to confirm these data.

Wider implications of the findings: The strength of the study is the definition of the impact of a dysmorphic uterus in IVF cycles with PGT-A. If confirmed in larger multicenter datasets, these data may select patients who could benefit from hysteroscopic metroplasty.

Trial registration number: not applicable

Abstract citation ID: dead093.067
O-057 Prevalence of T-shaped uterus among women undergoing fertility treatments based on ESHRE/ESGE and Congenital Uterine Malformation by Experts (CUME) criteria.

A. Bilgory[1], Y. Atzmon[1], N. Aslih[1], Y. Shibli[1], Abu Raya[1], M. Sharqawi[1], M. Shavit[1], E. Shalom-Paz[1]

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Study question: What is the prevalence of a T-shaped uterus among women undergoing fertility treatments based on ESHRE/ESGE (Grimbizis et al.,2013) and CUME (Ludwin et al.,2020)?

Summary answer: The prevalence of T-shaped uterus was 4.1% according to the ESHRE/ESGE, and according to the CUME, 1.1% T-shaped uterus and 0.9% borderline T-shaped uterus.

What is known already: The definition of a T-shaped uterus by the ESHRE/ESGE consensus (Grimbizis et al., 2013) was based on three-dimensional ultrasound (3D-US) images, defining it as a narrow uterine cavity caused by thickened lateral walls with a ratio of two-thirds uterine body and one-third cervix. This definition is subjective and therefore makes giving an objective diagnosis difficult. The CUME group (Ludwin et al., 2020) described practical diagnostic criteria for a T-shaped uterus according to 3D-US images. As there is no consensus regarding the definition, the overall prevalence according to different studies varied from 0.2 to 10.0%.

Study design, size, duration: A retrospective cohort study with prospective analysis of 3D-US images was conducted. All women who were admitted to our unit for fertility treatments and underwent 3D-US between 12/2017-12/2021 were included. Women were grouped according to infertility type. All 3D-US images of uteri suspected to be T-shaped according to ESHRE/ESGE were assessed according to the CUME criteria, based on the following three measurements: lateral indentation angle $\leq$130°, lateral indentation depth $\geq$7 mm, and T-angle $\leq$40°.

Participants/materials, setting, methods: The study was conducted in a single university-affiliated hospital. Women who underwent fertility treatments due to various indications were included. The exclusion criteria were women undergoing fertility preservation, egg donation and oocyte recipients. All 3D-US were performed by well-trained ultrasound technicians. We first screened all images and calculated the prevalence of T-shaped uteri in our population based on the ESHRE/ESGE consensus. Next, we performed the three measurements according to the CUME criteria.

Main results and the role of chance: Altogether 451 women were admitted to our fertility unit. Nine cases were excluded due to unsatisfactory 3D-US images because of technical difficulties. Finally, 442 women were included in the study and divided into in the following groups: anovulation 10.6% (n = 47), mechanical factor 11.1% (n = 49), male factor 38.7% (n = 171), and unexplained infertility 39.6% (n = 175).

The prevalence of T-shaped uterus according to the ESHRE/ESGE was 4.1% (n = 18). Among them, 3.2% (n = 3) were from the female factor groups (anovulation + mechanical), 4.1% (n = 7) from the male factor group, and 4.6% (n = 8) from the unexplained infertility group.

Afterwards, the 3D-US images were analyzed according to the CUME criteria. T-shaped uterus was defined when all three criteria were met, and borderline T-shaped uterus was defined when two out of three criteria were met. According to CUME criteria, the prevalence of T-shaped uterus was only 1.1% (n = 5), three being from the male factor group and two from the unexplained infertility group. No T-shaped uteri were found in the female factor groups. Additionally, 0.9% (n = 4) were considered borderline T-shaped.

Limitations, reasons for caution: The ESHRE/ESGE consensus is subjective and may lead to either under- or over-diagnosis. On the other hand, the CUME criteria are objective and well-defined, but due to their strictness, dysmorphic uteri with very narrow uterine cavities and thickened lateral walls may not be diagnosed as T-shaped uteri.

Wider implications of the findings: Diagnosis and management of women with T-shaped uteri are very controversial topics. Therefore, making the diagnosis more precise and objective can help us to screen for the most significant cases and tailor the management accordingly. The similar prevalence among different infertility groups reflects the incidental nature of this diagnosis.

Trial registration number: Not applicable.

INVITED SESSION
SESSION 19: CSRM EXCHANGE SESSION
Monday 26 June 2023 Hall D5 14:00 - 15:00

Abstract citation ID: dead093.068
O-058 Metabolism, histone modification and early embryo development

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Cellular metabolism provides fundamental components for chromatin dynamics and epigenetics. However, metabolic features, and their impact on epigenetic remodeling during mammalian pre-implantation development, remain poorly understood. In this study, we established the metabolic landscape of mouse pre-implantation embryos from zygote to blastocyst, and quantified their absolute carbohydrate metabolites. We found that amino acid/carbohydrate/lipid metabolism increased during pre-implantation development, whereas nucleotide metabolism increased at the 4- and 8-cell stages. We further demonstrated that oxidized nicotinamide adenine dinucleotide (NAD^+) is indispensable for mouse pre-implantation development. Mechanistically, NAD^+ avoids excessive minor zygotic gene activation (ZGA) by cooperating with deacylase SIRT1 to remove zygotic H3K27ac. In human, NAD^+ supplement can promote the removal of zygotic H3K27ac and benefit preimplantation development. Our findings demonstrate that precise and timely regulation of minor ZGA is controlled by metabolic dynamics, and enhance our understanding of the metabolism of mammalian early embryos.

Abstract citation ID: dead093.069
O-059 ntermetabolites of cholesterol synthesis in granulosa cell and oocyte aging

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With the development of society, there has been a significant delay in female fertility and an increasing desire for childbearing among females of advanced

maternal age in recent decades. Nevertheless, women over 35 years old have more risk of infertility and miscarriage compared to young women, due to decreased oocyte quality. These changes are mainly caused by meiotic defects, especially aneuploidy. Therefore, exploring the mechanisms of aging-related meiotic defects and aneuploidy in oocytes is of great significant for improving the pregnancy outcomes of aged women.

Oocytes are enveloped by granulosa cells (GCs) to form follicles, which constitute the reproductive units in ovaries. GCs form a metabolic community with oocytes for oocyte growth and maturation. Numerous studies have reported that metabolic coupling between GCs and oocytes could affect the process of oocyte meiosis resumption, spindle assembly, and chromatin arrangement. Maternal aging is related to disruption of oocyte-GC interactions that leads to disorders of the oocyte meiotic process. Therefore, abnormal metabolic coupling between GCs and oocytes is an important reason for decreased quality in aged oocytes.

To further clarify the metabolic coupling changes between GCs and oocytes during ovarian aging, we systematically characterized the dynamic changes in the overall transcriptomic landscapes of oocytes and GCs from young and aged mice throughout oocyte meiosis (during the growing oocyte [GO], full-grown oocyte [FGO], metaphase I [MI] oocyte [MIO], and metaphase II [MII] oocyte [MIIO] phases). Data revealed that the mevalonate (MVA) pathway, an essential metabolic pathway that uses acetyl-CoA to produce cholesterol and isoprenoids, was specifically highly expressed in GCs with the resumption of oocyte meiosis, and the expression of MVA pathway in GCs decreased with age. Atorvastatin-mediated inhibition of MVA metabolism in GCs decreased the first polar body extrusion (PBE) rate, and increased oocyte meiotic defects and aneuploidy in young cumulus-oocyte complexes (COCs). Further studies showed that the effect of the MVA pathway on oocyte meiosis was mainly regulated by protein isoprenoylation mediated by the inter-metabolites of cholesterol synthesis. Importantly, upregulation of the MVA pathway in aged GCs could ameliorate the depletion of ovarian reserve, decrease oocyte meiotic defects, and improve oocyte quality.

In conclusion, metabolite in granulosa cell is an important factor determining the oocyte quality; MVA pathway in GCs is a critical regulator of meiotic maturation and euploidy in oocytes, and age-associated MVA pathway abnormalities contribute to oocyte meiotic defects and aneuploidy; Supplementation of inter-metabolites of cholesterol synthesis is a new therapeutic target for clinical intervention to improve oocyte quality.

Trial registration number: XXXX

POSTER DISCUSSION SESSION

SESSION 20: ENDOMETRIOSIS AND ENDOMETRIAL DISORDERS

Monday 26 June 2023 Hall D2 14:00 - 15:00

Abstract citation ID: dead093.070
P-313 Dysregulation of endometrial stromal serotonin homeostasis impairs decidualization in patients with recurrent implantation failure via phosphatidylcholine metabolism

X. Sheng[1], J. Tian[1], G. Yan[1], H. Sun[1]

[1]*Nanjing University Medical School, Nanjing Drum Tower Hospital, Nanjing-Jiangsu, China*

Study question: Does abnormal serotonin homeostasis contribute to impaired endometrial decidualization in patients with recurrent implantation failure (RIF)?

Summary answer: Abnormal serotonin homeostasis in patients with RIF, which is accompanied by decreased expression of monoamine oxidase (MAO), affects decidualization of endometrial stromal cells.

What is known already: Previous studies have found that the expression of MAO, which metabolizes serotonin, is reduced in the endometrium of patients with RIF, and serotonin can induce disruption of implantation in rats. However, whether abnormal serotonin homeostasis leads to impaired decidualization in patients with RIF and the mechanism remains unclear.

Study design, size, duration: Endometrial samples from 31 patients with RIF and 31 fertile patients were used to investigate the expression levels of MAOA, MAOB, and serotonin. We isolated human endometrial stromal cells to investigate the role of MAOA, MAOB and serotonin in inducing decidualization in vitro and further explored the mechanism using RNA-seq and LC/MS analyses.

Participants/materials, setting, methods: The levels of serotonin in the endometrium of patients with RIF were detected by ELISA and immunohistofluorescence, and the key genes of abnormal serotonin metabolism were analyzed combined with single-cell sequencing data. The effects of MAO on the decidualization of stromal cells were investigated using human endometrial stromal cells in vitro induced decidualization model and mouse artificially induced decidualization model. The potential mechanisms of MAO regulating decidualization were explored by RNA-seq and LC/MS analysis.

Main results and the role of chance: We found that women with RIF have abnormal serotonin metabolism in the endometrium and attenuated MAO in endometrial stromal cells. Endometrial decidualization was accompanied by increased MAO in vivo and in vitro. Attenuated MAO caused increased local serotonin content in the endometrium, impairing stromal cell decidualization. RNA-seq and LC/MS analyses showed that abnormal lipid metabolism, especially phosphatidylcholine metabolism, was involved in MAO-deficiency induced defective decidualization. Furthermore, decidualization defects were rescued by phosphatidylcholine supplementation.

Limitations, reasons for caution: This study found that impaired serotonin metabolic homeostasis and abnormally reduced MAO expression were one of the reasons for repeated implantation failure. However, the source and other potential function of serotonin in the endometrium remain to be further explored.

Wider implications of the findings: This study shows new insights into the mechanisms of serotonin homeostasis in human endometrial decidualization and provides new biomarkers or targets for the treatment of patients with RIF.

Trial registration number: not applicable

Study funding: No

Funding source: Funding by national/international organization(s)

Abstract citation ID: dead093.071
P-340 Endometrial microbiome - is every dysbiosis an inflammation?

E. Morawiec[1], A. Bednarska- Czerwinska[2], M. Czerwinski[2], A. Pudelko[1], A. Strychalska[1], M. Borowiec[1]

[1]*Gyncentrum Clinic, Genetic Laboratory, Katowice, Poland*
[2]*Gyncentrum Clinic, Gyncentrum Fertility Clinic, Katowice, Poland*

Abstract: Study question: The endometrial microbiome, in addition to other parameters of inflammation (CD138 presence, NK bright fraction presence), may be a complementary marker of ongoing local inflammation.

Summary answer: Presented results of the microbiome in comparison with the CD 138 marker may indicate inflammation in the endometrium, which is caused by dysbiosis.

What is known already: The study of the microbiome and the assessment of its impact on female reproductive system on embryo implantation, pregnancy maintenance and the success of the in vitro fertilization (IVF) procedure is an increasingly popular research topic. The protective role of *Lactobacillus* (other than *L.iners*) is known, and probably its low number may be related to the impossibility of embryo implantation. Dysfunctional mycobiome may be the cause of chronic inflammation of the endometrium, including

endometriosis and recurrent implantation failures. Importantly, the dysbiotic profile of the endometrial microflora often does not cause any clinical symptoms.

Study design, size, duration: The clinical study included 182 women in child-bearing age, who were patients of the Gyncentrum Clinic (Poland), and who were qualified for the IVF procedure. In this group of patients, 201 samples combined were tested, obtaining 19 negative results due to the low quality of swabs, and the small amount of microbiota impossible to efficiently isolate. In the study, samples were analysed from January to December 2022 collection.

Participants/materials, setting, methods: To analyze the assumed parameters, patients treated at the Gyncentrum Clinic underwent a routine hysteroscopy procedure, during which endometrial swabs (determination of the molecular microbiome using the Next Generation Sequencing NGS method) and tissue fragments (determination of CD138 immunohistochemically and NK fractions by flow cytometry) were collected. Nucleic acids were isolated from the swabs for the preparation of libraries, based on the Illumina-16S Metagenomic Sequencing Library Preparation.

Main results and the role of chance: The microbiome study and other inflammation parameters in the group of our patients allowed us to observe some trends. Due to the microbiological endometrium profile, the patients were divided into 3 groups: 1) normal microflora (domination of *Lactobacillus* other than *L.iners* and/or *Bifidobacterium*;n =135), 2) moderate dysbiosis (presence of *Lactobacillus* and/or *Bifidobacterium* and/or *L. iners*-max. 50 % of all sample- and also other potentially harmful species e.g. *Enterobacteriaceae* group, *Streptococcus* group, *Veilonella*;n =28); 3) dysbiosis (with/without low number of *Lactobacillus* and *Bifidobacterium*, strong dominance of harmful species listed above or *L.iners* dominance;n =19). We observed that in the group of endometrial dysbiosis patients, an increased CD138 index ($\geqslant$1/10 high power fields HPF) is more often noted - in the group of dysbiotic patients with CD138 = 0/10 HPF 7.21 %, in the group of dysbiotic patients with increased CD 138 15.49 %. We didn`t notice any trends comparing the results of CD138 to the NK bright fraction (CD56 + +/CD16-) tested in endometrial specimens, or the relationship of this fraction to the microbiome. Observed trend indicates a certain relationship in the presence of abnormal microflora and inflammation expressed by CD138, but it requires confirmation on a larger study group and obtain information about the success of pregnancy.

Limitations, reasons for caution: The conducted research require broader analysis on a larger group of patients. It is necessary to obtain full information about the success of pregnancy in all analyzed patients. Identification of taxa using the NGS method is not the same as the identification of microorganisms living in the studied microenvironment.

Wider implications of the findings: Presented results should be treated as a starting point for further analyses, important in the context of research based on infertility, focusing on relationships between the microbiome and the IVF effectiveness. It seems interesting to extend the molecular study of the microbiome to its other elements - viruses, fungi, protozoa.

Trial registration number: 161/KBL/OIL/2021

Study funding: No

Funding source: Funding by hospital/clinic(s)

Abstract citation ID: dead093.072

P-342 Uterine contractile patterns in adenomyosis patients normalize under hormonal contraception use: the WAVES study

C. Klaassen[1], C. Rees[1], Y. Huang[2], S. Thomas[1], A. de Boer[1], B. Zizolfi[3], V. di Foreste[3], A. di Spiezio Sardo[3], N. Christoforidis[4], H. van Vliet[1,5], M. Mischi[2], B. Schoot[1,5]

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[5]*University Hospital Ghent, Department of Reproductive Medicine, Ghent, Belgium*

Abstract: Study question: Comparison of uterine contractility (UC) in adenomyosis patients (AP) with and without hormonal contraception (HC) compared to controls with HC, measured by transvaginal ultrasound (TVUS).

Summary answer: AP with HC show better contraction coordination compared to untreated AP. AP with HC show comparable UC compared to controls with HC.

What is known already: Adenomyosis is a disease of the uterus that can cause dysmenorrhoea, menorrhagia, dyspareunia and subfertility. These symptoms could be explained by the different contraction patterns in women with adenomyosis compared to healthy controls. Therapeutic use of hormonal contraception reduces the symptoms experienced by women with adenomyosis. This could be explained by the normalization of contraction patterns, which has not yet been objectively quantified due to the absence of a suitable measurement tool. A novel speckle-tracking and strain analysis by 2D TVUS recordings has recently been used to assess differences in contraction coordination, contraction frequency, velocity and direction in healthy women.

Study design, size, duration: This study is part of an ongoing multi-centre prospective observational cohort study investigating UC on TVUS. Our study includes the TVUS recordings of 23 women with adenomyosis without hormonal contraception treatment, 15 women with adenomyosis undergoing hormonal contraception treatment, and 17 women with healthy uteri undergoing hormonal contraception treatment. Patients were included in 3 centres from 2017 to 2023 (Catharina Hospital Eindhoven, Fertility Clinic Thessaloniki and University Federico Napels).

Participants/materials, setting, methods: 23 women with sonographic suspicion of adenomyosis without HC, 15 women with adenomyosis undergoing HC treatment and 17 women with healthy uteri with HC were included. HC included oral combined HC, progesterone only pill and hormonal IUD. UC frequency, amplitude, velocity and coordination were assessed by applying a dedicated speckle-tracking and strain analysis to 2-4-minute TVUS recordings in midsagittal section. AP with HC were compared to AP without contraception and to healthy controls with HC.

Main results and the role of chance: Age, BMI, parity and uterus volume were significantly higher in the women with adenomyosis compared to the healthy controls ($p < 0.05$). The adenomyosis group with contraception showed more contraction coordination compared to the adenomyosis group without hormonal contraception treatment (0.23 $\pm$0.10 vs. 0.29 $\pm$0.11, $p = 0.041$). There was a tendency towards higher contraction frequency (1.53 $\pm$0.21 vs. 1.42, $p = 0.153$) and lower amplitude (0.56 $\pm$0.04 vs. 0.65 $\pm$0.04, $p = 0.159$) in the adenomyosis group with hormonal contraception treatment compared to the adenomyosis group without hormonal contraception treatment. There were no significant differences in uterine contractility between the adenomyosis group with hormonal contraception treatment compared to the healthy group with hormonal contraception treatment.

Limitations, reasons for caution: No sub-analysis was done to assess effects of additional adenomyosis and contraception characteristics due to this being an ongoing study. Women with extensive adenomyosis were not included due to impossibility to perform analysis of ultrasound recordings. AP were older, had higher BMI and larger uterus volumes than healthy controls.

Wider implications of the findings: The normalization of UC under therapeutic use of HC compared to untreated AP and the lack of differences in UC between AP and healthy controls with HC, confirms the therapeutic effect on adenomyotic symptoms. This presents a new therapeutic efficacy marker for adenomyosis.

Trial registration number: NL52466.100.15

Study funding: Yes

Funding source: Funding by commercial/corporate company(ies)

Abstract citation ID: dead093.073

P-368 Clinical impact of personalized embryo transfer (pET) guided by endometrial receptivity analysis (ERA) in patients with ≥ 1 failed transfers

M. Ruiz-Alonso[1], T. Stankewicz[2], J.A. Castellón[3], C. Gómez[3], E. De La Fuente[3], E. Gómez[1], C. Simón[4,5,6,7], D. Valbuena[3]

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[6]*Harvard University, Beth Israel Deaconess Medical Center, Boston, U.S.A*
[7]*University of Valencia, Obstetrics & Gynecology, Valencia, Spain*

Study question: Does pET following ERA increase pregnancy (PR), ongoing pregnancy per transfer (OGPR) and live birth rates (LBR) in patients with ≥ 1 previous failed transfer?

Summary answer: In this group of patients, pET guided by ERA obtained a significantly higher PR, OGPR and LBR versus standard embryo transfer.

What is known already: pET consists of synchronizing embryo transfer with the optimal endometrial receptivity status of the patient by the transcriptomics identification of her personalized window of implantation (WOI). Controversial results have been published regarding the clinical usefulness of this precision medicine diagnosis in a range of clinical indications. Here we present data from patients with ≥ 1 previous failed transfer undergoing pET guided by ERA.

Study design, size, duration: Multicenter retrospective study involving 526 patients with at least one previous failed embryo transfer (ET) between 2017 and 2021. These patients were divided into two groups: cases (pET guided by ERA) (n = 385), or controls (standard blind ET) (n = 141). Clinical outcomes compared were PR, OGPR and LBR for each group.

Participants/materials, setting, methods: Age, body mass index (BMI), previous failed attempts, proportion of embryos with PGT-A and transferred embryo quality were compared between the two groups. ERA test was performed in HRT cycles and subsequent embryo transfer was carried out replicating the same conditions and following the recommendation given for pET. Standard ET was also performed in HRT cycles. Wilcoxon rank sum test was applied to quantitative comparations. Chi-square test was done for categorical variables.

Main results and the role of chance: In this retrospective analysis, age and number of previous failed ET was higher in the ERA group ($p < 0.05$). Clinical results in terms of PR, OGPR and LBR were significantly higher when pET guided by ERA was performed when compared to the non-ERA group.

	pET guided by ERA	Standard embryo transfer	p-value
Number of patients	385	141	-
Age (mean ± SD)	36.21 ± 4.94	34.54 ± 3.40	0.0007*
BMI (mean ± SD)	23.10 ± 3.18	23.07 ± 3.38	0.67
Previous failed attempts (mean ± SD)	2.51 ± 1.73	1.89 ± 1.42	0.0000026*
Embryo Quality ICM (%)			0.59
Optimal (A+B)	87.5%	85.6%	
Suboptimal (C+D)	12.5%	14.4%	
Embryo Quality TE (%)			0.6
Optimal (A+B)	72%	74.43%	
Suboptimal (C+D)	28%	25.57%	
Embryos with PGTA (%)	200/385 (51.95%)	70/141 (49.65%)	0.64
PR (%)	227/385 (58.96)	64/141 (45.39)	0.007*

(continued)

Continued

	pET guided by ERA	Standard embryo transfer	p-value
OGPR (%)	168/385 (43.64)	47/141 (33.33)	0.042*
LBR (%)	161/378 (42.59)	45/139 (32.37)	0.045*

*Significant different ($p < 0.05$).

Limitations, reasons for caution: This is a retrospective study, having limitations of its nature.

Wider implications of the findings: These results show that pET guided by ERA in patients with ≥ 1 failed transfers increased significantly PR, OGPR and LBR versus standard blind ET.

Trial registration number: Hospital Clínico de Valencia Ethic Committee approval n°. 2021/018.

SELECTED ORAL COMMUNICATIONS

SESSION 21: INSIGHTS INTO NUCLEIC ACIDS FOR EMBRYO DEVELOPMENT AND SELECTION

Monday 26 June 2023 Hall A 15:15 - 16:30

Abstract citation ID: dead093.074

O-060 Single-cell DNA sequencing reveals that current bulk DNA sequencing methods lead to an underestimation of chromosomal mosaicism in human blastocysts

E.A. Chavli[1], S. Klaasen[2], D. Van Opstal[3], J. Laven[1], G. Kops[2], E. Baart[1]

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[2]*Hubrecht Institute-KNAW and University Medical Centre Utrecht, Hubrecht Institute-KNAW and University Medical Centre Utrecht, Utrecht, The Netherlands*
[3]*Erasmus MC, Department of Clinical Genetics, Rotterdam, The Netherlands*

Study question: What is the incidence of chromosomal mosaicism in trophectoderm and inner cell mass from good quality human blastocysts after NGS-based single-cell molecular karyotyping?

Summary answer: Single-cell analysis showed that almost all human blastocysts are mosaic, involving low level mosaicism and/or abnormal cells resulting from reciprocal error events.

What is known already: Chromosome segregation in early human embryos is considered to be error prone. While meiotic errors affect all cells within embryos, mitotic errors lead to chromosomal mosaicism, the presence of cytogenetically different cells within an embryo. The reported incidence of mosaicism in blastocysts after bulk DNA sequencing of multicellular trophectoderm (TE) biopsies performed for preimplantation genetic testing for aneuploidy (PGT-A) varies between 2-19%. However, bulk DNA analysis can only assess net average chromosome gains or losses with a detection limit of 20-30%, and it makes distinguishing true mosaicism from technical artefact problematic.

Study design, size, duration: Observational study in human good quality surplus embryos which were donated for research purposes (CCMO, NL82597.000.22). The embryos were thawed and cultured until the blastocyst stage. From the blastocysts with at least a morphology grade 3BB, the ICM was dissociated from the TE. From 55 embryos, both samples were successfully disaggregated into single cells and manually placed in 384 well plates. For the cytogenetic analysis, a validated method for single-cell molecular karyotyping was used (scKaryo-seq).

Participants/materials, setting, methods: Embryos with at least two cytogenetically different cells were considered mosaic. To investigate if bulk DNA sequencing would have detected this mosaicism, we performed an *in-silico* reanalysis on our single-cell data. For each embryo, TE cells with the same mitotic abnormality were quantified. Next, we determined the number of mosaic embryos in which these abnormalities were present in at least 20% of TE cells, as a cut-off for what PGT-A methods are expected to detect.

Main results and the role of chance: On average 42% of the total number of cells that could be isolated per embryo were successfully karyotyped, giving a total of 1057 karyotyped cells (522 normal, 535 abnormal) from 55 embryos. Six embryos (11%) were normal, four (7%) were uniformly abnormal and 45 (82%) were mosaic. From these, 14 (26%) embryos were aneuploid mosaic with cytogenetically different abnormal cells and 31 (56%) were diploid-aneuploid mosaic with normal and abnormal cells. Here cytogenetically different abnormal cells were also frequently observed, with products of reciprocal events detected in 20 embryos.

The *in-silico* reanalysis was performed on 38 embryos with a mosaic TE. It predicted that only 11% of the mitotic abnormalities observed in TE cells through scKaryo-seq would have been detected by bulk DNA sequencing. In 19 out of 38 embryos at least one mitotic abnormality affected more than 20% of the TE cells, meaning that only 50% of the embryos would have been recognized as mosaic with bulk DNA analysis.

Bulk DNA sequencing methods used for PGT-A lead to an underestimation of mosaicism, since low-level mosaicism that affects a few cells within an embryo and products of reciprocal events without a net gain or loss remain undetected.

Limitations, reasons for caution: Isolation and cytogenetic analysis of viable single cells at the blastocyst stage is technically challenging and only 42% of the cells per embryo could be successfully karyotyped. Furthermore, it is unknown to what extent in vitro culture conditions and the freezing-thawing process influenced our findings.

Wider implications of the findings: These single cell observations support the notion that mosaicism is a common biological phenomenon in human blastocysts. Any selective mechanism eliminating embryos or cells with chromosomal abnormalities is still not fully active at this stage. The underestimation of mosaicism by bulk DNA analysis has potential implications for current PGT-A practices

Trial registration number: not applicable

Abstract citation ID: dead093.075
O-061 Single cell atlas of small RNAs in the human preimplantation embryo reveals the miRNA dynamics of lineage segregation

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Study question: How does the small non-coding RNA (sncRNA) expression profile of the human embryo change from day three (E3) to seven (E7) of preimplantation development?

Summary answer: From E3 to E7, micro-RNA expression becomes more dynamic, targeting genes important for lineage segregation.

What is known already: The drivers of lineage specification [SP1] in the human embryo remain unknown. SncRNAs regulate gene expression in most biological systems studied to date, with critical roles in cell development, differentiation, and disease. The most well-studied class of sncRNA, microRNAs (miRNAs), are required for embryo development to the peri-implantation stage in animal models, and their expression regulates embryonic stem cell differentiation in vitro.

Study design, size, duration: Eighty-seven E3-E5 embryos donated for research to the CReATe Fertility Centre (Veritas IRB#16580) between 2002 and 2021 were thawed and cultured to E3-E7. A total of 1279 cells were profiled for sncRNAs. Of these, 463 had known gender and 189 cells were co-sequenced for their mRNA complement.

Participants/materials, setting, methods: Good-quality embryos by morphology were enzymatically and mechanically dissociated into single cells and subjected to small and large RNA sequencing, as outlined in Petropoulos et al. 2016 and Hagemann-Jensen et al. 2018. Gene expression was analyzed with the 'Seurat' package. MiRNA targeting and pathway analysis was performed with Mienturnet.

Main results and the role of chance: We identified the complete complement of small RNAs present in the human preimplantation embryo, with piRNAs being the most abundant in E3 and miRNAs increasing in diversity and abundance to E7. Uniform Manifold Approximation and Projection (UMAP) analysis revealed progression with developmental stage and lineage. Split-cell co-sequencing identified canonical transcriptional markers of preimplantation development, allowing the classification of the small RNA profiles of 16-cell, early blast, inner cell mass (ICM), mural TE, and polar TE populations. Enriched miRNAs in the E5 ICM included miR-302b-5p and miR-302c-3p, which have known functions in stem cell programming. Conversely, TE cells were enriched for miR-519b-3p, miR-519c-3p, and miR-516a-5p, all members of the imprinted primate-specific microRNA gene cluster (C19MC), which is required for placenta formation. Targeting analysis identified miRNA-gene networks involving the Hedgehog, Hippo, and FoxO signalling pathways, all of which are developmentally significant.

Limitations, reasons for caution: This study was performed on embryos from a single center – embryo handling and culture conditions may influence sncRNA expression. Furthermore, the ability of these embryos to implant was unknown, therefore we likely profiled some non-viable embryos.

Wider implications of the findings: The epigenomic changes which drive lineage segregation are poorly understood, and there are vast differences in embryonic development between conventional model systems and humans. We report the first sncRNA profile of single cells between human E3 and E7, providing a resource for further exploration of their role in preimplantation development.

Trial registration number: not applicable

Abstract citation ID: dead093.076
O-062 MicroRNAs in Blastocoel Fluid: a molecular signature for predicting human embryo implantation potential

R. Battaglia[1], A. Caponnetto[1], C. Ferrara[1], A. Fazzio[1], C. Barbagallo[1], D. Barbagallo[1], M.E. Vento[2], L. Carli[3], E. Kasapi[4], S. Palini[5], W. Sierka[6], D. Valerio[7], S. Longobardi[8], T. D'Hooghe[9], C. Di Pietro[1]

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Study question: Can microRNA expression in blastocoel fluid (BF) predict embryo implantation potential?

Summary answer: Up-regulation of miR-106, miR-373, miR-301, miR-320 and miR-525-3p represents the molecular signature characterizing the embryos with high implantation capability.

What is known already: The discovery of DNA within the BF and in spent embryo culture media, has caused increased interest in the non-invasive pre-implantation testing application for monogenic disorders and aneuploidies. Aneuploid embryos have a lower implantation potential, however, chromosomal abnormalities represent only a tiny percentage of the causes of implantation failure. MiRNAs can control all cellular pathways and are involved in pluripotency, self-renewal, and stemness, and their altered regulation affects different human diseases.

Study design, size, duration: From September 2018 to March 2022, 112 BFsampleswere collected from human embryos on the fifth day of development, before the blastocyst cryopreservation. The samples were classified according to blastocyst grade and the data on implantation outcome and term births were collected for the transferred embryos. We compared the expression profiles of 89 miRNAs, previously identified in BF, between 33 BF from implanted embryos and 30 from non-implanted ones, regardless of blastocyst grade.

Participants/materials, setting, methods: By custom-designed TaqMan Low-Density Array card (TLDA), we analyzed the expression of 89 miRNAs in 4 different BF samples simultaneously. Differentially expressed (DE) miRNAs were identified by Volcano plot and Significance Analysis of Microarrays (SAM) tests. Bioinformatic analysis was performed to identify the biological role of the DE miRNAs. To evaluate miRNA's ability for predicting implantation, Pearson's correlation analyses, classical univariate Receiver Operator Characteristic (ROC) curve analysis, and the optimal cut-off value determination were performed.

Main results and the role of chance: We found five miRNAs, miR-106, miR-373, miR-301, miR-320 and miR-525-3pup-regulated in BF from implanted blastocysts. The identified miRNAs perform an important role during the first phases of embryo development suggesting that their up-regulation may reflect embryo health. Moreover, four of the five miRNAs showed significant correlation coefficients in both implanted and non-implanted blastocysts, indicating that their expression changes in the same way in the single sample and reflects the potentiality of the embryo to implant. Finally, ROC curve analysis confirmed that our miRNAs could be considered potential biomarkers for implantation.

Limitations, reasons for caution: Successful implantation requires a close dialogue between the embryo and the endometrium, mediated by different proteins and miRNAs produced by both the embryo and maternal tissues. Determining the quality of the embryo and its implantation potential is not sufficient to predict successful pregnancy outcomes.

Wider implications of the findings: This study represents the first report correlating miRNA profiles in BFand implantation and suggests that miRNA signature could become an accurate tool to evaluate embryo quality. It could be associated or replaced with the PGT-A to choose the most competent embryo and improve the outcome of assisted reproduction cycles.

Trial registration number: The study has been approved by theEthical Committee of Azienda Ospedaliero Universitaria Policlinico "G.Rodolico -San Marco" Catania.

Abstract citation ID: dead093.077
O-063 Accurate mitochondrial DNA quantification clarifies the clinical value of measuring mtDNA in trophectoderm biopsy specimens

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Study question: Can accurate mitochondrial DNA (mtDNA) quantification of trophectoderm (TE) biopsy specimens provide insights into the biology and viability of blastocyst-stage human embryos?

Summary answer: mtDNA quantity in TE cells is correlated with embryo morphology and shows alterations associated with aneuploidy. However, measurement does not significantly improve embryo viability assessment.

What is known already: Mitochondria are essential organelles, responsible for producing ATP. Changes in the amount of mtDNA in blastocysts biopsy specimens have been reported in association with embryo implantation potential, leading to proposals that mtDNA quantification might serve as a useful biomarker of embryo viability. However, results from clinical studies to explore this possibility have yielded contradictory data, due in part to deficiencies of the molecular methods used for mtDNA measurement. We sought to clarify what quantification of mtDNA can tell us about embryo biology and viability by developing and applying a method that we believe to be the most accurate ever devised.

Study design, size, duration: This study involved the analysis of samples collected during the course of routine preimplantation genetic testing for aneuploidy (PGT-A). The IVF treatments and embryo biopsies were undertaken at two different clinics, while chromosomal analyses were carried out at a single reference laboratory. Mitochondrial data was subsequently analysed in a university setting. The embryos analysed were derived from a broad population of patients referred for PGT-A (average age 38.7 years; range 25-47).

Participants/materials, setting, methods: 651 blastocysts from 133 couples underwent trophectoderm biopsy on day-5 or day-6. The specimens were analysed using a highly validated real-time PCR method, which was used to quantify three distinct sites in the mtDNA and 198 loci in the nuclear genome. The measurement of multiple independent loci provided outstanding sensitivity and accuracy. The nuclear loci were used to normalise the mtDNA data, adjusting for differences in the number of cells in the biopsy specimens.

Main results and the role of chance: The method developed displayed extraordinary sensitivity and accuracy when quantifying mtDNA. Lower mtDNA quantities were associated with day-6 biopsy ($p < 0.0001$), extent of blastocyst expansion ($p < 0.0001$), and superior TE morphology, although the latter was not statistically significant ($p = 0.09$). mtDNA levels were higher in aneuploid embryos ($p < 0.0001$), independent on patient age. However, the difference was not sufficient to be considered diagnostic. There was no correlation between mtDNA level and the chances of blastocyst implantation in this dataset. Lower mtDNA levels, previously reported to be associated with higher probabilities of embryo implantation, were most often observed in embryos of excellent morphological grade and likely reflect the increased TE cell numbers of such embryos. Little if any mtDNA replication occurs during preimplantation development and consequently the mtDNA content is divided amongst an ever-growing number of cells, meaning less mtDNA per cell. In this context, mtDNA quantification of blastocyst biopsy specimens provides a highly sensitive measure of TE cellularity, but probably provides little additional benefit for embryo selection beyond conventional morphological grading. However, the fact that higher mtDNA quantities were observed in aneuploid embryos, may indicate that subtle differences in TE cellularity exist in abnormal embryos, which are not fully captured by traditional morphological assessment.

Limitations, reasons for caution: Previous studies suggested that some blastocysts have greatly elevated mtDNA levels and that such embryos are not viable. In this study, only 5% of embryos were considered outliers in terms of mtDNA quantity. Unfortunately, none of these embryos were transferred, so the potential of these embryos could not be assessed.

Wider implications of the findings: The quantification of mtDNA in trophectoderm biopsies has sometimes been used for the prioritisation of embryos for transfer. While our results confirm existence of biologically interesting associations between mtDNA and aneuploidy, and a relationship with certain aspects of embryo morphology, measurement of mtDNA seems unlikely to significantly improve embryo selection.

Trial registration number: not applicable

Abstract citation ID: dead093.078

O-064 Genome-wide analysis of meiotic recombination reveals a subset of oocytes with reduced frequency of recombination and increased risk of aneuploidy.

Z. Gunes[1], D. Wells[2]

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[2]Juno Genetics, Clinical Genetics, Oxford, United Kingdom

Study question: Are there differences in recombination rates between male and female embryos, and is the frequency of recombination associated with the risk of aneuploidy?

Summary answer: There is more recombination in female meiosis than in males. Aneuploidy is associated with fewer recombination events. Genome-wide reduction in recombination predisposes oocytes to aneuploidy.

What is known already: Aneuploidy is believed to be the primary explanation for embryo implantation failure and miscarriage and is, therefore, of great relevance to IVF. Despite the high frequency and clinical importance of aneuploidy, the mechanisms causing a gamete to become chromosomally abnormal are only partially understood. One factor known to influence the risk of aneuploidy is meiotic recombination. An absence of recombination or atypical positioning of chiasmata predisposes to chromosomal malsegregation. Most data on the subject of meiotic recombination and aneuploidy comes from miscarriages and aneuploid births, but this provides an incomplete view since most aneuploidies are lethal at earlier developmental stages.

Study design, size, duration: ~300,000 polymorphisms scattered across the genome were genotyped in DNA from 125 couples and 543 blastocysts that they produced. The inheritance of individual alleles was tracked from parents to embryos, allowing the parental origin of each chromosome to be determined. This strategy also permitted the detection of abnormal numbers of chromosomes, allowed deduction of which meiotic division aneuploidy had arisen in, and revealed the sites of meiotic recombination on each chromosome in the embryos analysed.

Participants/materials, setting, methods: Embryo analysis involved trophectoderm biopsy, followed by multiple displacement amplification. Parental DNA and amplified embryo samples were then tested using a microarray. 22,133 individual recombination events on 14,561 chromatids were evaluated. The relationship between aneuploidy and recombination frequency was considered. The average distance between recombination events was calculated by dividing the size of each autosome (in Mega bases – Mb) by the number of detected recombination events. An unpaired t-test was conducted for statistical analysis.

Main results and the role of chance: A quarter of all embryos were found to carry one or more chromosome abnormalities (altogether, 221 chromosome abnormalities were detected). Of these, 82% had a maternal (oocyte) origin, while 18% were paternal. Chromosomes 16 and 22 were the most frequently affected by aneuploidy, together accounting for 33% of all maternal chromosome abnormalities. Analysis of recombination events in maternally and paternally inherited chromosomes confirmed that female meiosis is associated with a significantly higher frequency of recombination (41.4 ±7.7 recombination events compared with 24.4±4 in chromosomes of paternal origin). Chromosomes 21 and 22 showed the lowest number of recombination events, consistent with their small size (average of 0.6 and 0.7 recombination sites per chromosome, respectively). As expected, a complete failure of recombination occurred more frequently for chromosomes with lower numbers of recombination events. Interestingly, embryos with aneuploidies of maternal origin showed evidence that the oocytes that produced them had lower recombination rates in general (not restricted to the affected chromosomes) than those producing euploid embryos (P = 0.01). Across the genome, average distances between recombination events were ~10% lower for euploid embryos compared with aneuploid embryos (72.4 +/-14 Mb versus 81+-18 Mb) (P = 0.0004).

Limitations, reasons for caution: It is possible that the pattern and frequency of recombination sites that exist in sperm and oocytes might show some differences from the blastocysts tested during this study, considering that more than half of all oocytes either fail to fertilise or produce embryos that arrest before the blastocyst stage.

Wider implications of the findings: As expected, a higher aneuploidy rate and more recombination events were seen in female meiosis compared with male meiosis. Interestingly, aneuploidy was associated with lower levels of recombination across the whole genome, not only for the affected chromosomes, suggesting that unusually low recombination activity predisposes some oocytes to chromosome malsegregation.

Trial registration number: not applicable

SELECTED ORAL COMMUNICATIONS
SESSION 22: NEW CONCEPTS: POOR RESPONDERS
Monday 26 June 2023 Hall D3 15:15 - 16:30

Abstract citation ID: dead093.079

O-065 A multi-center randomized controlled trial of intraovarian injection of platelet rich plasma for women with poor ovarian response

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Study question: Does intraovarian injection of autologous platelet-rich plasma (PRP) improve oocyte yield in young patients with poor ovarian response (POR)?

Summary answer: Intra-ovarian PRP injection did not result in increased number of oocytes retrieved or improvement of any other relevant measured outcomes.

What is known already: POR is one of the major hurdles to overcome in patients undergoing controlled ovarian hyperstimulation (COH) for in vitro fertilization (IVF). Several methods have recently emerged to attempt follicular reactivation in these patients with a poor prognosis. One of them, the intra-ovarian injection of autologous PRP, demonstrated promising results in retrospective and prospective cohort studies.

Study design, size, duration: This was a multi-center, randomized controlled trial (RCT) to evaluate the efficacy of PRP in improving IVF outcomes in women with POR. Patients who met inclusion criteria (<38 years, two or more prior cycles with < 3 oocytes retrieved, and without single gene disorders, prior ovarian surgery, endometriomas, BMI >35, or severe male factor infertility) were randomized to either receive an autologous intra-ovarian PRP injection (PRP group) or no intervention (control group) prior to COH.

Participants/materials, setting, methods: A total of 83 patients were randomized to PRP group (n = 41) or control group (n = 42). After treatment, patients underwent COH, oocyte retrieval, intracytoplasmic sperm injection (ICSI), preimplantation genetic testing for aneuploidy (PGT-A), and single frozen euploid embryo transfer. Number of MII oocytes obtained was the primary outcome. Secondary outcomes included ovarian reserve tests (antral follicle count [AFC] and anti-Mullerian hormone [AMH]), blastocyst and euploid blastocyst yields, and sustained implantation.

Main results and the role of chance: No significant differences were observed in number of MII oocytes retrieved (3.1 ± 3.3 vs 2.8 ± 2.4 in PRP vs control, respectively; p = 0.9), blastocysts (1.3 ± 2.1 vs 1.0 ± 1.3, p = 0.8), or euploid blastocysts (0.9 ± 1.6 vs 0.8 ± 1.1; p = 0.5) per cycle. Similarly, no differences were observed in the likelihood of obtaining at least one euploid blastocyst (37 vs 45%, p = 0.4; relative risk [RR], 95% confidence interval [CI] 0.9, 0.6-1.2) or the rate of sustained implantation (29 vs 31%, p = 0.9; RR 1.0, 0.7-1.3). Pre- and post-treatment AFC in both groups (PRP: from 5.2 ± 3.2 to 7.9 ± 4.5, p < 0.0001; control: from 5.6 ± 3.3 to 6.8 ± 4.8, p = 0.02) were statistically significantly different, whereas AMH was

different in the PRP group only (PRP: 0.73 ± 0.46 to 0.99 ± 0.98, p = 0.02; control: 0.70 ± 0.59 to 0.73 ± 0.57, p = 0.59).

Limitations, reasons for caution: Oocyte retrieval took place the cycle immediately following treatment; therefore, we could not evaluate long-term effects of PRP on ovarian reserve or response. Lack of a placebo group would limit interpretability had a difference between groups been observed. The effect of PRP on patients >38 years old was not studied.

Wider implications of the findings: Intra-ovarian PRP injection did not improve reproductive outcomes by any relevant measure. Our findings do not support wide utilization of PRP to improve IVF outcomes in women with POR.

Trial registration number: NCT04163640

Abstract citation ID: dead093.080

O-066 Transdermal testosterone prior to ovarian stimulation for in vitro fertilization in women with poor ovarian response. A multicenter multinational double-blind placebo-controlled randomized trial (The T-TRANSPORT)

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Study question: Does administration of 2 months of transdermal testosterone prior to ovarian stimulation for IVF/ICSI increase clinical pregnancy rates in Bologna criteria poor ovarian responders?

Summary answer: Pre-treatment with 5.5mg testosterone for 2 months prior to ovarian stimulation for IVF/ICSI does not increase clinical pregnancy rates as compared with placebo.

What is known already: The role of androgens in the treatment of infertile women has been extensively investigated over the last 20 years. Early animal studies and small human studies supported a potentially beneficial role of androgen pretreatment for the management of poor ovarian responders. Still, available evidence regarding androgen administration remains inconclusive, and clinical guidelines do not support their use in infertile women, mainly due to the lack of robust evidence regarding efficacy, the small sample sizes, the lack of data regarding safety, and the lack of uniform dose and duration of testosterone pretreatment for poor ovarian responders.

Study design, size, duration: T-TRANSPORT is the first multicenter multinational superiority phase III, double-blind placebo-controlled randomized trial aiming to detect differences in clinical pregnancy rates between testosterone transdermal gel or placebo for the treatment of poor responders. A sample size of 400 women was calculated, with an interim analysis prespecified to be performed after 70% of recruitment, to determine continuation, sample size re-calculation, or early termination for futility, based on the conditional power reached (≥80%, 40-80% and <20%, respectively).

Participants/materials, setting, methods: T-TRANSPORT included women 18-43 years old from 10 centers and 4 European countries (Spain, Belgium, Denmark and Switzerland) randomized between 2015-2022. Women with poor ovarian response according to the Bologna criteria were randomly assigned 1:1 (stratified by center and age group (<36, 36-39 and ≥40 years old)) to receive either 5.5mg of transdermal testosterone or placebo for ~60 days prior to initiation of ovarian stimulation for IVF/ICSI.

Main results and the role of chance: Overall, 316 poor responders were included and 290 patients were randomized. Based on the prespecified interim analysis, recruitment stopped when 70% of the patients have completed their treatment and blinded data were analyzed. Patients were categorized as group A and Group B until the decision of the DSMB board to break the randomization code is taken.

Ovarian stimulation outcomes did not differ between groups, with a (mean ± SD) number of oocytes retrieved 3.42 ± 2.25 vs. 3.69 ± 2.72, number of MII oocytes 2.75 ± 2.06 vs. 2.83 ± 1.91 and number of embryos 1.46 ± 1.22 vs. 1.90 ± 1.92, for the comparison of Group A vs. Group B, respectively.

Clinical pregnancy rates were comparable between groups, Group A: 17.42% vs. Group B 16.30%, RR (95%CI):1.07(0.64-1.79). Analysis of the results per-age strata also failed to demonstrate significant differences between groups in women <36 (Group A:24% vs. Group B: 17.9%), 36-39 (A: 21.4% vs. B:20%) and ≥40 years old (A:10% vs. B:12.3%)

Finally, no severe adverse events were observed in any of the groups that led to treatment discontinuation. Comparison of androgenic adverse events showed differences for hirsutism A:7.10% vs. B:14.07% and acne A:16.13% vs. B:21.48%, whereas very few cases of alopecia A:1.29% vs. B:2.22% and voice deepening (A:1.29% vs. B:0%) were reported.

Limitations, reasons for caution: All patients, per protocol, underwent D3 embryo transfer.

In addition, the study duration exceeded 7 years, owing to a temporary halt during the COVID-19 pandemic.

Finally, the results presented here are blinded data which will be unblinded by the time of presentation based on the interim analysis performed.

Wider implications of the findings: Testosterone pre-treatment prior to IVF/ICSI does not increase clinical pregnancy rates in poor ovarian responders.

Planned posthoc analyses within the T-TRANSPORT trial aim to investigate whether testosterone may affect ovarian reserve markers, follicular fluid biomarkers, cumulus cells gene expression, and libido in women with poor ovarian response.

Study funding: Yes.

Funding source: Funding by commercial/corporate company(ies). The study received Unrestricted grants by Ferring Pharmaceuticals, BESINS international and Roche Diagnostics. Testosterone and placebo gel were provided by BESINS INTERNATIONAL.

Trial registration number: NCT02418572

Abstract citation ID: dead093.081

O-067 Does the measurement of different AMH isoforms in poor responder patients improve the prediction of ovarian response?

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Study question: Does the measurement of AMH isoforms in low ovarian reserve patients improve the prediction of the number of oocytes after ovarian stimulation (OS)?

Summary answer: AMH assays measuring a broader range of AMH isoforms combined with antral follicle count (AFC) improve the prediction of oocytes collected after OS.

What is known already: Granulosa cells secrete AMH as a non-active prohormone (proAMH). After cleavage, it forms a complex (AMHN,C) which activates the receptor (AMHR2). Circulating AMH is a mixture of isoforms (proAMH, AMHN,C and other sub-fragments) after proteolysis, targeted by the monoclonal antibodies included in the AMH assays commonly used in clinical practice, potentially affecting the quantification of the hormone. Novel AMH assays include antibodies directed towards a broader range of the

molecule. They may provide more accurate and reliable results, especially important in poor responders, as results may involve clinical decisions on whether or not to proceed with OS.

Study design, size, duration: Prospective observational study measuring AMH isoforms using five different AMH assays, including 72 women with low ovarian reserve performing OS for IVF/ICSI at a tertiary referral fertility center, from February 2019 to December 2021. Low ovarian reserve was defined as AMH serum levels <1.1ng/ml (Elecsys, Roche), following Bologna criteria. All patients performed OS for IVF/ICSI with antagonist protocol.

Participants/materials, setting, methods: On day 2/3 of cycle, prior to initiating the OS, AFC, serum FSH, LH, estradiol, progesterone and AMH levels were measured using Elecsys assay (Roche). Extra serum samples were frozen at -20C for subsequent analysis, using four different AMH assays (AnshLabs, Texas): AL-196 (PCOCheck ELISA), AL-124 (PicoAMH ELISA), AL-105 (US-AMH ELISA) and AL-133 (Total Mature-AMH ELISA). Ethical approval was obtained (Research Ethics Committee-REFA033c) and informed consent was signed by all participants.

Main results and the role of chance: Patient characteristics were [Median(IQR)]: age:39(36-42)years, BMI:28.51(26.1-30.1) Kg/m2, AFC:5.5(4-7), FSH:8.23(6.4-12.5) mIU/mL, LH 6.54(4.51-8.91) mIU/mL, Estradiol: 41.18(28.47-56.44)pg/mL, AMH-Elecsys: 0.64(0.29-0.81)ng/mL, AL-196:0.64(0.39-0.99)ng/mL, AL-124:0.79(0.49-1.24ng/mL, AL-105: 0.89(0.57-1.39)ng/mL, AL-133:1.06(0.63-1.59)ng/mL. Stimulation outcomes were: follicle number on the day of trigger (Fdot):5(3-7), cumulus-oocyte-complexes (COC's):3(2-4.5) and metaphase II oocytes (MII):3(1-4).

Spearman correlation was performed between AMH assays, AFC and OS outcomes. Elecsys revealed a good correlation with the other AMH assays ($rs = 0.60$-0.63, $p<0.001$), however, it was higher among ELISA assays ($rs = 0.954$-0.993, $p<0.001$). All AMH assays showed a significant positive correlation with AFC, Fdot, COC's and MII. However, AL-196 showed the highest correlation ($rs = 0.524$, $rs = 0.615$, $rs = 0.594$, $rs = 0.595$, respectively; $p<0.001$). Different models combining one AMH assay plus AFC were created to predict COC's and MII: AFC+AL-196 revealed the best prediction (Adjusted R2=0.474[$p < 0.001$] and 0.485[$p < 0.001$], respectively) when compared to the same AMH assay alone. AUROC analysis was performed to investigate which model predicted better <=3COC's after OS. Although all models demonstrated fair values, AUROC was better for AFC+AL-196 model (AUROC:0.796), yet no significant differences were seen when compared to AUROC for AFC+AMH-Elecsys ($p = 0.39$). AFC and AMH were good predictors for COC's collected [RR(95%CI):1.49(1.11-2.04),$p<0.01$; RR(95%CI):1.26(1.13-1.49),$p<0.001$, respectively). AFC+AMH-Elecsys predicted COC's better than AFC alone (R2=0.47 vs 0.29, respectively;Performance-Score:42.56% vs 13.21%, respectively). AFC+AL-196 had the highest performance (R2=0.599;Performance-Score:90.12%).

Limitations, reasons for caution: Although clinical assessment was performed in the same centre following the same methodology, inter-observer variability for AFC is a limitation. Besides, fresh AMH serum samples were analysed using Elecsys assay, whereas frozen serum samples were shipped to Ansh Lab (Texas) for batched analysis.

Wider implications of the findings: For patients with serum AMH<1.1ng/mL, a wide range of oocytes might be expected after OS. Novel AMH assays, targeting a wider broad of the molecule, together with AFC, can be used for these patients to obtain a better prediction for clinical outcomes and anticipate very poor response.

Trial registration number: Not applicable

Abstract citation ID: dead093.082

O-068 Transdermal testosterone gel (TTG) pre-treatment duration in improving ivf outcome in patients with poor prognosis (poseidon group 3 and 4): A randomised controlled trial.

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Study question: To compare the most effective duration of pre-treatment with transdermal testosterone gel in improving IVF outcomes using GnRH antagonist protocol in poor prognosis patients.

Summary answer: Pre-treatment with testosterone gel for 28 days had higher clinical pregnancy rate, more mature oocytes in lesser days and lower total dose of gonadotropins.

What is known already: Poor prognosis patients undergoing IVF exhibits subnormal ovarian response resulting in higher IVF cycle cancellation and lower clinical pregnancy. Amongst various adjuvants regimens use of androgen supplementation improves clinical pregnancy and probability of live birth in such patients. Androgen stimulates early stages of follicular growth, increase the number of primary, pre-antral and antral follicles. It increases FSH receptor expression in granulosa cells, in turn leading to better oocyte yield and pregnancy rate. We aim to compare the most effective duration of pre-treatment with transdermal testosterone gel in improving IVF outcomes using GnRH antagonist protocol in poor prognosis patients.

Study design, size, duration: A prospective, randomised controlled trial was carried out from 1st September 2021 to 31st August 2022 at a tertiary infertility centre in India. 100 poor prognosis patients POSEIDON group 3 and 4 (age 21-40 years, AFC <5 and AMH ≤1.2 ng/ml) were enrolled. They were randomized into 4 groups of 25 each by computer based program.

Participants/materials, setting, methods: Group 1, control received no pretreatment, Group 2, Group 3 and Group 4 received 1% TTG one actuation (12.5mg testosterone daily) for 2, 3 and 4 weeks respectively before start of ovarian stimulation. Patients received a starting dose of HP HMG dose 300 IU and dose was adjusted. Inj. Cetrorelix 0.25 mg started when lead follicle was 14 mm and Oocyte retrievals were performed 35 hours after 250 mcg r-HCG trigger.

Main results and the role of chance: All groups were comparable with regards to baseline FSH, AFC and AMH levels. Only one cycle was cancelled in control group because of lack of response to gonadotropins till day 5 of stimulation. Requirement of total days (9.68 Vz 8.95 days) and total dose of gonadotropins was significantly lower in group 4 as compared to control group 1. This showed that cases with pre-treatment of transdermal testosterone gel for longer duration, required less amount of gonadotropin (2826 IU Gp 1 Vz 2329 IU Gp 4) treatment ($p < 0.01$) and also the mean number of mature oocytes retrieved (0.80 Gp 1 Vz 3.38 Gp 4) were significantly higher ($p < 0.01$). Day 3 fresh embryo transfers were performed for all. Incidence of clinical pregnancy rate was 4.2%, 8%, 12% in group 1, 2, and 3 respectively which increased to 20% in group 4 (p-0.33). Miscarriage rate was 4.2% , 4%, 12% and 4% in group 1 ,2, 3 and 4 respectively (p-0.71). Ongoing pregnancy rate was 4% in group 1, while it was 7.7 %, 10.6 % and 19.2% in group 2,3 and 4 respectively (p-0.33).

Limitations, reasons for caution: The study was done at a single centre with small sample size, replication with more subjects and in different centers is needed. The long term effects of testosterone pretreatment requires further studies.

Wider implications of the findings: Pre-treatment with testosterone gel in poor prognosis patients improves response to gonadotrophin, reducing amount and days to complete stimulation and results in higher number of mature oocytes and improved clinical pregnancy rates. Transdermal testosterone treatment is advantageous as it is not first metabolized in the liver, convenient to the patient.

Trial registration number: CTRI/2022/03/040793

Abstract citation ID: dead093.083

O-069 Double vs. single stimulation in young poor prognosis patients followed by a fresh embryo transfer: a randomized controlled trial.

A. Racca[1], I. Rodriguez[1], S. Garcia[1], N.P. Polyzos[1]

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Study question: Does double stimulation, followed by a fresh ET (DUO-STIM fresh) improve outcomes as compared with a single stimulation in young poor prognosis patients?

Summary answer: Compared to single stimulation, DUO-STIM fresh leads to significantly higher number of good quality embryos, without hindering fresh embryo transfer outcomes.

What is known already: Dual Stimulation (ovarian stimulation both in the follicular and luteal phase of the same cycle) is an innovative strategy to retrieve higher number of oocytes in a shorter time frame, thus it is particularly appealing for poor ovarian responders. Three current limitations of Dual-Stimulation are: (1) it is unclear whether outcomes of the second (luteal) wave results from the second stimulation, or a carry-over effect from previous follicular stimulation; (2) the desynchronization between endometrium and ovaries and, (3) lack of robust evidence. No previous studies explored Dual Stimulation starting from the luteal phase, and with a fresh embryo transfer (DUOSTIM-fresh).

Study design, size, duration: This study is a prospective, randomized, controlled, single-center, superiority clinical trial comparing two different ovarian stimulation protocols: a double stimulation cycle vs. a single stimulation cycle followed by fresh ET. The primary outcome was the number of good quality embryos obtained, while secondary outcomes included results from fresh embryo transfer (clinical pregnancy, miscarriage). A total of 120 women were enrolled in this study between October 2020 and October 2022, with a 1:1allocation.

Participants/materials, setting, methods: Only young (<40 years old) poor-prognosis (AMH<1.2 ng/mL) patients were recruited in the Fertility Clinic within a tertiary University Hospital. In the investigational group, DUOSTIM-fresh, the 1st stimulation was initiated in the luteal phase followed by a 2nd stimulation 5 days post first OPU, initiated in the follicular phase and a fresh ET of the best embryo generated (1st or 2nd cycle). The control group performed a follicular phase single stimulation cycle with fresh ET.

Main results and the role of chance: Overall, 107 patients were randomized, 53 in the investigational (DUO-STIM fresh) and 54 in the control arm (single stim).

DUO-STIM fresh resulted in a significantly higher number of good quality embryos as compared to single stim (difference of mean 0.81, 95% CI 0.12-1.49). The mean percentage of embryo transfer was comparable (62.3 and 51.9, respectively for double vs single stimulation). No significant differences were found for fresh embryo transfer outcomes (ongoing pregnancy rates: 24.5 and 22.2, respectively for DUO-STIM fresh vs conventional IVF).

Of interest comparisons between different stimulation cycles (A: luteal phase DUOSTIM-fresh, B: follicular phase DUOSTIM-fresh and C: single stim didn't demonstrate any significant difference in terms of ovarian response with the mean (SD) number of mature oocytes being (A: 3.3 (2.9) B: 3.4 (3.4) C: 3.5 (2.9), respectively).

Limitations, reasons for caution: Study sample size was calculated to detect differences on the mean number of good-quality embryos. Therefore, results for secondary outcomes (embryo transfer rates and clinical pregnancy rates) should be interpreted with caution as exploratory findings that warrant future investigations.

Wider implications of the findings: Although DUOSTIM-fresh results in higher number of blastocysts as compared with a single stimulation in young poor-prognosis patients, the decision of performing dual stim should be evaluated with caution considering that whether this may improve embryo transfers rate and pregnancy outcomes is still unclear. Results on Cumulative-Live-Birth-Rate are warranted.

Trial registration number: NCT04446845

SELECTED ORAL COMMUNICATIONS

SESSION 23: AGE, LIFESTYLE FACTORS AND SPERM FUNCTION

Monday 26 June 2023 Hall D1 15:15 - 16:30

Abstract citation ID: dead093.084

O-070 Paternal age is associated with mitochondrial vulnerability to sperm cryopreservation

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Study question: Is paternal age associated with lower mitochondrial activity in fresh and cryopreserved semen?

Summary answer: Paternal age does not appear to severely compromise sperm mitochondrial activity in fresh semen but determines higher mitochondrial vulnerability to sperm cryopreservation.

What is known already: The impact of paternal age on male fertility has not been fully clarified although growing evidence suggests age-related loss of sperm quality. Mitochondrial activity has been recognized as a reliable marker of sperm functionality, reflecting motility, vitality and fertilization competence. Sperm cryopreservation has been largely utilized in fertility preservation and ART schemes, despite its potential harm on cell membranes including those of the mitochondria. The influence of advanced paternal age (APA) on sperm vulnerability to cryopreservation-induced cell damage is still not known.

Study design, size, duration: Twenty three normospermic patients, 11 of which $\leq$ 35 (non-APA) and 12 $\geq$ 42 years old (APA), provided semen samples by masturbation after 1-5 days of abstinence, between April and August of 2022. A 250µl semen aliquot was frozen. Sperm concentration, motility, vitality, morphology and mitochondrial functionality were compared in fresh semen from non-APA vs. APA patients, while motility and mitochondrial functionality were compared in thawed semen from both groups with the two-tailed Student t-test.

Participants/materials, setting, methods: Fresh and thawed semen samples were provided by patients under evaluation for couple infertility treatment in our fertility center. Semen was cryopreserved with CryoSperm (Origio) and analyzed after rapid thawing and washing/dilution in Sperm Preparation Medium (Origio). Sperm vitality was assessed with VitalScreen (FertiPro) and mitochondrial functionality was evaluated with MitoTracker Red CMXRos (Cell Signalling Technology), through the percentage of stained cells and fluorescence intensity measured in 75 spermatozoa per patient with the ImageJ software.

Main results and the role of chance: Mean ages for non-APA and APA patients were 32.6 and 45.3 years, respectively. Age groups did not differ for any of the parameters assessed in fresh semen (volume: 2.3 ± 1.1 vs. 2.6 ± 1.2mL; concentration: 58 ± 32 vs. 47 ± 20 x$10^{6/mL}$; rapid progressive motility 5 ± 5 vs. 5 ± 8%; slow progressive motility 40 ± 10 vs. 36 ± 10%; non-progressive motility 10 ± 5 vs. 10 ± 3%; immotile: 44 ± 9 vs. 49 ± 13%; normal morphology: 6 ± 3 vs. 5 ± 1%; vitality: 71 ± 8 vs. 66 ± 15%, for non-APA and APA, respectively). However, non-APA patients presented a higher percentage of spermatozoa with rapid progressive motility (5 ± 7 vs. 0%; p = 0.04) and a lower percentage of immotile spermatozoa (76 ± 12 vs. 88 ± 9%; p = 0.02) after thawing. Regarding mitochondrial functionality, no differences were observed in fresh semen from different age groups [93 ± 11 vs. 92 ± 7% stained cells; 3.33 ± 1.57 vs. 3.35 ± 1.49 (fluorescence arbitrary units), for non-APA and APA, respectively). In cryopreserved semen, however, although age groups did not differ for the percentage of stained

spermatozoa (92 ± 10 vs. 90 ± 5%), fluorescence intensity was higher in spermatozoa from non-APA patients (2.53 ± 1.63 vs. 1.76 ± 0.54; $p < 0.01$).

Limitations, reasons for caution: Our study is limited by the potential interference of confounding factors not equally distributed in age groups. The conclusions from this study must be confirmed in other patient populations with different race/genetics and habits.

Wider implications of the findings: Our study sheds light on the impact of paternal age on sperm quality, a topic highly relevant to reproductive medicine still not fully understood. We provide evidence that APA is associated with higher vulnerability of mitochondria to the stress induced by cryopreservation and its metabolic consequences.

Trial registration number: not applicable

Abstract citation ID: dead093.085
O-071 Paternal body mass index affects early embryo development but does not impact clinical outcomes: a timelapse study of 7,656 embryos in oocyte donation cycles

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Study question: Does paternal body mass index (BMI) affect preimplantation development and clinical outcomes in oocyte donation cycles?

Summary answer: High paternal BMI was associated with delays in early development and poorer embryo quality on day 3, but this did not adversely affect pregnancy outcomes.

What is known already: Maternal obesity is known to negatively affect preimplantation development and clinical outcomes. While oocyte competence plays a major role in early embryogenesis, male obesity has been linked to poorer semen parameters and epigenetic alterations in sperm. Such characteristics may inherently compromise preimplantation development and ultimately impair reproductive success. Only a few reports have assessed the effects of male BMI on the progression of cell divisions and embryo morphokinetic patterns. However, current findings remain contradictory, while the effects of male obesity on fertility continue to be understudied.

Study design, size, duration: This retrospective study included 7,656 embryos from 1,342 oocyte donation ICSI cycles, performed between April 2018 and December 2021 across two fertility centres. Sperm originated from the partner (n = 1087) or donor (n = 255) and was either fresh (n = 460) or frozen (n = 835). Only the first fresh (n = 1,140) or frozen (n = 202) embryo transfer (ET) was considered. Our database included 1,089 single and 238 double ETs performed at either the cleavage (n = 424) or blastocyst stage (n = 903) of development.

Participants/materials, setting, methods: Males were stratified according to BMI, based on WHO guidelines, defined as: normal weight (BMI<24.9, n = 677); pre-obese, (BMI=25.0–29.9, n = 533); and obese (BMI>30, n = 132). Patients with severe male factor and preimplantation genetic testing cycles were excluded. Univariate analyses and logistic regression were used to evaluate associations between BMI, embryo morphokinetics and clinical outcomes. Analyses were adjusted for various confounders pertaining to patient demographics, sperm and cycle parameters. A p-value of < 0.05 was considered significant.

Main results and the role of chance: Overall, obese men displayed poorer sperm motility and underwent more day 2-4 embryo transfers and freeze-all cycles, compared to pre-obese and normal weight men ($p < 0,05$). Fertilization rates were not affected by male BMI (77.0% ± 18.9 normal weight; 77.4% ± 18.1 pre-obese; 75.3% ± 19.9 obese; $p = 0.62$), also in our adjusted analysis. Several morphokinetic parameters were delayed in embryos from pre-obese and obese patients, including pronuclei fading (tPNf, $p = 0.0064$) and division timings from the 2 to 5-cell stage (t2-t3-t4-t5, $p < 0,05$). Successively, the number of good quality cleavage embryos decreased significantly with higher BMI (normal weight, 62%; pre-obese, 59%; obese, 50.8%; $p = 0.02$). In contrast, developmental events past the 5-cell stage, including time to 8-cells (t8), blastulation (tSB) and expanded blastocyst (tB) were comparable amongst the groups ($p > 0.05$). Similarly, we observed no effect of male BMI on blastocyst quality (normal weight, 70.2%; pre-obese, 73.4%; obese, 75.5%; $p = 0.06$). Despite early developmental delays, we observed no significant effects on biochemical pregnancy (normal weight, 64.7%; pre-obese 64.1%; obese, 58.7%; $p = 0.45$), ongoing pregnancy (normal weight, 60.0%; pre-obese, 58.8%; obese, 52.9%; $p = 0.35$) and live birth rate (normal weight, 41.9%; pre-obese, 42.0%; obese, 34.9%; $p = 0.27$). Our adjusted analysis corroborated these findings.

Limitations, reasons for caution: The main limitation of this study is its retrospective design, which may not account for all confounders. Moreover, lifestyle factors, such as smoking or alcohol consumption were not considered. As such, results cannot be generalized to all patient populations.

Wider implications of the findings: Obese males show lower motile sperm counts and delayed cleavage development. While these changes impaired day 3 embryo quality, they did not affect blastocyst development and quality. That translates into comparable clinical outcomes, suggesting that specific counselling is not required in obese patients.

Trial registration number: not applicable

Abstract citation ID: dead093.086
O-072 Lack of meaningful impact of male body mass index (BMI) on reproductive outcomes measured by cumulative live birth rates (CLBR) in 80830 IVF-ICSI treatments

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Study question: Does men's BMI influence the reproductive success measured as CLBR per embryo transfer(ET), embryos replaced(EmbR) and oocytes utilized, if female BMI is controlled?

Summary answer: Male's BMI do not affect reproductive outcomes, although obese males seem to need slightly more ET to reach the first live birth.

What is known already: Obesity is a systemic, chronic and multifactorial disease present worldwide, involving all ages, ethnicities and social classes, and associated with hormonal alterations that may lead to a decrease in seminal quality and reproductive outcomes. The exact mechanisms involved, between excess body fat and reproductive disturbances, if any, are complex and unknown. This leads us to investigate the effect of BMI on reproductive outcomes to carry out a better counseling of couples who go to a clinic for assisted reproduction treatment. The recent improvement of measuring reproductive success by cumulative rates has never been applied to many risk factors, as obesity.

Study design, size, duration: This retrospective observational multicentric study has evaluated the results from 80830 IVF-ICSI treatments, 298422 oocytes and 215357 embryos transferred performed in Spanish IVIRMA fertility clinics between January 2008 and December 2020 by couples using their own sperm sample and oocytes.

Participants/materials, setting, methods: Couples attending IVI clinics. Male BMI was categorized in: underweight (<18.5 kg/m^2) (U), normal weight (18.5-24.99 kg/m^2) (N), overweight (25-29.99 kg/m^2) (OV) and obese (≥ 30 kg/m^2) (OB) patients, and CLBR were calculated using Kaplan Meier methods, by Cox regression to control women's BMI and age, and male's age. Reproductive success was calculated by CLBR perET, EmbR and utilized oocytes until the first LB. Data were expressed as % with corresponding 95% confidence intervals.

Main results and the role of chance: After 3 ETs, CLBR per ET, were, for groups U,N,OV and OB, respectively, 47.4%(44.3-50.3), 48.1%(47.4-48.9), 47.8%(46.2-49.4) and 47.1%(44.1-49.9), increasing after 5 ETs to 64.6(59.7-69.0), 65.1%(63.9-66.2), 62.8%,(60.2-65.3) and 59.2%(54.5-63.4).

There were statistically significant differences between Obesity and Normal weight groups ($p = 0.03$), hazard ratio [HR]: -0.01 on the Cox regression adjusted by female's age and BMI, and male's age.

Considering EmbR, after 3, CLBR were 38.8%(36.2-41.26), 35.7%(35.1-36.3), 35.2%(34.0-36.4) and 33.7%(31.5-35.9), and after 6 EmbR, 62.7%(58.5-66.5), 59.8%, (58.9-60.7), 59.8%(57.7-61.8) and 58.0%(54.1-61.5), for U, N, OV, and OB respectively, with no significant differences among groups, also confirmed by the comparable results adjusted Cox regression.

Concerning CLBR per oocyte used, with 8 oocytes, results were 38.3%(35.2-41.3), 34.7%(33.9-35.4), 32.0%(30.6-33.4) and 29.9%(27.4-32.3), and after 12 oocytes used, 54.0%(50.6-57.3), 53.5%(52.7-54.3), 49.9%(48.3-51.5) and 46.8%(43.9-49.6), for the above mentioned ordered IMC groups.

Considering 16 oocytes used, results on U, N, OV and OB males were also comparable: 69.6%(65.9-72.8), 66.7%(65.9-67.5), 62.6%(60.8-64.3) and 62.1%(58.8-65.2), respectively, also confirmed by Cox regression adjusted estimates.

Limitations, reasons for caution: The retrospective nature of this study leads to biases derived from the clinical practice and the presence of missing/incomplete or imprecise data, together with the possibility of not having controlled by all possible confounding factors.

Wider implications of the findings: Different male's BMI, when controlled by main confounders, show comparable results in the number of oocytes needed, EmbR to get the first child, and, although we confirmed a minor influence on ET needed, the message is that male's weight seems not affecting reproductive outcomes in IVF/ICSI treatments.

Trial registration number: not applicable

Abstract citation ID: dead093.087

O-073 Delta-9-THC acts on the calcium channel CatSper and alters human sperm function

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Study question: Do the main psychoactive phytocannabinoid delta-9-tetrahydrocannabinol (THC) and its non-psychoactive analog cannabidiol (CBD) affect human sperm function?

Summary answer: THC reduces the ligand-dependent activation of sperm-specific Ca^{2+} channel CatSper and affects human sperm functions *in vitro*.

What is known already: Marijuana (*Cannabis sativa*) is one of the most commonly used recreational drugs worldwide. Although the impact of phytocannabinoids on reproductive health has been investigated, there is no evidence of a direct effect of THC on CatSper function.

Study design, size, duration: We studied the effects of the main psychoactive phytocannabinoid, THC, its non-psychoactive analog, CBD, as well as their major metabolites on Ca^{2+} influx via CatSper in human spermatozoa. THC and CBD were selected to further evaluate their action on progesterone-, prostaglandin-, and pH-induced activation of human CatSper. The effects of THC and CBD on hyperactivation, progressive motility in viscous media, and acrosomal exocytosis were also assessed.

Participants/materials, setting, methods: The impact of phytocannabinoids on CatSper activity was investigated on sperm samples from healthy volunteers using kinetic Ca^{2+} fluorimetry. Motility assessment was performed using Computer-Assisted Sperm Analysis (CASA). Sperm penetration into viscous media was assessed using a modified Kremer test. Acrosomal exocytosis was evaluated by flow cytometry using *Pisum sativum* agglutinin-stained spermatozoa.

Main results and the role of chance: Both THC and CBD suppress natural ligand-induced calcium influx via CatSper. In particular, THC inhibits progesterone-induced Ca^{2+} influx via CatSper at pharmacologically relevant concentrations in a non-competitive manner and reduces the pH-induced activation of CatSper. In addition, THC impaired sperm hyperactivation and penetration into viscous media and induced spontaneous acrosomal exocytosis *in vitro*.

Limitations, reasons for caution: This is an *in vitro* study. Future studies are needed to test the physiological relevance *in vivo* and whether THC can disrupt human sperm function.

Wider implications of the findings: The action of THC on CatSper in human sperm might impair the fertilization process. Healthcare providers, especially fertility clinicians, should be aware of the potentially negative effects of cannabis consumption on sperm physiology

Trial registration number: not applicable

Abstract citation ID: dead093.088

O-074 THC reduces sperm mitochondrial membrane potential, but does not affect the acrosome reaction or in-vitro embryo developmental rates.

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Study question: Does THC alter sperm function and early embryo development following in vitro fertilization with THC-exposed sperm?

Summary answer: THC reduces sperm mitochondrial membrane potential (MMP), mainly through cannabinoid receptor agonism, but does not alter the acrosome reaction or rates of embryo development.

What is known already: Cannabis is the most commonly used recreational drug among males of reproductive age. THC is the main psychoactive component of cannabis and can cross the blood-testis barrier, disrupting the endocannabinoid system (ECS) in sperm. However, the mechanisms by which THC affect sperm function and early embryo development are unclear. In sperm, endogenous cannabinoids influence the acrosome reaction and MMP, which are critical for motility, viability and, ultimately, fertilization. To date, no studies have investigated how sperm exposed to physiologically relevant doses of THC may impact in-vitro early embryo development, and cannabis use recommendations remain unclear for patients undergoing IVF procedures.

Study design, size, duration: Bovine sperm and cumulus oocyte complexes (COCs) were used as a translational model for humans. Cryo-thawed sperm was separated using a Percoll gradient and incubated for 6-hours in one of five treatment groups: control, vehicle (0.01% ethanol), low-THC (0.032μM), mid-THC (0.32μM), and high-THC (4.8μM) – concentrations equivalent to THC plasma levels following therapeutic and mid-high recreational use, respectively (Whan et al., 2006). A minimum of 30,000 sperm and 60 COCs were analyzed per group.

Participants/materials, setting, methods: Sperm were treated for 6-hours prior to all experiments. Flow cytometry using sperm ($1\text{-}2x10^6$) stained with propidium iodide (PI) and FITC-conjugated peanut agglutinin (FITC-PNA) or JC-1 was used to measure either the acrosomal reaction or MMP, respectively. COCs were aspirated from slaughterhouse ovaries and matured in-vitro for 24-hours. IVF was performed with THC-treated sperm ($1x10^6$/mL/drop) for 10-hours. Presumptive zygotes were cultured in-vitro for 8-days. Cleavage and blastocyst rates were measured 48-hours and 8-days post-fertilization, respectively.

Main results and the role of chance: Measuring PI and FITC-PNA fluorescence showed the percent of sperm that were acrosome-reacted and either alive or necrotic. There were no significant differences in acrosome-reacted sperm among groups ($n = 9$). Measuring JC-1 fluorescence showed the percentage of sperm with high MMP. Results indicate a significant reduction in sperm with high MMP (37%) following high THC (4.8μM) exposure ($p = 0.002418$, $n = 4$). No significant differences in MMP were observed among other groups. To elicit the mechanism by which THC was reducing

sperm MMP, we repeated experiments using only JC-1-stained sperm exposed to high THC treated with SR141716 and SR144528, which are CB-1 and -2 antagonists, respectively. Additional groups included: SR141716 (4.8.μM), SR144528 (4.8μM), THC + SR141716 and THC + SR144528. Results indicate a significant reduction in sperm with high MMP (38%) in the THC group (4.8μM) compared to control (51%) ($p = 0.0417$, $n = 6$), vehicle (54%) ($p = 0.0069$, $n = 6$), and SR141716 (65%) ($p < 0.0001$, $n = 6$). There were no significant differences in sperm with high MMP in THC + SR141716 or THC + SR144528 groups, indicating that THC is acting agonistically, primarily at CB1 receptors. Following IVF with THC-treated sperm, there were no significant differences in cleavage or blastocyst rates among treatment groups.

Limitations, reasons for caution: The use of bovine instead of human cells could be considered a limitation. However, as studying effects of THC-treated sperm on IVF outcomes would not be possible using human samples, it is actually an advantage. The similarity between bovine and human gametes makes bovine an ideal translational model for humans

Wider implications of the findings: As cannabis use and THC concentrations increase, this research addresses the growing concern of how cannabis impacts fertility and, ultimately, pregnancy outcome. Understanding how THC affects sperm function and embryo development will provide physicians with science-based recommendations concerning cannabis use for patients trying to conceive, pregnant, or undergoing fertility treatments.

Trial registration number: not applicable

SELECTED ORAL COMMUNICATIONS

SESSION 24: THE GENETIC CONTENT OF PREIMPLANTATION EMBRYO: PREDICTION, DETECTION AND REPAIR

Monday 26 June 2023 Hall D4 15:15 - 16:30

Abstract citation ID: dead093.089
O-075 Deficiency of DNA double-strand break repair in human preimplantation embryos revealed by CRISPR-Cas9

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Abstract under embargo

Abstract citation ID: dead093.090
O-076 Update on the International Registry of Mosaic Embryo Transfers

F. Spinella[1,10], E. Greco[2], I. Listorti[2], P.F. Greco[2], A. Biricik[3], M. Surdo[3], N. Meschino[4], A. Alteri[5], V. Bianchi[6], I. Bravo[6], S. Madjunkova[7,10], A.G. Besser[8,10], M. Viotti[9,10], IRMET(10)

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[10]Registry of Mosaic Embryo Transfers (IRMET)

Study question: Which are the results obtained by the International Registry of Mosaic Embryo Transfers?

Summary answer: An update of clinical outcomes and post-natal results of mosaic embryos with low and high-level of mosaicism is provided.

What is known already: Chromosomal mosaic embryos are characterized by the presence of chromosomally different cell lines within the same embryo. While the transfer of these embryos is now offered as an option for women who undergo in vitro fertilization (IVF), several concerns remain. For instance, the limited data on pregnancy outcome and the possibility that intra-biopsy mosaicism in the TE is a poor predictor of the ploidy status of the ICM. Therefore, some argue that mosaicism should be not reported until a clear classification of such embryos in relation to their reproductive potential has been defined.

Study design, size, duration: We collected the clinical outcomes of 2045 mosaic embryos transferred in women who undergoing IVF between May 2019-May 2022. All embryos were cultured to blastocyst stage; trophectoderm (TE) biopsy was performed on Day-5 of development or Day6/7 for slow-growing embryos. The clinical outcomes obtained after the transfer of mosaic embryos with the different chromosomal constitutions were compared. Prenatal and post-natal outcome was collected for available cases.

Participants/materials, setting, methods: Preimplantation genetic testing for aneuploidies (PGT-A) was performed using high-resolution next-generation sequencing (NGS) methodology. TE biopsies were classified as mosaic if they had 20%-80% abnormal cells. For statistical analysis, mosaic embryos were divided into groups based on mosaic levels and chromosomal constitution detected in TE: single mosaic aneuploidy (monosomy/trisomy; SM), double mosaic chromosomes (monosomy/trisomy or combination, DM), complex mosaic aneuploidy (>2 different aneuploidies; CM) and mosaic segmental aneuploidy (single and double deletion/insertion >5Mb, MS).

Main results and the role of chance: Embryos classified as 'low-mosaic' by NGS-based PGT-A have a higher likelihood of achieving implantation compared to 'high-mosaic' embryos (48% vs. 39.%; $p < 0.05$), as well as ongoing pregnancy/live birth (40% vs. 29%; $p < 0.05$). Chromosomal composition of mosaicism abnormalities dictates the success rate of mosaic embryo transfers, with low and high segmental mosaics being preferable over low- and high-mosaics involving whole chromosomes. For 550/670 pregnancies, parental tests and post-natal data were available. The majority (99.75%) of the babies were largely healthy by routine physical inspection by neonatologists (no gross abnormalities in babies from mosaic embryos, $n = 495$). Prenatal testing performed on pregnancies from mosaic embryo transfers were generally normal. The mosaicism detected at the embryonic stage by PGT-A was reflected in prenatal testing in only 5 out of 550 pregnancies (0.9%) in which the mosaicism identified with PGT-A at the blastocyst stage was reflected in gestation by prenatal chromosomal testing as true fetal mosaicism.

Limitations, reasons for caution: Additional clinical data must be obtained to evaluate the contribution of each different chromosome before this approach can be evaluated as an additional tool to choose mosaic embryos for transfer.

Wider implications of the findings: The International Registry of Mosaic Embryo transfers continues to grow in sample size, in turn increasing the power of analysis. The findings of the mosaic embryo transfer registry can help educate the management and selection of embryos in the clinic.

Trial registration number: Not applicable

Abstract citation ID: dead093.091

O-077 Time-lapse imaging analysis of segmental aneuploid embryos: a multicenter study identifies morpho-kinetic patterns associated with chromosomal mosaicism

M. Figliuzzi[1], L. Bori[2], S. Caroselli[1], L. Picchetta[1], M. Reverenna[3], M. Poli[1], A. Campbell[4], R. Smith[4], G. Coticchio[5], D. Cimadomo[6], L. Rienzi[6], M. Meseguer[2], A. Capalbo[1]

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Study question: Do segmental aneuploid (SA) embryos display unique morpho-kinetic patterns at early developmental stages?

Summary answer: The first three cell division cycles of embryos carrying segmental aneuploidies are significantly slower than euploid and whole-chromosome aneuploid (WCA) embryos.

What is known already: Segmental aneuploidies in blastocyst-stage human embryos prevalently originate (i.e., 70% of cases) from mitotic errors. Their clinical management is challenging because it is impossible to establish their meiotic/mitotic origin from PGT-A analysis of a single trophectoderm biopsy. Morpho-kinetic analysis based on Time-lapse microscopy (TLM) has been proposed as a valuable tool for systematic embryo evaluation, although it is unclear whether this approach alone can predict chromosomal abnormalities. This study aims to pinpoint morphokinetic patterns associated with the occurrence of segmental aneuploidies and/or mitotic errors, possibly improving DNA-based diagnostic interpretation and their clinical management.

Study design, size, duration: This is a retrospective multicenter study including a total of 7,693 embryos from 2,370 IVF cycles and 2,127 couples, cultured between 2016 and 2021 in 4 European IVF centers. Embryos with no more than 4 chromosomal alterations were considered in the analysis, resulting in 3,288 euploids, 3,155 WCA, 715 SA, and 535 complex aneuploids (CA). Overall, the dataset contained 3,742 distinct euploid-SA embryo sibling pairs.

Participants/materials, setting, methods: Standard morpho-kinetic features were annotated using various TLM systems. Blastocysts were subjected to comprehensive chromosomal screening via PGT-A. Morpho-kinetic timings across different embryo groups were compared using Kolmogorov-Smirnov (KS) test and binomial test, and associations with cleavage features were assessed via two-sided Fisher Exact (FS) test. Multi-center and center-specific logistic regression models were calibrated using a subset of data (70%), and their predictive performance was evaluated on independent test data using Area-Under-ROC curve (AUROC) metrics.

Main results and the role of chance: SA embryos cleaved at significantly slower rate than their euploid siblings in the first 3 cell cycles, from the appearance of the second blastomere t2 (average delay delta=0.49h, KS $p = 0.006$, fraction of pairs in which SA was delayed compared to euploid sibling $F = 54.2\%$, binomial $p = 0.002$) up to t8 (delta=2.16h, KS $p = 4e\text{-}4$, $F = 57.9\%$, binomial $p = 1e\text{-}8$). The developmental delay was partially compensated at full blastocyst stage tB (delta=1.71h, KS = 0.0008, $F = 57.5\%$, binomial $p = 5e\text{-}8$). SA timings also differed from WCA ones: SA embryos were significantly slower than their WCA siblings during early cell cycles, with maximum delay reached at t8 (delta=1.67h, KS $p = 0.009$, $F = 55.4\%$, binomial $p = 0.003$), but they fully caught-up at tB, with no significant difference observed. These morpho-kinetic patterns were consistently observed in each center separately, but with different effect sizes. The presence of segmental aneuploidies was significantly associated with multinucleation (OR = 1.99, FS test $p = 0.05$) and morula cell exclusion (OR = 2.69, $p = 0.006$). A logistic model based on morpho-kinetic data and cleavage features from a single center dataset and regressed against embryo aneuploidy class (euploid vs. SA embryos) displayed adequate predictive performance on independent data from the same center (AUROC=0.70), although predictivity diminished when tested on data from other centers (AUROC=0.52-0.55).

Limitations, reasons for caution: We cannot rule out inter-center variability in the annotation procedures of morpho-kinetic parameters. Standardized and automated annotation would increase the performance of predictive models and their transferability. Further work is needed to assess

whether the morpho-kinetic signal is driven by mitotic errors and if an association with clinical outcomes exists.

Wider implications of the findings: These findings might help uncovering biological events contributing to preimplantation mosaicism. Moreover, the developed predictive framework might help improving decision-making in PGT-A cycles, providing interpretation for segmental aneuploidies and possibly helping in the evaluation of embryos showing intermediate copy number values for whole chromosomes that are suggestive of mosaicism.

Trial registration number: Not applicable

Abstract citation ID: dead093.092
O-078 Mosaic embryos – are they accurately detected by PGT-A?

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Study question: Following quantitative NGS for PGT-A, do current thresholds for mosaicism calling give a true reflection of the presumed mitotic origin of mosaic errors?

Summary answer: A large proportion of embryo mosaicism diagnosed following quantitative NGS-based PGT-A does not correlate with a true and expected mitotic origin, showing instead meiotic signatures.

What is known already: Mosaicism is a true biological phenomenon that can affect ongoing embryo development. However, the reproductive potential of mosaic embryos in assisted reproduction varies greatly across publications and is not fully understood. While the transfer of mosaic embryos could result in healthy live births, embryos containing meiotic errors almost never result in healthy live births. Since the clinical implications of meiotic and mitotic errors are very different, it is critical to accurately differentiate between the two. However, diagnosing mosaicism using current NGS-based PGT-A is not always accurate due to technical and biological limitations which may misclassify mosaic embryos.

Study design, size, duration: Follow-up analysis was carried out on 159 embryos previously categorised as mosaic by NGS-based PGT-A. To identify the origin of mosaic calls, SNP genotyping and karyomapping were performed on the same amplified DNA used for the clinical NGS analysis. Trophectoderm biopsy samples were obtained from embryos on days 5,6 or 7 of development from a single IVF centre between 2018-2022. For all PGT-A tested embryos, the reported mosaicism rate at the IVF centre was 12%.

Participants/materials, setting, methods: Samples were analysed using PGTai from CooperSurgical with a 30-80% CNV mosaicism range. Patients with mosaic embryos consented to karyomapping follow-up using sibling embryo reference DNA. The origin of mosaic events was defined by SNP inheritance patterns for the affected chromosome. Meiotic errors were defined by the presence of both haplotypes from one parent for trisomy, and the absence of a parental haplotype for monosomy. Mitotic errors were inferred following a result of biparental inheritance.

Main results and the role of chance: A total of 154 diploid embryos identified as mosaic by NGS-based PGT-A were re-analysed by SNP-genotyping. Of these, 30.5% (n = 47) displayed at least one error of meiotic origin following SNP genotyping.

Among the 154 embryos, 189 mosaic chromosomal abnormalities were identified. After SNP-genotyping, 30.7% of chromosomal abnormalities (n = 58) showed evidence of meiotic signatures: 52 whole-chromosome (24 gains, 28 losses), and 6 segmental errors (5 gains and 1 loss). Of 73 segmental errors detected in total, only 8.2% of them (n = 6) were identified to be of meiotic origin.

Following PGT-A, 7.8% of embryos (n = 12/154) displayed ≥3 chromosomal abnormalities in the mosaic range. Of these embryos, 58.3% (n = 7/12) displayed one or more meiotic aneuploidies. Overall, embryos showing multiple mosaic events were significantly more likely to have a least one error of meiotic origin than embryos containing one abnormality (McNemar's test p-value= 1.171e-14).

Among mosaic calls above 50% CNV, 56.7% (n = 55/97) showed evidence of meiotic origin. In contrast, but importantly, in the low-moderate mosaicism range <50% CNV, 3.3% of errors (3/92) also displayed meiotic signatures, including two whole chromosome errors (chr21 gain at 32% CNV and chr13 loss at 46% CNV), and one segmental error (dup(6)(pter-p12.1) at 33% CNV).

Limitations, reasons for caution: Since karyomapping relies on recombination events to identify meiotic trisomy, the true origin of intermediate copy-number changes cannot always be identified. Therefore, errors without recombination may be missed, potentially underestimating meiotic gains, especially segmentals. The absence of a parental haplotype cannot entirely rule-out a mitotic error from a biopsy sample.

Wider implications of the findings: Quantitative NGS methodologies are unable to accurately distinguish the origin of mosaic errors in PGT-A samples. Since the clinical impact of meiotic and mitotic errors differs greatly, this limitation could explain mosaic embryos' reduced viability. Reliable identification of meiotic aneuploidies in presumed mosaic embryos is likely to improve clinical outcomes.

Trial registration number: not applicable

Abstract citation ID: dead093.093
O-079 Perinatal and postnatal outcomes up to the third year of life after the transfer of mosaic embryos compared with euploid embryos

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Study question: Are perinatal and postnatal outcomes different following the transfer of blastocysts diagnosed as low-grade mosaic compared to euploid embryos?

Summary answer: Our study did not show any significant difference between children conceived after transferring low-grade mosaic versus euploid embryos regarding perinatal outcomes and physical health.

What is known already: A considerable percentage of embryos are classified as mosaic in IVF-cycles with preimplantation genetic testing for aneuploidy (PGTA). Although societies, such as Preimplantation Genetic Diagnosis International Society (PGDIS), have proposed recommendations for the transfer of these embryos, there is no worldwide consensus on the classification, especially in terms of the level of mosaicism, and their clinical management. There are studies on clinical outcomes in terms of implantation and pregnancy, and some of them have reported the birth of healthy children. However, studies evaluating perinatal outcomes and, physical health and development during childhood are limited.

Study design, size, duration: This retrospective cohort study includes 172 children born after the transfer of a single embryo analysed with PGT-A using next-generation sequencing (NGS), between October-2017 and August-2022. Children were divided into two groups, according to whether they were classified as euploid (n = 115) or mosaic embryo (n = 57) after PGT-A. All mosaic embryos carried a level of mosaicism below 50%. Data related to the level of mosaicism, number of chromosomes involved or type of alteration were reviewed.

Participants/materials, setting, methods: This study analyses clinical parameters/outcomes during gestation, birth and postnatal period, including prenatal test, pregnancy and delivery complications, type of delivery, breastfeeding, incubator/neonatology stay, congenital anomalies, maternal and gestational age, birth weight, height and head circumference, Apgar-score and health problems or chronic diseases until today. The oocyte/sperm age and their origin were also analysed. The differences between groups were evaluated by R (4.2.0). In children from mosaic embryos, postnatal karyotyping was offered to the parents.

Main results and the role of chance: According to mosaicism characteristics, 61.4% of embryos had 25-39% of mosaicism and 38.6% had 40-50%,

82.5% had one chromosome affected and 17.5% two chromosomes. No significant differences were observed between the euploid and mosaic groups in pregnancy and delivery complications, type of delivery and gestational age (39.22 ± 1.92 weeks in euploid group vs 39.00 ± 1.78 in mosaic), however maternal age was higher in mosaic group (40.07 ± 3.32y vs 38.27 ± 3.06y, $p < 0.001$). Regarding newborn parameters, no differences were reported in birth weight, height and head circumference in euploid group compared to mosaic group (3222 ± 581 vs 3227 ± 530g, 49.92 ± 2.67 vs 50.13 ± 2.65cm, 34.50 ± 1.87 vs 34.50 ± 1.86cm; $p > 0.05$), neither in the Apgar-score (8.58 ± 2.48 vs 8.96 ± 1.79, $p > 0.05$) nor in the incubator/neonatology stay. With regard to congenital anomalies, their incidence was similar in both groups (8.7%, euploid vs 7.0%, mosaic) and in all cases were minor anomalies. No health problems/chronic diseases were recorded in either group, with the average age of the child at present being 3.48 ± 0.81y in euploid group and 2.92 ± 1.32y in mosaic. Prenatal testing was higher in the mosaic group, being carried out in 49.1% of pregnancies (23 non-invasive test and 4 amniocentesis) with normal result. Moreover, postnatal karyotype was performed in 6 children from mosaic group with normal result.

Limitations, reasons for caution: This study was limited by the sample size. Further larger studies are needed to ensure that the health outcome of children conceived after low-grade mosaic embryo transfer is comparable with children conceived after euploid embryo transfer.

Wider implications of the findings: Data following the transfer of blastocysts diagnosed as mosaic remain limited, especially in terms of neonatal/early-childhood outcomes. Our study suggests that the transfer of low-grade mosaic embryos lead to healthy children and provides further perinatal and postnatal clinical data. The normal prenatal/postnatal karyotype in the analyzed cases provides further reassurance.

Trial registration number: Not applicable

SELECTED ORAL COMMUNICATIONS

SESSION 25: FEMALE FERTILITY PRESERVATION: LESSONS LEARNED SO FAR

Monday 26 June 2023 Auditorium 10-12 15:15 - 16:30

Abstract citation ID: dead093.094

O-080 An umbrella review of meta-analyses regarding the incidence of female specific malignancies following fertility treatment

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Study question: To investigate the validity of the association between the development of female specific malignancies, including ovarian, endometrial, breast and cervical cancer following fertility treatment.

Summary answer: There is a statistically significant increase in the incidence of ovarian cancer and borderline ovarian tumours associated with fertility treatment.

What is known already: Approximately 1.3 million IVF cycles have been performed since 1991. The association between fertility treatment (FT) and female-specific cancers however, remains contentious, largely due to the conflicting outcomes published in various meta-analyses.

Study design, size, duration: A thorough literature search of the Cochrane Database of Systematic Review, EMBASE, Google Scholar and Pubmed was carried out to identify relevant systematic reviews and meta-analyses, from inception up until April 2022.

Participants/materials, setting, methods: The inclusion criteria included original studies stating the incidence of cancer in FT and control groups (non-FT). The principal outcome of interest was the incidence of cancer (breast, endometrial, cervical, and ovarian) in FT and non-FT groups. The effect estimates, Hazard Ratios and Odds Ratios (OR) were extracted, or calculated de novo. The strength of evidence and extent of potential biases were summarised.

Main results and the role of chance: In total, 3129 publications were identified, and 11 meta-analytical reviews were included. This umbrella review synthesised 324 meta-analyses, including data from over 20 million patients. The incidence of ovarian cancer (OR 1·21;95% CI 1·00-1·45) and BOTs (OR 1.87;95%CI 1·18-2.97) was higher in the FT compared to non-FT group. The meta-analyses related to ovarian cancer was statistically significant ($p < 0.05$), using a random-effects model. There was no significant association demonstrated between FT and the incidence of cervical, breast or endometrial cancer.

Limitations, reasons for caution: Although it has been demonstrated that exposure to fertility treatment does significantly increase the risk of ovarian cancer and BOTs, further studies are required in order to draw accurate conclusions.

Wider implications of the findings: Understanding the potential long-term sequelae of FT is fundamental to guide counselling and enable individuals to make informed decisions about their reproductive future. Clinicians should engage in appropriate patient selection, with those deemed to be at a higher risk, selected for more personalised fertility treatment and careful preventative follow-up.

Trial registration number: NA

Abstract citation ID: dead093.095

O-081 A 10-year follow-up of reproductive outcomes in women returning after elective oocyte cryopreservation

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Abstract under embargo

Abstract citation ID: dead093.096

O-082 15-year-experience in oncological fertility preservation: Impact on disease survival and reproductive outcomes

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Study question: What is the impact of fertility preservation (FP) procedures and cancer treatment on relapse, survival, ovarian damage and pregnancy outcomes in oncological patients?

Summary answer: FP technique (including ovarian stimulation for oocyte vitrification) does not affect relapse or survival rates even in hormone dependant tumours.

What is known already: FP has become a crucial part of oncological evaluation of young women facing cancer given the high survival rates achieved. Oocyte vitrification (OV) and ovarian cortex cryopreservation (OCC) are the main techniques offered to these patients at the moment. However, information on the true incidence of premature ovarian insufficiency (POI), return rates to use the cryopreserved material and the natural pregnancy rates achieved in these patients is still limited. Moreover although there is some data about the safety of these techniques the impact of FP on disease survival is yet to be definitely assessed.

Study design, size, duration: Prospective cohort study. 695 patients enrolled since 2001 until 2016. Patients referred to FP unit in a public hospital setting (Hospital Peset Valencia 2001-2006 and University Hospital La Fe 2007-2016). After evaluation 556 patients received a FP technique (OV, OCC or embryo vitrification) and 139 patients did not receive any due to medical reasons or patient's choice. Minimum follow-up 5 years after enrolment

Participants/materials, setting, methods: Baseline characteristics including type of cancer and previous chemotherapy at diagnosis and prior to FP technique were recorded followed by risk of chemotherapy treatment received, relapse, survival, POI and poor ovarian reserve (POR) occurrence and pregnancy outcomes. Primary outcome was median survival time after FP in months. Secondary outcomes included relapse rate, POI and POR incidence, usage FP rate, clinical pregnancy and live birth (LB) rates naturally and after FP use.

Main results and the role of chance: There were no differences in survival comparing patients undergoing FP versus no FP (median 89.67 vs 92.81 months, $p = 0.3$). However, patients that used their cryopreserved material survived more than those who did not (97.3 vs 89.5, $p = 0.012$). When assessing survival rates comparing patients that had approval to get pregnant versus those who did not we found a higher survival in the former (98.84 vs 84.79 months, $p < 0.001$). Breast cancer patients with hormone dependent tumors undergoing ovarian stimulation for OV vs OCC had no differences in survival (95.62 vs 87.38 months, $p = 0.37$). POI incidence was 20.29% ($N = 141$). POI patients were significantly older (32.28 vs 29.63, $p < 0.001$) and had received high-risk chemotherapy more frequently (31.74% vs 2.27%, $p < 0.001$). Ovarian damage incidence (including also POR) was 48.06% ($N = 334$). Eighty-six patients (15.47%) used their cryopreserved material. Among the patients with pregnancy wish ($N = 266$) there were 84 spontaneous live births (31.58%). Patients that conceived naturally were significantly younger (30.71 vs 33.46, $p < 0.001$) and the chemotherapy received was more frequently low-risk (43.20% vs 23.52%, $p = 0.018$). There were 37 LB after use of FP (37/86, 43.02%). Patients with a higher ovarian reserve percentile had a higher chance of achieving a natural LBR (OR 1.016, 1.005-1.027, $p = 0.004$)

Limitations, reasons for caution: The higher survival found in patients using their cryopreserved material is mediated by the prognosis of the disease itself that limits the chance of pregnancy to those with a stable disease.

Wider implications of the findings: FP does not have a negative impact on survival even if ovarian stimulation is used. Almost half of the patients had ovarian damage (POI or POR) as a result of treatment; this is higher than previously reported. Among the patients with fertility wish around one third achieved a LB naturally.

Trial registration number: not applicable

Abstract citation ID: dead093.097

O-083 Ovarian reserve, Reproductive function and Pregnancy outcomes among Female survivors of childhood Hodgkin lymphoma: results from the DCOG LATER-VEVO Later study

S. Broer[1], K. Drechsel[2], F. Stoutjesdijk[2], J. Twisk[3], M. Van den Berg[3], E. Van Dulmen - den Broeder[3], G. Kaspers[2], M. Veening[2]

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Abstract under embargo

Abstract citation ID: dead093.098

O-084 Fertility outcomes several years after urgent fertility preservation (FP) for breast cancer

M. Peigné[1], P. Mur[2], L. Laup[3], C. Sifer[4], A. Mayeur[5], F. Eustache[6], S. Sarandi[7], C. Vinolas[3], S. Rakrouki[3], A. Benoit[2], M. Grynberg[8], C. Sonigo[8]

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Study question: What are the fertility outcomes of women who tried to conceive after breast cancer treatment and FP?

Summary answer: Three to-ten years after FP for breast cancer, less than half of patients tried to conceive but 39% of them had a child, mainly spontaneously.

What is known already: Breast cancer is the most common cancer in women of childbearing age. Because of the gonadal toxicity of treatments and temporary contraindication to pregnancy, fertility after cancer treatment is often impaired. Different FP techniques can be offered. Although it is well established that pregnancy in breast cancer survivors is safe, little is known on the incidence of pregnancies after breast cancer treatments in women having undergone FP, the way those patients conceived and outcomes.

Study design, size, duration: This is a retrospective observational, bicentric cohort study. All patients having undergone FP before breast cancer treatment (oocyte and/or embryo vitrification after controlled ovarian stimulation (COS) or *in vitro* maturation and/or ovarian tissue cryopreservation) between January 1, 2013 and July 31, 2019 were included (n = 844). Patients for whom data on post-cancer pregnancy attempt was missing (n = 195) were excluded from the analysis of pregnancies.The cut-off date was March 1, 2022.

Participants/materials, setting, methods: For women who got pregnant, the time to conception was calculated between the 1st FP consultation (CS1) and the day of the estimated conception. For those who did not conceived, we considered the time between the CS1 and the cut-off date or the date of patient's death. Cumulative incidences of pregnancy and live birth were calculated. A logistic regression Cox model was performed to study the predictive factors of pregnancy and live birth.

Main results and the role of chance: Among the 649 patients with available data on post-cancer pregnancy attempt, 255 (39.3%) tried to conceive. Overall, 135 (52.9%) of them achieved a pregnancy, mainly spontaneously (79.3%), and 99 (38.8%) reported a live birth. At the CS1, 76.3% of women were in couple and 42.3% already had at least a child. The mean age at CS1 was 32 ± 4.2 years. Invasive ductal carcinoma was the diagnosis in 95.8% of women and 45.7% had COS for FP. In our cohort, 48 months after CS1, the cumulative incidence of pregnancy was 33.1% (CI95% [27.6-37.9]). After adjustment on age, parity, type of chemotherapy and hormonotherapy, only multiparity at diagnosis and absence of chemotherapy were found to be positive predictive factors of pregnancy after cancer. Only multiparity was found to be negatively associated with live birth (RR0.47 [0.33-0.67]). Of the 793 patients who vitrified oocytes/embryos, 68 reused them (26.6% of the patients who tried to conceive) and obtained 8 live births. Women using their cryopreserved biological material were older at CS1, had lower ovarian reserve parameters and had vitrified embryos. Ovarian reserve parameters and number of cryopreserved oocytes were higher while age at CS1 was lower in patients reporting a live birth after oocyte/embryo thawing.

Limitations, reasons for caution: Due to the retrospective nature of the study, some data is missing. Even if the median follow-up is more than 4 years, a longer follow-up is necessary

Wider implications of the findings: Although pregnancy rates after breast cancer are reduced, most of conceptions are achieved spontaneously. Our findings provide useful information to advise women on the different techniques of FP, their efficacy and safety.

Trial registration number: Not applicable

SELECTED ORAL COMMUNICATIONS

SESSION 26: MACHINE LEARNING AND MOBILE APP HELP TO PERSONALIZE ART

Monday 26 June 2023 Hall D5 15:15 - 16:30

Abstract citation ID: dead093.099
O-085 Patients support through a mobile application improves ART outcomes across all age groups

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Study question: Assess the effectiveness of fertility treatment companion mobile application, which provides tracking, adherence tools, education and community support, in improving ART outcomes

Summary answer: Results showed that patients use of mobile application during treatment had significantly improved ART outcomes compared to the CDC average, across all age groups

What is known already: Growing numbers of adults use digital health tools to diagnose and manage their health conditions. Direct-to-consumer, interactive, diagnostic apps with information and personalization capabilities beyond those of static search engines are rapidly growing in use (M. Millenson et al. 2018).

A very limited amount of research has been conducted on mobile application's ability to impact ART outcomes (I. Robertson, etc. 2021). Existing studies include small control groups and limited data.

To date, though sparse, most research has focused on mobile applications' and internet-based technologies' ability to mitigate the psychological effects of infertility (Meyers & Domar, 2021).Study design, size, duration:

Retrospective analysis conducted using Embie mobile application's database (EMU) between Jan 1, 2021 and Dec 31, 2022. Study group included 6449 cycles by 5067 users self-reported in real-time.

EMU stimulation cycle cancelation rates and pregnancy outcomes, at week 20 of pregnancy, were compared to CDC 2020 preliminary report of LBR and stimulation cycle cancellation rates, divided into 4 age groups; <35, 35-37, 38-40 and >40.

EMU population included 53% U.S., 47% out of U.S.

Participants/materials, setting, methods: Participants are fertility clinic patients globally who voluntarily downloaded the mobile application via the App or Play stores.

The application provides ART cycle protocol tracking and monitoring, eggs retrieved, embryo, transfer and pregnancy results, etc, along with educational articles and community support.

Self-reported data is entered in real-time by the user. We cleared, merged and analyzed using python, then compared the CDC report by age group. Chi square significance performed comparing two proportions (with continuity correction).

Main results and the role of chance: 4426 EMU of stimulation cycles analyzed 2319, 903, 683, 521 by age respectively.

EMU stimulation cancelation rates 2.76%, 3.54%, 6.00%, 7.49% respectively.

2073 EMU transfer cycles analyzed 1078, 431, 336, 228 by age respectively.

Ongoing EMU pregnancy at 20 weeks was observed in 618, 252, 170, 75 respectively.

Ongoing EMU pregnancy rates at 20 weeks 57.33%, 58.47%, 50.60%, 32.89% respectively.

Delta CDC/EMU stimulation cycle cancellation rate 5% vs 2.76%, 7.20 vs 3.54%, 10.10% vs 6.00%, 14.20% vs 7.49% respectively.

Delta CDC LBR per transfer data vs EMU ongoing pregnancy 49.80% vs 57.33%, 45.50% vs 58.47%, 40.40%vs 50.60%, 23.90% vs 32.89% respectively.

Delta CDC LBR per transfer data to EMU U.S. users' only ongoing pregnancy 49.80% vs 62.86%, 45.50% vs 65.77%, 40.40% vs 58.17%, 23.90% vs 43.62% respectively.

Statistically significant analysis by chi square score showed all delta measurements to be significant ($p < 0.0001$).

Limitations, reasons for caution: Self-reported data may not be as reliable as data collected in a clinical setting. However, the use of a large cohort reduces the impact of a single error. Additionally, LBR were compared to ongoing pregnancy at 20 weeks, which can influence the outcomes at a low rate.

Wider implications of the findings: Our results suggest that fertility treatment mobile applications can significantly improve ART outcomes and patient's experience. Clinics should consider adopting such tools as an adjunct to care.

The ability to collect and analyze big data globally in real-time will impact future ART research, medical understanding and improvement of patient's fertility treatment.

Trial registration number: not applicable

Abstract citation ID: dead093.100
O-086 Co-development of Orchid mobile application for supporting fertility intentions and reproductive choices

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Study question: What does co-development bring to the design of a mobile application (app) for supporting fertility intentions and reproductive choices?

Summary answer: Co-development of the app for supporting fertility intentions and reproductive choices informed the design and content, from expressed preferences, ensuring acceptability by the end user.

What is known already: We sought to develop a digital tool to support people of reproductive age to understand their pregnancy preferences and fertility intentions; helping them to prepare for pregnancy, improving pregnancy outcomes, preventing unplanned pregnancies and reducing inequalities in maternal health. Orchid encourages the person to consider that they have a choice about their fertility intentions and to reflect on their personal reproductive goals and how this may fit with other life goals. We aimed to co-develop a mobile app via a person-centred approach, which draws on behaviour change theory.

Study design, size, duration: The app development was conducted via University College London. Co-development was conducted online over five rounds (four online surveys and one online group discussion) from November 2022 – February 2023. Round 1 – Content mapping, Round 2 – App layout, Round 3 – Online group discussion of content and layout, Round 4 – App design, and Round 5 - Functioning pilot app.

Participants/materials, setting, methods: Participants were invited via a database of potential research participants and were purposefully selected to ensure diversity and inclusion. The co-development group was made up of 10 women from across the UK; aged 19-44 years with a range of relationship status' (married, divorced, cohabiting and single), ethnicities (White n = 8, Asian n = 1, Mixed n = 1) and family sizes.

Main results and the role of chance: We gained feedback from our co-development group through online surveys, made up of a combination of structured and open text questions. We also held an online group session which allowed us to have an in-depth discussion about the content and look of the app. As a result of the co-development feedback, we redesigned key elements of the app; altering the language used throughout, including the wording of the pregnancy prediction, adding extra information about the reproductive preference groups and including a wider range of resources. The overall look of the app also changed considerably, with feedback from the co-development group helping to make decisions regarding the colour scheme, the number of screens, the layout and presentation of information. Final user testing was also conducted by the co-development group to verify functionality of the app.

We performed multiple iterations of co-development – with a survey at every development stage – and used the feedback to inform our decisions throughout the development process to ensure acceptability to the end user.

Limitations, reasons for caution: There was a limited number of participants involved in this, the first stage of developing a Minimum Viable Product (MVP) of the app. A larger group of even more diverse individuals will be needed for the next stage, which will likely increase the complexity of the co-development process.

Wider implications of the findings: We co-developed an MVP app with a sample population, representative of our end-user. Participants were involved in every stage of the development process and provided a positive evaluation of the product. People must be viewed less as consumers and more as contributing partners in their care.

Trial registration number: not applicable

Abstract citation ID: dead093.101

O-087 Patient-centric, machine learning (ML)-based personalised prognostics supports fertility specialists to improve access to assisted reproductive technology (ART) and increase overall live birth (LB) outcomes

M. Yao[1], E.T. Nguyen[1], M.G. Retzloff[2], K. Cadesky[3], L.A. Gago[4], S. Copland[5], J.E. Nichols[6], J.F. Payne[6], B.A. Ripps[7], M. Peavey[5], J. Meriano[8], B.W. Donesky[9], J.S. Bird-III3rd[9], J. Groll[10], X. Chen[1], J.S. Bird Jr.[9], D. Walmer[11], T. Swanson[1], M. Menabrito[12]

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Study question: Does the use of patient-centric, ML-prognostics counselling report (Univfy® PreIVF Report) affect assisted reproductive technology (ART) conversion (first ART cycle usage) and LB rate (LBR)?

Summary answer: The use of patient-centric, ML-prognostics counselling report (Univfy® PreIVF Report) by fertility specialists is associated with higher ART conversion and LBR among new patients.

What is known already: ART is a highly effective and safe treatment for clinical infertility. However, ART remains vastly underutilised resulting in missed opportunities to help more people build families. Commonly used age-based trends often do not address patients' perceived risks including their own ART success probability and ART cost burden as related to their personalised LB probabilities. We previously reported the use of artificial intelligence (AI)/ML to generate patient-centric counselling reports based on ART success prediction models developed and validated for each fertility centre to address their local patient populations in ways that are personalised, relevant and actionable.

Study design, size, duration: Retrospective cohort analysis. Eight fertility centres from 22 locations across 9 states (US) and Ontario, Canada contributed to the research design, compilation of outcomes data, and interpretation of results. Five centres provided ART utilisation and outcomes data for 15,289 new patients seen in each centre's study period when the Univfy® PreIVF Report was available and data were submitted for aggregated research analysis. Each centre provided 4-6 years of data within the period 2016-2022.

Participants/materials, setting, methods: The effect of Univfy or No-Univfy Group on ART conversion was analyzed by Chi square tests using aggregated data and separately for each centre's data, for 3 timed analyses, 180-Day, 360-Day and "Ever" (no restriction) after new patient visit. Patients who received the Univfy® PreIVF Report prior to IUI or ART conversion, or had no such conversion after receiving it were placed into the Univfy Group. The No-Univfy Group comprises patients who did not receive a report.

Main results and the role of chance: Univfy report usage was associated with higher conversions to Direct-ART (by 2.6-, 2.4-, 1.9-folds) and Any-ART (by 2.9-, 3.0-, 2.4-folds) in the aggregated data when analyzed for 180-Day, 360-Day and Ever, respectively; p-value < 0.001. Direct-ART is ART conversion without prior IUI(s); Any-ART conversion includes ART conversion with or without prior IUI(s).

In the centre-specific analyses, the fold increase in Direct-ART and Any-ART conversions ranged from 1.8 to 4.5 and 2.2 to 4.7, respectively, in the 360-Day period; p-value <0.001. Univfy® PreIVF Report usage was associated with an increase in estimated LBR ranging from 2.1 to 1.3 folds for the Univfy Group compared to No-Univfy Group (360-Day analysis, $p < 0.001$) based on conservative versus liberal scenarios. Similar ART conversion and LBR results were observed for 180-Day and Ever analyses, $p < 0.001$.

We used conservative to liberal assumptions for IUI-LBR and NC-LBR because IUI and NC outcomes were not readily available. (Conservative: IUI-LBR 15%, natural conception (NC)-LBR 5%; Liberal: IUI-LBR 25%, NC-LBR 20%). Estimated LBR for ART used clinical ongoing pregnancies and documented live births as LBs and the following LBR assumptions: freeze-all with no transfers yet (50%); gestational carrier ART (45%); ART with unknown outcomes (0%).

Limitations, reasons for caution: This study was not prospective or randomised. The intended report usage was to support physicians when counselling patients. Although we observed comparable report and ART usage across predicted ART-LB probabilities, there is potential unintentional bias towards higher report or ART utilisation among patients with more favorable clinical characteristics.

Wider implications of the findings: These results represent our retrospective experience in diverse geographies in North America. We endeavor to collaborate with additional centres to test the reproducibility of AI/ML-driven, validated personalized IVF prognostics on improved overall live birth outcomes and ART access when counselling patients about treatment options.

Trial registration number: not applicable

Abstract citation ID: dead093.102

O-088 Social Networks (SNs) in reproductive medicine: the experience of the 5 Young ESHRE Ambassadors 2022 (5YEA)

A. Anastasi[1], R. Einenkel[2], I. Gazzo[3], P. Makwana[4], T. Penna[5]

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[4]Vasundhara Hospital Ltd, ART department, Jodhpur, India
[5]University of Turin, Department of Law, Turin, Italy

Study question: The 5YEA, experienced how SN can promote dissemination of evidence based scientific education and counter misinformation for both scientific community and general public.

Summary answer: An incorrect use of SNs can lead to the massive dissemination of incorrect information but if used correctly they can help scientists, patients and general-public.

What is known already: SNs have proved to be a very powerful weapon. SNs are applications available on the Internet, a global computer network that uses the Web as a service to navigate through documents. Their rise, in conjunction with the development of mobile phones, has affected all professional sectors, including the scientific community.

Social networks have multiple uses from facilitating scientists to communicate and make their research more accessible, to healthcare professionals generating interest and helping the general public be more aware about their health & wellbeing, all this while countering the the uncontrolled dissemination of misinformation.

Study design, size, duration: From ESHRE annual meeting 2022, the 5YEA shared scientific messages and personal considerations on research and topics presented at the meeting. Through the various SNs(mainly Twitter-Instagram-Facebook-LinkedIn) they have directed many participants to follow the topics from the comfort of their homes and, generating curiosity for people even outside the scientific community. Even today, the topics shared on SNs are followed by users both from the scientific world and from the whole social world.

Participants/materials, setting, methods: The 5YEA actively participated during the 5 days of the annual meeting. The sharing of their messages on the SN involved thousands of registered users across various platforms. The conspicuous increase in the number of users who have begun to follow them has allowed us to express the considerations of this abstract.

Main results and the role of chance: Scientific integrity, defined in particular by the methodological rigor and honesty of the researcher, is essential for the validation of his work by the scientific community. SNs, accessible to research professionals, on the one hand, and to the general public, on the other, have given specialists a voice to raise awareness of scientific integrity in these two populations. SNs are also a forum for discussing methodological weaknesses or distorted results of some studies. But they are also an educational tool to educate their readers about scientific fraud.

SNs has increased the massive diffusion of fake news. Raising scientists' awareness of the use of these powerful communication tools seems to be a necessity, as these tools can interfere with the visibility of their research work but also, sometimes even, with their private life.

Today on SNs there is therefore a struggle going on, difficult to ignore, which questions the credibility of scientific discourse and its integrity and for which very few researchers are prepared. It is a fight for which it is necessary to attract the attention of all generations of scientists. This fight must not be lost to restore and preserve the still fragile trust between science and society.

Limitations, reasons for caution: New SN platforms are born every day. Some are reserved to scientific professionals (high scientific rigor), others not (general public creates confusion by indulging in inappropriate comments). Given limitations to text length, information is always condensed, while users are pushed to share information frequently to get a greater reach.

Wider implications of the findings: With all the emerging and sometimes misinformed discussions taking place on social media, experts should spend more time clarifying them. However, the quantification and corresponding appreciation of this work is currently lacking.

Trial registration number: not applicable

Abstract citation ID: dead093.103

O-089 Using ChatGPT to answer patient questions about fertility: the quality of information generated by a deep learning language model

K. Beilby[1], K. Hammarberg[2]

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[2]*Monash University, Global and Women's Health- Public Health and Preventive Medicine, Melbourne, Australia*

Study question: What is the quality of information provided by ChatGPT when using common patient questions as prompts?

Summary answer: Overall, the quality of the information generated by ChatGPT was high with little evidence of commercial bias.

What is known already: People seeking fertility-related information rely on internet sources when deciding on reproductive planning and assisted conception. The quality of information within the commercial landscape of infertility treatment is poor. ChatGPT, a variant of Generative Pre-trained Transformer v3 (GPT-3), is a language model that uses deep learning to generate human-like text. Given prompts, it generates answers by predicting the next word in the sequence based on patterns learned from training data. The training data for GPT-3 is not curated, but a snapshot of the Web, which includes all kinds of information, including biases that may exist within sources.

Study design, size, duration: Ten common patient questions were used as prompts. Three questions related to fertility awareness (impact of female/male age on fertility and fertile window in the menstrual cycle), one to the chance of success with IVF, one to elective egg freezing, one to the benefits of add-ons, one to PCOS and pregnancy, one to choosing a fertility clinic, and one to how many IVF cycles should be attempted.

Participants/materials, setting, methods: Two experts independently scored the quality of the information generated by the ChatGPT using a scoring matrix with a range of 0 to 7 where higher scores indicate higher quality. Text was rated against humanistic answers based on how well it corresponded (0-3), evidence of commercial bias or controversial claims (no = 1, yes=0), use of accurate proportions/ statistics and whether it was stated that medical advice should be sought (yes=1, no = 0).

Main results and the role of chance: The scores returned by the two experts were closely aligned with only one point difference for one of the answers. This discrepancy was resolved through discussion. While none of the answers received the maximum score of 7, 6/10 scored 5 or more and 3 received a score of 3-4. Only one answer, the answer to the question about the benefits of add-ons, scored less than 3. This was also the only question where the response had evidence of commercial bias and one of only two that made claims that could be considered controversial.

Limitations, reasons for caution: The scoring method used in this study has not been validated and is exploratory in nature as this area of evaluation is emerging. However, the use of expert evaluation is common when assessing the performance of machine learning models and often used to fine-tune their parameters and improve their performance.

Wider implications of the findings: It is known that people seeking fertility-related information rely heavily on online sources such as clinic websites, consumer advocacy organisations, patient support groups and social media. Our findings suggests that ChatGPT may be a useful tool for patients seeking factual and unbiased information regarding fertility and fertility treatment.

Trial registration number: Not applicable

POSTER DISCUSSION SESSION
SESSION 27: IMPLANTATION AND EARLY PREGNANCY

Monday 26 June 2023 Hall D2 15:15 - 16:30

Abstract citation ID: dead093.104

P-468 Personalized embryo transfer guided by transcriptomic endometrial receptivity analysis (ERA) in infertile patients: a systematic review and meta-analysis

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[4]*Institute for Clinical Effectiveness and Health Policy IECS- Center for Research in Epidemiology and Public Health- National Scientific and Technical Research Council CONICET, Argentine Cochrane Centre, Buenos Aires, Argentina*

Study question: What is the effectiveness of a personalized embryo transfer (pET) guided by an endometrial receptivity test in assisted reproduction?

Summary answer: We have not found evidence supporting the routine use of a personalized embryo transfer guided by an endometrial receptivity test in any case yet.

What is known already: Currently, the outcomes of good quality embryo transfers are far from ideal. Clinical focus is shifting towards other actors that could play a key role in the good-quality embryos implantation failure, such as the endometrial preparation and the achievement of an embryo-endometrium synchrony, so that the embryo has a better chance to implant during the window of implantation (WOI). Today, there is a wide range of different endometrial receptivity tests that use different sets of genes to identify patients with displacements of their WOI in order to adjust the individual length of progesterone exposure in a personalized embryo transfer (pET).

Study design, size, duration: This is systematic review and meta-analysis were conducted following the Cochrane methods and the GRADE approach to rate the certainty of evidence (CoE). The protocol has been sent for registration to PROSPERO (CRD42022299827). We included studies that compared women that were tested with transcriptomic endometrial receptivity tests versus those that were not tested. We only included comparative studies, including randomized controlled trials (RCTs), and retrospective and prospective cohorts.

Participants/materials, setting, methods: Participants were women undergoing an infertility treatment (both with own or donor gametes), with or without repeated implantation failure (RIF), with or without preimplantation genetic testing for aneuploidies (PGT-A). Each of the above-mentioned populations was analyzed separately as different subgroups. Electronic searches in CENTRAL via the Cochrane Register of Studies Online (CRSO), PubMed, and Embase were performed from inception to October 2022. Screening, study inclusion and data extraction was performed by pairs of independent reviewers.

Main results and the role of chance: We included 35 studies. Two of them were RCTs and 25 were cohort studies that compared pET guided by an endometrial receptivity tests guided versus sET in women with non-tested endometrium. Besides, we included 16 studies that compared women with sET with a receptive endometrium versus pET in women with non-receptive endometrium. And we included three studies that compared pET guided by an endometrial receptivity test in a specific population of women (RIF or adenomyosis) versus a control population. The two RCTs included women with no history of RIF. In non-RIF women, no important differences (moderate-CoE) were found in live birth rate (LBR) and clinical pregnancy rate (CPR). In that population, cumulative CPR may be higher with pET (RR 1.15, 95%CI 1.00-1.34; low-CoE) but no differences were found in cumulative LBR. Seven cohort studies that adjusted by confounding were meta-analyzed. In RIF, pET may improve the CPR (OR 2.50, 95% CI 1.42-4.40; low-CoE) but, in agreement with the RCTs, no benefits were found in non-RIF. In summary, routine use of pET guided by endometrial receptivity tests is not supported by current published evidence in non-RIF women. However, low-CoE suggests that it may be useful in RIF.

Limitations, reasons for caution: Most data come from non-RCTs which increases the risk of bias. Besides, the large heterogeneity found in the population, interventions and comparisons led to large imprecision due to the few studies per analyzed strata. It is important to avoid the extrapolation of the results to any different setting.

Wider implications of the findings: Routine use of pET guided by endometrial receptivity tests is not supported by current published evidence in non-RIF women. However, low-CoE suggests that it may be useful in RIF. Better designed studies are needed to find out if any of these tests work in any specific population.

Trial registration number: not applicable

Abstract citation ID: dead093.105

P-480 An endometrial receptivity scoring system evaluated by ultrasonography in patients undergoing frozen-thawed embryo transfer: a prospective cohort study

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[4]*Reproductive & Genetic Hospital CITIC-Xiangya, Imaging Department, Changsha, China*

Study question: Can a scoring system based on ultrasound indicators evaluate the endometrial receptivity (ER) in a noninvasive and effective manner?

Summary answer: A noninvasive ultrasound scoring system was proposed, through which, a patient's ER situation can be assessed in a noninvasive, efficient and accurate manner.

What is known already: Endometrial receptivity (ER) refers to the receptiveness of the endometrium to embryonic implantation and subsequent development. The endometrium plays a crucial role in embryo implantation, especially when the embryo quality is good. Ultrasound technology has become a routine method for ER evaluation. The independent evaluation value of various ultrasonic indicators is controversial. Some researchers designed a multi-index prediction system, but the prediction results vary. To further understand ER, we performed this prospective cohort study to help evaluate ER in a noninvasive and efficient manner.

Study design, size, duration: This prospective study included 197 infertile women undergoing FET from April 2019 to July 2021. Transvaginal three-dimensional ultrasound was performed on the transfer day to evaluate ER, including endometrial thickness, morphology, volume, movement, blood flow and flow index.

Participants/materials, setting, methods: This study was conducted at the Reproductive and Genetic Hospital of CITIC-Xiangya. The primary outcome of this study was the clinical pregnancy rate. All included patients were divided into a pregnant group and a nonpregnant group according to whether clinical pregnancy was achieved.

Main results and the role of chance: A total of 197 FET patients with 139 pregnancies (70.5%) were analysed. Primary infertility (adjusted odds ratio (aOR), 1.98; 95% confidence interval (CI), 1.01–3.882; $P = 0.047$) and more frequent endometrial peristalsis (aOR, 1.33; 95% CI, 1.028–1.722; $P = 0.03$) were protective factors against clinical pregnancy. Scores of 1 to 2 were given according to the relationship between different groups of ultrasonic indices and the clinical pregnancy rate (CPR). A patient's ER score was the sum of the scores of the 6 items. The ER score was significantly higher in the pregnant group than that in the nonpregnant group (7.40 ± 1.73 versus 6.33 ± 1.99, $P = 0.001$). The CPR increased as the ER score increased; the CPR significantly improved for the group with total ER scores of < 6 to ≥ 6 (45.5% versus 75.6%, $P = 0.001$).

Limitations, reasons for caution: First, this study only included natural cycles of FET. The application for other populations remains to be verified. Second, perhaps with the progress of ultrasonic technology, there will be new and more direct indicators. Third, we did not further explore the relationship between ER and the live birth rate.

Wider implications of the findings: The scoring system provides a broader idea for guiding clinical practice to perform more efficient transplantation and provide a noninvasive evaluation of ER in the future.

Trial registration number: None

Abstract citation ID: dead093.106

P-534 Serum progesterone levels on the day of embryo transfer do not affect pregnancy outcomes after euploid blastocyst transfer: data from artificial cycles with intramuscular progesterone

W. Chen[1], Y. Xu[1], Y. Xu[1], F. Gu[1]

[1]*The First Affiliated Hospital- Sun Yat-sen University- Guangzhou- Guangdong-China, Reproductive Medicine Center, Guangzhou- Guangdong, China*

Study question: Do serum progesterone levels on the day of embryo transfer (ET) have an impact on implantation and pregnancy outcomes?

Summary answer: Serum luteal progesterone levels were not associated with pregnancy outcomes under the condition of utilizing 60mg intramuscular progesterone for luteal support in artificial cycles.

What is known already: It was recognized that circulating progesterone level might be associated with frozen-thawed embryo transfer (FET) success, and a serum progesterone level above a certain threshold at the time of blastocyst transfer improved live birth rates (LBR) and reduced risk of miscarriage in artificial FET cycles. However, this hypothesis is controversial as the previous findings were based on data using vaginal progesterone as luteal phase support with various doses and timing of administration. Studies evaluating euploid-only FET cycles with intramuscular progesterone were lacking.

Study design, size, duration: The study incorporated infertile couples undergoing (preimplantation genetic testing, PGT) from 1st January 2018 to 31st July 2021. It is a retrospective cohort study including 771 patients who underwent single frozen-thawed euploid blastocyst transfer after an artificial endometrial preparation cycle with 60mg intramuscular progesterone daily for luteal support. Each patient only contributed one cycle per cohort.

Participants/materials, setting, methods: Patients with recurrent implantation failure, endometrium 7.0 mm and cycles with gonadotrophin releasing hormone analogue down regulation were excluded. Serum progesterone measurements were taken on the day of ET, approximately 20 ± 2 hours after the last injection of progesterone. The primary outcome was LBR based on serum progesterone levels. Secondary outcomes were the relationship between serum progesterone levels and clinical pregnancy rate, miscarriage rate as well as obstetrical and neonatal outcomes.

Main results and the role of chance: The median of serum progesterone levels was 11.80 ng/ml [9.95, 14.60] (median/IQR) and the overall LBR, clinical pregnancy rate and miscarriage rate were 55.6%, 64.33% and 10.69% respectively. Patients with the lowest centile of serum progesterone level (≤P10, ≤8.2ng/ml) had a similar clinical pregnancy rate (62.0% vs 64.6%), live birth rate (50.6% vs 56.2%) and miscarriage rate (12.2% vs 10.5%) compared with the rest of patients. After dividing patients in deciles according to serum progesterone levels, no differences in LBR were observed among groups and no correlations were found between progesterone levels and the other pregnancy outcomes. Multivariate regression analysis confirmed that serum progesterone levels did not affect the LBR after adjusting for possible confounders (age, body mass index, PGT indication, primary infertility, history of miscarriage, endometrium thickness, day of blastocyst and embryo quality) with adjusted (OR) 0.99, 95%CI 0.96-1.03), while the day of blastocyst (D6 vs. D5: aOR 0.47, 95%CI 0.33-0.65) as well embryo quality (good quality embryo vs. available embryo: aOR 1.88, 95%CI 1.38-2.58) were associated with LBR independently. Similarly, serum progesterone in their lowest levels did not negatively impact other pregnancy outcomes (ex. clinical pregnancy rates, miscarriage rates) and the perinatal outcomes.

Limitations, reasons for caution: This is a retrospective cohort study and the results only apply for patients under artificial cycles with intramuscular progesterone. Moreover, the time interval between the last administration of progesterone and the blood test was not controlled in the present study.

Wider implications of the findings: Serum progesterone level may not influence the FET outcomes independently in an artificial cycle with intramuscular progesterone. A daily dose of 60mg progesterone intramuscularly for luteal support is sufficient and the measurement of serum progesterone does not need to be performed in advance for most of the patients.

Trial registration number: not applicable

Abstract citation ID: dead093.107

P-537 Intentional endometrial injury significantly improves ongoing pregnancy rates of an oocyte donation program in patients without recurrent implantation failure (RIF): a randomized controlled trial.

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[5]IVI-RMA Madrid, Reproductive Medicine Unit, Valencia, Spain
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Study question: Is endometrial scratching a useful add-on to be applied in assisted reproduction patients without recurrent implantation failure (RIF)?

Summary answer: Endometrial scratching results in significantly higher clinical and ongoing pregnancy rates per intention to treat (ITT) among women undergoing oocyte donation without RIF.

What is known already: The effect of endometrial injury or endometrial scratch (ES) on reproductive outcomes has been widely studied by randomized controlled trials but from the information obtained regarding its benefit on improving endometrial receptivity remains controversial. Oocyte donation programs (OD) provides with the ideal setting for this research, as the recipient's endometrial priming guarantees the homogeneity of the endometrium avoiding a deleterious differential effect of the stimulation on endometrial receptivity and makes comparable the quality of the oocytes and transferred embryos, then limiting the confounding factors involved in studies with autologous oocytes and their frozen surplus embryos, better addressing the research question.

Study design, size, duration: A multicentric, open-label, randomized controlled trial has been conducted in a private setting from Oct 2013-Nov 2022. Eligible recipients were randomly assigned in a 1:1 ratio to either ES (by pipelle biopsy in the luteal phase of the menstrual cycle prior to the embryo transfer, n = 303) or no intervention (NES, n = 310), through a computer-generated randomization list, and embryo transfers (ET) performed in the cycle following the intervention.

Participants/materials, setting, methods: 18-44 years aged ovum recipients with preserved ovarian function, 19-29.9 kg/m2, first/ second OD fresh embryo transfer, endometrial thickness > 6 mms, and 1-2 optimal quality blastocysts transferred. Exclusion: any adverse condition, RIF with OD, ET not performed in the cycle following the intervention. Outcomes included ongoing implantation rate (ongoing sacs/ embryo transferred), biochemical, clinical and ongoing pregnancy rates analyzed per ITT basis and per protocol strictly completed (PP, treatment correctly received, embryo transfer achieved).

Main results and the role of chance: A total number of 458 recipients underwent OD-embryo transfer, 226 in scratching arm and 232 in the NES group.

No differences existed in mean age and BMI of recipients and oocyte donors, oocytes retrieved and microinjected, fertilization rates, blastocysts transferred, endometrial thickness and estradiol /progesterone levels after their endometrial priming, nor other clinically relevant parameters.

The biochemical pregnancy rate was 62.6%95%CI(56.6-68.3) and 55.6%95%CI(49.7-61.3) in the ES arm and in NES arm on the ITT analysis (OR 1.073 95%CI(0.990-1.163), p = 0.088); and 75.3%95%CI(69.0-80.7) vs. 70.7%95%CI(64.3-76.4), respectively PP (OR1.048 95%CI(0.965-1.137), p = 0.266.

The clinical pregnancy rate was 57.1%95%CI(51.0-63.1) and 47.5% 95%CI(41.7-53.3) in the ES arm and in NES arm on the ITT analysis (OR = 1.102 95%CI(1.015-1.196); p = 0.021), and in the per protocol analysis, 68.6% 95%CI(62.0-74.6) vs 60.3%95%CI(53.7-66.6) respectively, OR = 1.086 95%CI(0.995-1.186), p = 0.066.

The ongoing pregnancy rate was 45.1%95%CI(39.1-51.2) and 36.3% 95%CI(30.8-42.1) in the ES arm and in NES arm on the ITT analysis (OR = 1.092 95%CI(1.007-1.183); p = 0.033), and in the per protocol analysis, 53.8%95%CI(47.0-60.5) vs 46.1%95%CI(39.6-52.8) respectively, OR = 1.080 95%CI(0.985-1.184), p = 0.101.

The ongoing implantation rate was in the PP analysis, 61.4%95%CI(55.1-67.8) in ES, and 56.5%95%CI(50.1-68.2) in NES respectively, p = 0.276, while in the ITT analysis, 51.3% 95%CI(45.4-57.2) vs 44.4% 95%CI(38.7-50.1), p = 0.098 in the ES arm and in NES arm.

Limitations, reasons for caution: The number of patients not fulfilling the complete procedure probably avoided to find significant differences on the PP analysis, and during the study, there may be some clinical and/or laboratory

changes through the years, that should have been affecting outcomes on both groups.

Wider implications of the findings: This RCT shows that ES in the luteal phase of the cycle preceding the OD embryo transfer in recipients without RIF would pose a significant benefit thus its consideration for all IVF patients can be advised at this point.

Trial registration number: NCT01955356

Abstract citation ID: dead093.108
P-544 Biochemical markers as a predictor of clinical pregnancy on day 6 after embryo transfer

P. Głodek[1], M. Krajewski[1], D. Drzyzga[1], K. Gross-Tyrkin[1], K. Zieliński[1,2], M. Kunicki[1,3]

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[3]*Medical University of Warsaw, Department of Gynecological Endocrinology, Warszawa, Poland*

Study question: Is it possible to predict clinical pregnancy using Machine Learning models on day 6 after embryo transfer to an acceptable degree of certainty?

Summary answer: Using selected biochemical markers it is possible to build a machine learning model to predict clinical pregnancy.

What is known already: Human chorionic gonadotropin (β-hCG) is considered the main early predictor of pregnancy. Due to the fact that the highest percentage of spontaneous abortions occurs in the first trimester predicting clinical pregnancy outcomes at the early stages of pregnancy is particularly difficult. The level of B-hcg measured at the early stages of implantation and the dynamics of its increase can help predict clinical pregnancy and predict its further development. However, the specific cut-off points at which pregnancy can be determined vary from study to study, and a different factor is often suggested to increase the certainty of β-hCG-based predictions.

Study design, size, duration: 1474 single frozen embryo transfers (1205 patients between 23 and 47 years of age), January 2019 -December 2022 were used for data modelling and statistical testing. The dataset consisted of endocrinal markers:β-hCG, PRG (progesterone), E (estradiol) measured on the 6th day after ET and other practitioner-chosen data: age, PGD results, embryo quality status, RIF or RPL history, embryo maturation days as well as pregnancy outcomes. Exclusion criteria were: PRG > 50ng/ml, β-hCG >70 mIU/ml.

Participants/materials, setting, methods: A machine learning model for predicting clinical pregnancy was trained using the gradient boosting technique. The model used 100 decision trees with 8 leaves, maximal depth of 2 nodes and a learning rate of 0.1. The performance of the model was measured using the accuracy score metric and area under ROC (AUC). Feature importance was based on Shapley values. Additional analysis was performed using statistical testing using a two-sided Student's t-test.

Main results and the role of chance: A Machine Learning naive model employed chosen variables to predict clinical pregnancy. The most important variables turned out to be the serum level of β-hCG and PRG on day 6 after embryo-transfer. The final model, limited to those variables, performs with an accuracy of around 83 % (accuracy score = 0.834), the area under ROC (AUC) was 0.927. Based on Shapley values, β-hCG followed by PRG are the most relevant for the model's predictions. Low PRG concentrations increased the chances of clinical pregnancy. The findings were confirmed by additional statistical analysis. Observations were divided into two groups for which the cut-off point was the level of PRG of 24 ng/ml – the median for the whole group. The probability of clinical pregnancy is higher in the group where the serum PRG is below 24 ng/ml even if β-hCG levels are the same. A statistically significant difference between these groups regarding β-hCG (P value < 0.001) was noticed. In order for serum β-hCG to be used as a more accurate clinical pregnancy predictor its level should be interpreted in the context of PRG serum levels.

Limitations, reasons for caution: Since in the dataset, only data regarding frozen embryo transfers were included, fresh ETs need to be explored further. The model was based on endocrinal markers as measured on day six after ET hence it cannot be used to interpret those markers as measured on any other date.

Wider implications of the findings: In order to most accurately inform patients regarding the possible outcomes of embryo-transfers, practitioners should not limit themselves to monitoring β-hCG serum levels but also include PRG as an important prognostic of clinical pregnancy.

Trial registration number: Not Applicable

INVITED SESSION
SESSION 28: THE ROLE OF ENDOCRINE DISRUPTOR CHEMICALS IN REPRODUCTION

Monday 26 June 2023 Hall D3 17:00 - 18:00

Abstract citation ID: dead093.109
O-090 Endocrine disruptors

J. Flaws[1]

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Female mammals, including women, are exposed to endocrine disrupting chemicals (EDCs) on a daily basis. This is of concern because many EDCs inhibit ovarian follicle growth and steroidogenesis, processes that are critical for maintaining female fertility. Our research focuses on the effects of EDCs known as phthalates on female fertility. Phthalates are of interest because they are ubiquitous synthetic chemicals used as plasticizers and stabilizers in a myriad of consumer products, including everything from shower curtains to children's toys to cosmetics and personal care products such as perfumes, nail polish, deodorants, and lotions. Phthalates are also used in pesticides, wood finishes, adhesives, solvents, lubricants, defoaming agents, and in medical devices including tubing, blood bags, surgical gloves, and dialysis equipment. Despite the widespread use of phthalates and ubiquitous human exposure, limited information is available about the effects of environmentally relevant phthalate mixtures on female reproduction. Thus, we tested the hypothesis that prenatal exposure to an environmentally relevant phthalate mixture affects female reproduction in the F1, F2, and F3 generations of mice. To test this hypothesis, pregnant CD-1 mice were orally dosed with vehicle control (corn oil) or a phthalate mixture (20μg/kg/day-500mg/kg/day) daily from gestational day 10 to birth. The mixture was based on urinary phthalate metabolite levels measured pregnant women in the United States, and consisted of 35% diethyl phthalate, 21% di(2-ethylhexyl) phthalate, 15% dibutyl phthalate, 15% di-isononyl phthalate, 8% diisobutyl phthalate, and 5% benzylbutyl phthalate. Adult F1 females born to these dams were used to create the F2 generation by mating them with unexposed males and F2 females were used to create the F3 generation by mating them with unexposed males. At selected times, estrous cyclicity and fertility indices were monitored and ovaries and sera were collected for analysis. Our data indicate that prenatal exposure to the phthalate mixture significantly decreases anogenital distance, testosterone levels, and litter size, but it significantly increases abnormal estrous cyclicity, uterine weight, the number of cystic ovaries, follicle-stimulating hormone levels, and luteinizing hormone levels compared to control in the F1 generation. Further, our data indicate that prenatal exposure to the phthalate mixture increases body weight, uterine weight, the number of cystic ovaries, and time to pregnancy and it decreases testosterone levels compared to control in the F2 generation. In addition, our data indicate that prenatal exposure to the phthalate mixture increases body weight, uterine weight, the number of cystic ovaries, and abnormal cyclicity and it decreases anogenital distance and the number of live pups compared to control in the F3 generation. Collectively, these data suggest that prenatal exposure to an environmentally relevant mixture of phthalates adversely affects reproductive

function in a multi- and transgenerational manner in female mice. These findings have increased our understanding of the mechanisms by which EDCs cause female reproductive toxicity. This information eventually may lead to the development of novel targets for the prevention or treatment of infertility induced by EDCs.

Abstract citation ID: dead093.110
O-091 The impact of endocrine disruptors on early pregnancy and clinical outcomes in IVF

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The impact of endocrine disruptors on early pregnancy and clinical outcomes in IVF

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Since more than 40 years, there are annual increasing of studies, meta-analysis reporting and discussing the potential associations between endocrine disrupting chemicals (EDCs) and human fertility potential declining. Today it's accepted that a substantial number of environmental and exposition factors affect the fertility and fecundity capacity of couples during the peri and post conception period. It's accepted that most of EDCs interfere with or mimic steroid hormone action; predominantly by affecting estrogen, androgen and thyroid hormones signaling pathways and disturbing specific molecular process on relation with single or multiple biological functions.

Some of EDCs can interact with the male and female reproductive system and lead to endocrine disruption in the testis and ovary. they exert their effects mainly via binding transcription factor receptors, EDCs can alter endocrine function through a variety of mechanisms. At fertility age EDCs may alter the expression and/or activity of enzymes required for synthesis and/or catabolism of testicular and ovarian sex steroids and the expression of hormone receptors and/or their ability to bind their and endogenous ligands.

For example, in male, the literature reported a negative correlation between disrupted spermatogenesis and lifestyle factors (environmental-professional expositions) such as alcohol consumption, cigarette smoking, drug use, and obesity caused by high-energy diet. In female infertility diseases, the negative impact of chemicals ED has been studied in animals. Many disorders have been described, such as low ovarian weight, impaired folliculogenesis, a high aneuploidy rate and the acceleration of follicular atresia. Indeed, women exposed to some endocrine-disrupting pesticides (such as atrazine, lindane) have an elevated risk of long menstrual cycles or anovulation but the most serious consequence is POF and endometriosis pathophysiology's.

In our IVF experience in Picardie region (France), we observed that various pesticides with an endocrine-disrupting action are associated with poor oocyte quality (maturation and competency), embryonic defects and poor IVF outcomes, and some pesticide compounds are linked to specific causes of female infertility, such as premature ovarian insufficiency, polycystic ovarian syndrome, and endometriosis. It was reported that EDCs can reduce embryo implantation chances and increase miscarriage, placenta and post-natal abnormalities. for endometrium receptivity and implantation failure EDCs can play by modifying keys elements of the immune response relevant to pregnancy, and disrupt immune tolerance required for robust placentation, optimal fetal development and be a as contributing risk factors in recurrent miscarriage, preeclampsia, preterm birth and related pathologic gestation

Environmental insults, including endocrine disrupting chemicals during critical periods of fetal development, can alter DNA methylation patterns, leading to inappropriate developmental gene expression and disease risks. Similar to environmental factors, endocrine-disrupting chemicals can influence gene expression without modifying the DNA sequence. It is commonly accepted that the transgenerational inheritance of parentally acquired traits is conveyed by epigenetic alterations risks also known as "epimutations".

In Conclusion, the negative impact of exposure to various endocrine-disrupting is becoming now a worldwide public health. Indeed, the community should be informed about the fertility decline, low ongoing pregnancy rates, and elevated risk of miscarriage associated with exposure to high doses of pesticides for example. We must keep in mind that humans are exposed to EDCs mixtures composed of hundreds of chemicals every day and not a single chemical in isolation.

INVITED SESSION

SESSION 29: LET'S TALK ABOUT THE CROSS-TALK!

Monday 26 June 2023 Hall D1 17:00 - 18:00

Abstract citation ID: dead093.111
O-092 Does embryo biosensing really determine endometrial fate decisions at implantation?

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Implantation depends on bi-directional communication between the blastocyst and endometrium. Encoded in this communication is information on maternal and embryonic fitness, which in turn can lead to the activation of species-specific reproductive suppression mechanisms. For example, blastocysts in over 130 mammals, although not humans, respond to the presence or absence of specific endocrine or endometrial cues by entering or exiting diapause, a state of suspended animation that leads to postponement of implantation. By contrast, embryo biosensing and selection depend on the endometrium first receiving and decoding fitness information from the conceptus and then either promoting implantation or initiating rapid tissue breakdown, thus limiting maternal investment into a failing pregnancy. Importantly, embryo selection relies on physiological mechanisms that sense deleterious (internal/external) cues, which is fundamentally different from reproductive failure caused by disease or trauma. Nevertheless, physiological embryo selection is easily conflated with pathology. For example, repeated implantation failure of low-fitness IVF embryos can lead to the diagnosis of 'recurrent implantation failure', an ill-defined clinical label, which often spurs uninformative investigations and ineffective ad-hoc treatments. On the other hand, pathological relaxation of embryo selection at implantation causes early pregnancy loss, with the frequency of affected cycles determining the age-independent recurrence risk of miscarriage. In this presentation, I will elaborate on the cellular mechanisms that control embryo biosensing and selection, highlight the clinical consequences, and discuss emerging strategies for pre-pregnancy optimisation of the endometrium.

Trial registration number: XXXX

Abstract citation ID: dead093.112
O-093 Endometrium-blastocyst cross talk: how far are we from understanding?

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The early events characterising implantation of the human embryo represent a critical step in which proper activation of orchestrated molecular pathways regulate the success of pregnancy. Understanding this complex network is considered by many the last frontier of human reproduction. Although outstanding research advancements have provided clinicians tools to select the best quality embryo, identification of the optimal timing in which the endometrium is ready to accommodate it remains a challenge. Over the last decade several studies have identified many key players involved in the preparation of the endometrium to implantation and several diffusible signals guiding the embryo in its journey toward the maternal endometrial tissue have been uncovered (Massimiani et al. 2020). However, pieces of the puzzle are still missing.

For convenience, the process of implantation is subdivided into several sequential processes, the completion of one being propaedeutic to the following. Once the blastocyst has arrived in close proximity to the uterine epithelium, the first event occurring is its escape from the zona pellucida. The molecular basis of blastocyst hatching remains poorly understood, and species-specific differences have been reported. Using the mouse model, we recently demonstrated that thyroid hormone up-regulates the expression of specific lytic enzymes (Isp1 and Isp2) known mediators of mouse embryo zona lysis. Interestingly, we observed that TH-mediated upregulation of Isp1 and Isp2 is strictly dependent on the presence of endometrial stromal cell feeder layer, clearly indicating that blastocyst–endometrium interaction is indispensable for TH-mediated increased expression of these proteases. So far, no molecular signals regulating hatching in humans have been identified, and only mechanical forces have been proposed to induce zona opening; however very recently the possibility that proteases may be involved also in humans has been suggested (Almagor et al., 2020), although further studies are needed to support this hypothesis.

Once free from the zona, the embryo contacts the endometrial epithelium and starts its journey to further proceed in the consolidation of pregnancy. Hormones, pro- and anti-inflammatory mediators, growth factors and their receptors, adhesion molecules, and proteases and protease inhibitors have been reported in the regulation of the process of embryo apposition, adhesion, and invasion (Massimiani et al., 2020). Among the many molecular signals, the Notch pathway has been recognized key in embryo-endometrium cross talk (Cuman et al., 2014). Transient activation of the Notch1 receptor has been demonstrated in the process of decidualization (Afshar et al., 2012), a limiting step to implantation, and blastocyst-conditioned medium has been shown to induce the expression of Notch family members in decidual cells (Hess et al., 2007). Expression of notch receptors and ligands has been reported in the blastocyst trophectoderm as well (Cuman et al., 2014). We previously demonstrated that the secreted factor epidermal growth factor-like domain 7 (EGFL7) is a novel modulator of the Notch pathway, it is expressed by trophoblast cells of the mouse blastocyst (Fitch et al., 2004; Lacko et al., 2014) and it regulates human trophoblast migration and invasion ability by activating the Notch pathway (Massimiani et al., 2015). Data will be presented demonstrating that EGFL7 is expressed in the human endometrium and its expression is mainly localized in the glandular epithelium throughout the menstrual cycle. EGFL7 is significantly upregulated in whole endometrial tissue during the secretory phase, particularly in the stromal compartment; this observation is further supported by in vitro studies showing its upregulation in decidualized endometrial stromal cells, suggesting a role for EGFL7 in implantation. Further support is provided by the evidence that its levels are strongly downregulated in the endometrium of women with impaired fertility. We propose EGFL7 as a novel player involved in the embryo-endometrium cross talk.

INVITED SESSION

SESSION 30: POLYCYSTIC OVARY SYNDROME: A REVISIT

Monday 26 June 2023 Hall D4 17:00 - 18:00

Abstract citation ID: dead093.113
O-094 Management of subfertility in women with PCOS: expectations, problems and solutions

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[2]*University of New South Wales, Obstetrics and Gynaecology, Sydney, Australia*

Polycystic ovarian syndrome (PCOS) is a common cause of subfertility particularly when anovulation is present. In this circumstance efforts are made to induce ovulation through lifestyle modification, medication, or a combination of both. Other patients with PCOS may be ovulatory but require IVF/ICSI for other causes, including male factor or tubal infertility. In all cases, attention needs to be placed on the underlying metabolic, psychological, and reproductive features that may complicate treatment and subsequent pregnancy. Pregnancy itself has a higher chance of complications and these can be reduced by adequate preconception evaluation and intervention.

Recently, the international evidence-based guideline for evaluation and treatment of PCOS has been updated (1) and the infertility section subjected to a literature integrity check to exclude publications that did not meet the highest criteria for incorporation into clinical management. A recent individual patient data meta-analysis has also clarified the relative efficacy of fertility treatments (2). The following conclusions are recommended;

1. Letrozole is the first line drug of choice for ovulation induction based on its efficacy and lower multiple pregnancy rate
2. Clomiphene is an effective drug particularly given a long experience of use and its lower cost
3. Metformin may be valuable for some patients particularly if glucose intolerance is present
4. A combination of clomiphene and metformin may be effective, particularly in overweight individuals
5. Gonadotrophins are an effective treatment in experienced clinics which the resources to undergo adequate monitoring
6. Laparoscopic ovarian surgery may assist some patients and is associated with a lower multiple pregnancy rate and may have long term benefits
7. IVF may be safely conducted with appropriate safeguards to reduce ovarian hyperstimulation syndrome in those where ovulation induction has failed.

It should be noted that some of these drugs are “off-label” in many countries and are not recommended in several healthcare systems.There are several areas where further information may lead to altered and improved practice:

1. IVF as a first line therapy may be more effective and allow better control of multiple pregnancy rates.
2. In vitro maturation (IVM) with IVF/ICSI shows increasing success with no or minimal FSH use (3).
3. Bariatric surgery is more readily available and may lead to significant weight loss related ovulation.
4. Newer GLP1 receptor agonists show dramatic weight loss and are being evaluated in PCOS.
5. First line gonadotrophin treatment has been advocated by some proponents.
6. Higher dose and longer use of standard oral agents may help in ovulation induction resistant patients.
7. Inositol and myoinositol are being evaluated as safe, low cost, over the counter alternatives.
8. Alternatives to hCG are available for ovulation induction in oral and IVF cycles.

9. Promising pharmacogenomic approaches may allow individualisation of treatment.

While the genomic and environmental aetiology of PCOS remains uncertain, the increasing emphasis on evaluation and assessment of effective therapies for subfertility is advancing rapidly.

References

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2. Wang R et al Hum Reprod Update. 2019 Nov 5;25(6):717-732.
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Abstract citation ID: dead093.114
O-095 Different phenotypes of PCOS: Implications to clinical practice

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Polycystic ovary syndrome (PCOS) is a very common, heterogeneous syndrome which presents with a spectrum of clinical and biochemical features. It is associated with both reproductive and metabolic dysfunction and has a negative impact on long term health, including mental health. Although there appears to be a common genetic background to PCOS, regardless of presentation, environmental factors, including diet, play an important part in determining the phenotype. The most commonly used diagnostic criteria, the Rotterdam criteria, takes account of the heterogenous nature of the syndrome but the spectrum of presentation can be categorised as four clinical and biochemical subtypes which comprise (A) ovulatory dysfunction, hyperandrogenism, polycystic ovaries (PCO), (B) ovulatory dysfunction, hyperandrogenism but without PCO, (C) hyperandrogenism without ovulatory dysfunction but with PCO and (D) ovulatory dysfunction with PCO but without hyperandrogenism. These are not rigid categories since some women may go from one to another, for example weight gain may lead to anovulation in a woman who previously had hirsutism but regular cycles. Nevertheless, the diagnostic sub-category has a significance influence on clinical management.

Women who have both hyperandrogenism and ovulatory dysfunction (A&B) are at higher risk of metabolic dysfunction which is exacerbated by obesity (and carries a high long-term risk of diabetes and cardiovascular disease) and so merit thorough investigation which includes assessment of glucose tolerance and lipids. Those who have predominantly ovulatory problems may require fertility treatment or regulation of menses but some who simply have irregular menses may need no specific treatment. Symptoms of hyperandrogenism (hirsutism, acne, alopecia) can be particularly distressing and may be best managed in collaboration with a dermatologist. The negative impact of PCOS on mental health is a very common problem, the high prevalence of which has only recently been appreciated. Not surprisingly, it is particularly relevant in those women who have moderate to severe symptoms of hyperandrogenism.

In summary, PCOS is a syndrome characterised by a variety of symptoms and endocrine abnormalities and it is important to tailor treatment individually, according to the need for symptom relief and with regard to concerns about long-term health.

SELECTED ORAL COMMUNICATIONS
SESSION 31: PREDICTIVE VALUE OF EMBRYO EVALUATION

Monday 26 June 2023 Auditorium 10-12 17:00 - 18:00

Abstract citation ID: dead093.115
O-096 Effect of Day-3 embryo morphology on blastocyst quality and ploidy: a machine-learning model of 34781 embryos

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Study question: What is the predictive value of using Day-3 (D3) embryo morphology and clinical factors to identify good quality euploid blastocysts?

Summary answer: Using a machine-learning model, D3-cell count and fragmentation helps identifying embryos most and least likely to develop into euploid blastocysts.

What is known already: It is well-known that the pattern of embryo cleavage affects blastocyst ploidy. Less has been elucidated in relation to embryo fragmentation. Published studies often used outdated biopsy techniques (D3-biopsy) and obsolete genetic technologies (FISH). Hence, the association between embryo fragmentation and ploidy status remains controversial. Existing hypothesis is that embryo fragmentation is a regulation event to maintain homeostasis and normalize genetic constitution. Literature is lacking studies investigating this association using newer genetic technologies such as Next Generating Sequencing (NGS). Moreover, a predictive model using D3-embryo characteristics and clinical factors can be useful for triage of laboratory resources.

Study design, size, duration: Retrospective observational single center study including 34781 embryos from 5701 cycles, between March 2017 and March 2020. Embryos were evaluated for degree of fragmentation and cell number on D3, 68 ± 1hrs post insemination. Female Age, AMH and BMI were annotated as patient characteristics. All blastocysts available on D5 or D6 with a quality ≥ BL3CC (n = 18361) were subjected to trophectoderm (TE) biopsy for Preimplantation-Genetic-Testing for aneuploidies (PGT-A) analysis with Next-Generation-Sequencing and ploidy rates were recorded.

Participants/materials, setting, methods: Degree of fragmentation was classified as follows: A(<=10%), B(11-25%), C(26-35%), D(>35%). Data on embryo fragmentation was stratified according to cell number category: <6cells, 6-10 cells, >10 cells and compacted stage. Morphology markers (cell-count and fragmentation rate), along with patient characteristics (age, anti-Mullerian hormone and body-mass index) was modeled using a gradient-boosting decision tree algorithm. Performance of the machine learning (ML) model was assessed with AUC values, calibration curves after partitioning the dataset 1:1 for training/validation.

Main results and the role of chance: Day-3 embryos consisting of < 6 cells (n = 5926), 6-10 cells (n = 23920), and >10 cells (n = 1612) were included, along with 3323 compacted embryos. The rate of good-quality blastocysts decreased with higher fragmentation rates (38.2%, 14.8%, 4.6%, and 1.6%, A, B, C, and D, respectively; P < 0.001). Day of biopsy was earlier for embryos with lower fragmentation rates (Day-5 biopsy: 24.2%, 9.7%, 4.0%, and 0.0%, P < 0.001). Among the biopsied blastocysts, euploidy rates were similar at 44.9%, 43.0%, 42.7%, and 66.7% in groups A vs B, C, and D, respectively (P = 0.075, 0.350 and 0.141). When stratified by cell number, euploidy rates were lowest in blastocysts derived from <6-cells, at 33.5%,

34.3%, 26.9%, and 50.0% in groups A vs B, C, and D, respectively (P = 0.803, 0.185 and 0.394).

The best performance ML model was provided by using fragmentation % and cell count along with female age, BMI and AMH. This resulted in a modest predictive accuracy for predicting euploid embryo development (AUC: 0.72, 95% CI: 0.71 to 0.73). The model was well- calibrated and correctly assessed the probability of D3-embryos developing into euploid blastocyst. For instance, embryos with a predicted probability of ≤ 2.5% rarely developed into euploid blastocyst (actual rate: 2.6%) and model had a predictive value of 97.4%.Limitations, reasons for caution:

The retrospective nature of the study and inter-observer variability in D3-embryo fragmentation scoring is a limitation. Nevertheless, embryologists performing the embryo scoring followed the same SOPs. Findings need to be validated in external cohorts.

Wider implications of the findings: The model can utilize clinical factors and D3-embryo morphology markers to estimate the probability of a D3-embryo developing into an euploid blastocyst. This model can be useful for patient information, mainly when a decision on embryo selection for PGT-A needs to take place.

Trial registration number: not applicable

Abstract citation ID: dead093.116

O-097 Predicting live birth rate using combined Day3-spent embryo culture medium metabolomics and cumulus cell gene expression based algorithm to optimize embryo selection in unexplained infertility

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Study question: Can combination of metabolomics of spent embryo culture medium (sECM) and cumulus cell (CC) genes judge live birth rate in unexplained infertility (UI)?

Summary answer: A combination of day3 metabolic profiling of sECM with CC gene expression yields an algorithm to predict live birth rate in UI.

What is known already: Optimizing embryo selection is rate-limiting for pregnancy success. The metabolomic profile of sECM can aid in advanced prediction of embryonic developmental potential. Recent transcriptomics studies proposed prokinectin 2 (PROK2), and pregnancy up-regulated nonubiquitous CaM kinase (PNCK) as two novel gene/s responsible for perifollicular vascularization and embryonic development respectively; two important events regulating bidirectional CC-oocyte signalling and implantation ability. Given that UI is contributory to impaired oocyte quality, we delineated if combined metabolic signature of CC gene expression encompassing metabolic, developmental competence, and extracellular matrix facet/s and sECM could address live birth rate in women with UI following single embryo transfer.

Study design, size, duration: A prospective cohort study was conducted at Institute of Reproductive Medicine between January 2022 and October 2022. 48 women (age: 30-39 years) diagnosed with UI undergoing oocyte-retrieval offered consented participation and were sub-stratified according to pregnancy outcome. Cleavage-stage embryos from intra cytoplasmic sperm injection (ICSI) cycles were scored according to combination of gene expression pattern of CC-samples and integral value of metabolite obtained from nuclear magnetic resonance (NMR) spectroscopy of sECM (range 0-3, 4-6, 7-9).

Participants/materials, setting, methods: Expression profiles of metabolic (LDHA, PFKP, PKM2), extracellular matrix (HAS2, PTX3, TNFAIP6, VCAN), and developmental competence (PNCK, PROK2) related genes were evaluated by real time-quantitative polymerase-chain-reaction (qRT-PCR) from retrieved CC. Day-3 sECM (30 μL) was collected for NMR from 43 single transferred embryos. Univariate and multivariate analysis was performed after NMR data acquisition. Live birth rate was evaluated by area-under-the-curve (AUC) values using combined score panel.

Main results and the role of chance: The groups were similar for patient age, BMI, AMH, infertility diagnosis, and stimulation protocol. A total of 43 cycles were included in the analysis [embryo transfer cancelled (n = 2), insufficient cumulus mass (n = 3)]. Clinical pregnancy was confirmed in 16 patients (37.21%). Mean mRNA levels for all genes were similar in CC associated with oocyte that fertilized normally (2PN) compared with those that either failed to fertilize or were abnormal (i.e. 1PN, 3PN). Similar results were observed in terms of speed of embryonic cleavage (≥7 cell on Day 3 vs. ≤6 cell on Day 3). Expression of VCAN, (OR: 1.102, 95% CI: 1.010-1.202, $p < 0.02$), PNCK (OR: 0.931, 95% CI: 0.916-0.947, $p < 0.001$) and PROK2 (OR: 1.273, 95% CI: 1.011-1.231, $p < 0.001$) was significantly higher ($p < 0.01$) in CC that achieved live birth compared with those that failed to result a successful pregnancy. Multivariate and univariate analysis revealed distinct metabolomic signatures between pregnant and non- pregnant group/s. Lactate, pyruvate and proline were the most significant ($p < 0.01$) altered metabolite in sECM of non-pregnant group when compared to their pregnant counterpart. The combination demonstrated a "good" (VCAN, pyruvate: AUC: 0.87), "fair" (PROK2, proline: AUC: 0.79) and "modest" (PNCK, glucose: AUC: 0.71) in terms of live-birth rate.

Limitations, reasons for caution: The study population is heterogeneous in terms of duration of infertility and previous infertility treatment outcome, limited by small sample size and subjective nature of embryo scoring. The presumable involvement of unknown factors in developmental competence of UI and impact of frozen cycle/s should also be addressed.

Wider implications of the findings: The study provides reliable accuracy measures to support relationship between live birth rate and combined score panel in patients with UI. It is envisioned integration of CC gene expression/s and metabolomics of sECM if combined with patient characteristics may tailor therapy by artificial intelligence to model the outcome in UI.

Trial registration number: Not applicable

Abstract citation ID: dead093.117

O-098 Improved pregnancy rates following day 5 eSET with early hatching/hatched blastocysts (116-118 hours) compared to expanded blastocysts in a strictly selected cohort: a prospective study

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Study question: To investigate whether blastocysts hatching early on day 5 following IVF are developmentally superior and lead to better clinical outcomes in non PGT-A eSET cycles.

Summary answer: Fresh eSET on day 5 with spontaneously early hatching blastocysts (116-118 hrs) results in significantly better pregnancy rates compared to top quality expanded blastocyst transfers.

What is known already: Most studies seem to indicate that transfer of blastocysts at more advanced stages of development result in higher pregnancy rates. However, most studies comparing results between full/expanded vs hatching/hatched blastocyst transfers are either retrospective, have relaxed inclusion criteria, or perform transfers following assisted hatching or extended culture. Moreover, there appears to be no data on proportion of blastocysts

that spontaneously hatch early on day 5, or how well they perform comparatively. Yet it has been reported that earlier time-points of blastocyst development are associated with higher euploidy. We felt the need for a well-designed prospective study to address these questions.

Study design, size, duration: This prospective study to compare pregnancy rates between early hatching/hatched blastocysts and top quality expanded blastocysts (4 BB or higher) in fresh, non PGT-A eSET cycles took place between October 2021 and October 2022 following institutional ethical clearance. 115 patients<37 years with AMH>1.5 undergoing 1st IVF cycles for tubal, mild male factor, unexplained infertility consented for the study and 94 patients completed it to eSET. Patients with PCOS, endometriosis or previous pelvic surgery were excluded.

Participants/materials, setting, methods: The study was conducted at a tertiary IVF centre. All patients underwent antagonist cycles, egg retrievals and IVF as per standard protocol. Blastocyst culture decision was taken at 2-PN examination. Embryos were examined at 116-118 hours as per our practise and decisions made to exclude from data analysis patients without at least one blastocyst at 4BB or above. eSETs performed at 120-122 hours with hatching/hatched blastocysts where available, expanded blastocysts in others. Luteal support initiated.

Main results and the role of chance: Demographic parameters of the patient cohort such as age (30.79 ± 4.06), BMI (25.53 ± 3.11) and AMH (3.23 ± 1.68) are presented as mean ± SD. Data was analysed using IBM SPSS V.26 statistical package. Differences in pregnancy rates between early hatching/hatched vs expanded blastocyst eSET groups were tested using Z test for proportions at 5% level of significance. Data was analysed for 94 patients of whom 62 had single expanded blastocyst transfers (22 achieved pregnancies) and 32 had eSET with early hatching/hatched blastocysts (of whom 18 became pregnant). 55 out of a total 261 blastocysts in the whole cohort were found to have initiated spontaneous hatching (n = 39) or fully hatched (n = 16) at 116-118 hours post-fertilisation resulting in an early hatching rate of 21.1% per blastocyst. Clinical pregnancy rate (CPR) defined as fetal heartbeat on ultrasound at 6 weeks of gestation and ongoing pregnancy rate (OPR) defined as pregnancy continuing beyond 10 weeks were found to be significantly superior in early hatching/hatched group vs expanded blastocyst group (CPR: 56.3% vs 35.5%, p = 0.002; OPR: 50% vs 30.6%, p = 0.003). Difference between miscarriage rate in the hatching/hatched group (11.1%, n = 2) and expanded blastocyst group (13.6%, n = 3) was not statistically significant.

Limitations, reasons for caution: Our sample size was relatively small in order to rigorously select a homogeneous group of good prognosis patients and adhere to strict study criteria- only 1st cycle, autologous, fresh, non PGT-A, day 5 eSET cycles were included. Larger controlled prospective studies would be valuable to confirm these findings.

Wider implications of the findings: To our knowledge this is the first report of improved pregnancy rates with early hatching blastocyst eSETs compared with expanded blastocysts. Basic science studies may shed more light on the biological underpinnings of these findings. Such transfer strategy may also prove useful to select the best blastocysts in non-PGT-A cycles.

Trial registration number: not applicable

Abstract citation ID: dead093.118

O-099 The inner cell mass (ICM) regions can be used to predict pregnancy with a similar accuracy as the original images of the entire embryo

H.M. Kim[1], T. Ko[2], J.Y. Jung[3], J.H. Park[3], J. Koo[4], S.H. Min[4], M.K. Chung[5], J. Kim[6], H. Lee[7]

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Study question: Can clinical pregnancy be accurately predicted using only ICM and TE regions in an embryo microscopy image?

Summary answer: The AI model accurately predicted pregnancy using ICM segmented images with a similar accuracy as using original embryo images.

What is known already: Previous research suggests that ICM and TE are key factors in determining embryo grade and clinical pregnancy. Grad-CAM images on the CNN models in various literature also highlight the significance of ICM and TE regions. However, there have been no studies to compare and assess the importance of the two regions in pregnancy prediction. This study aims to test the significance of each region by evaluating the performance of models after segmenting ICM and TE regions.

Study design, size, duration: We performed a retrospective study of single static images of 2,555 Day 5 blastocysts from seven in vitro fertilization (IVF) clinics between June 2011 and May 2022. The images were collected from standard optical light microscopes and matched with metadata such as pregnancy outcomes. ICM and TE regions were manually labeled and examined by 5 embryologists with over 10 years of experience.

Participants/materials, setting, methods: We defined a positive pregnancy indication as the presence of a gestational sac (G-SAC). We built 4 CNN models using original embryo images ("Original model"), ICM segmented images ("ICM model"), TE segmented images ("TE model") and ICM & TE segmented images ("ICM & TE model"). The performances were measured through 3-fold cross validation.

Main results and the role of chance: In 3-fold cross validation, the mean and the standard deviation of AUROCs were 0.753 ± 0.005 for Original model, 0.733 ± 0.003 for ICM model, 0.724 ± 0.016 for ICM & TE model and 0.690 ± 0.011 for TE model, respectively. The DeLong test showed significant differences between the AUCs of Original model and ICM model (p = 0.01), and ICM model and ICM & TE model (p = 0.0003). Positive correlations were found between prediction values with pearson correlation [Confidence interval] for Original image and ICM model (0.426 [0.343, 0.495]), Original model and ICM & TE model (0.345 [0.266, 0.419]) and Original model and TE model (0.223 [0.140, 0.304]). Original model showed the highest accuracy and TE model was the least accurate as expected. However, it is noteworthy that ICM model outperformed ICM & TE model. Although it is well known that TE plays a significant role in implantation, forcing the model to utilize both ICM and TE to predict pregnancy could have muddled the analysis. This study proved that pregnancy can be successfully predicted using ICM regions only as well as original embryo images.

Limitations, reasons for caution: This study has limitations due to its retrospective nature, using embryo images from seven IVF centers. Further study with a larger dataset is warranted to offset the differences in focus, magnification and color of embryo images.

Wider implications of the findings: We showed that the AI model using ICM segmented images predicted pregnancy with a similarly high accuracy as using original embryo images. However, the model using TE segmented images showed the lowest performances. Utilizing well-focused ICM images may enhance the performance of pregnancy prediction models.

Trial registration number: not applicable

INVITED SSESSION

SESSION 32: GUIDELINES SESSION: CHALLENGES IN ART, CAN ESHRE GOOD PRACTICE ADVISE BE OF ANY HELP?

Monday 26 June 2023 Hall D5 17:00 - 18:00

O-100 Recurrent implantation failure

N. Macklon[1], D. Cimadomo[2], MJ. de los Santos Molina[3], G. Griesinger[4], G. Lainas[5], N. Le Clef[6], DJ. McLernon[7], D. Montjean[8], B Toth[9], N. Vermeulen[6]

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[9]Gynecological Endocrinology and Reproductive Medicine, Medical University Innsbruck, Innsbruck, Austria

Study question: How should Recurrent Implantation Failure (RIF) in ART patients be defined and managed?

Summary answer: This is the first ESHRE good practice recommendations paper providing a definition for RIF and guidance on how to investigate causes and contributing factors and how to improve the chances of a pregnancy.

What is known already: RIF is a challenge in the ART Clinic, with a multitude of investigations and interventions offered and applied in clinical practice, often without biological rational or unequivocal evidence of benefit.

Study design, size, duration: This recommendations document was developed according to a predefined methodology for ESHRE good practice recommendations. Recommendations are supported by data from the literature, if available, the results of a previously published survey on clinical practice in RIF and the expertise of the working group. A literature search was performed in PubMed and Cochrane focusing on "recurrent reproductive failure", "recurrent implantation failure" and "repeated implantation failure".

Participants/materials, setting, methods: The ESHRE RIF Working Group included 8 members representing the ESHRE Special Interest Groups of Implantation and Early Pregnancy, Reproductive Endocrinology, and Embryology, and completed with an independent chair and an expert in statistics. The recommendations for clinical practice were formulated based on the expert opinion of the Working Group, while taking into consideration the published data and results of the survey on uptake in clinical practice. The draft document was then opened for online peer review to ESHRE members and revised in light of the comments received.

Main results and the role of chance: RIF describes the scenario in which the transfer of embryos considered to be viable has failed to result in a positive pregnancy test sufficiently often in a specific patient to warrant consideration of further investigations and/or interventions. The recommended threshold for the cumulative predicted chance of implantation to identify RIF for the purposes of initiating further investigation is 60%. When a couple have not had a successful implantation by a certain number of embryo transfers and the cumulative predicted chance of implantation associated with that number is greater than 60%, then they should be counselled on further investigation and/or treatment options. This term defines clinical RIF for which further actions should be considered. Nineteen recommendations were formulated on investigations when RIF is suspected, and 13 on interventions.

Limitations, reason for caution: While awaiting the results of further studies and trials, the ESHRE Working group recommends identifying of RIF based on the chance of successful implantation for the individual patient or couple and to restrict investigations and treatments to those supported by a clear rationale and data indicating their likely benefit.

Wider implications of the findings: This paper provides good practice advise, but also highlights the investigations and interventions that need further research. This research, when well-conducted, will be key to making progress in the clinical management of RIF.

Study funding and competing interest(s): Yes.

The other authors had nothing to disclose.

Abstract citation ID: dead093.120

O-101 Add-ons in the ART lab

K. Lundin[1], J. Bentzen[2], G. Bozdag[3], T. Ebner[4], J. Harper[5], N. Le Clef[6], A. Moffett[7], S. Norcross[8], Polyzos NP[9], S. Rautakallio-Hokkanen[10], I. Sfontouris[11], K. Sermon[12], N. Vermeulen[6], A. Pinborg[13]

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[12]Brussels University, Research Group Reproduction and Genetics, Brussels, Belgium
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Study question: Which laboratory based add-ons are effective and safe to use in ART treatment?

Summary answer: In total, more than thirty recommendations were formulated on the use of add-ons, with about a third being laboratory based tests or interventions

What is known already: Treatment add-ons are defined as not essential for the treatment, but optional additional treatments that are sometimes offered on top of routine fertility treatment. There are a wide range of treatment add-ons available, including diagnostic tests, drugs, equipment, holistic or alternative therapies, laboratory and surgical interventions, all claiming to potentiate the effect of the treatment and/or to lessen side effects, improve pregnancy or live birth rate, reduce the risk of miscarriage or shorten the time to pregnancy. Add-ons and can be at extra cost (or not), and evidence on efficacy and safety is often missing. Presumably they can be both beneficial or non-beneficial, as there may be absence of evidence to either.

Study design, size, duration: The ESHRE working group for good practice recommendations on add-ons in ART has produced recommendations useful for professionals, patients and policy makers. More than 30 add-ons were identified, and evaluated for efficiency and safety, and wherever possible also for cost. The literature was identified in a systematic search, reviewed and critically appraised. In the absence of any clear scientific evidence, judgement was based on the professional experience and consensus of the multidisciplinary development group. The guidelines are thus based on the best available evidence and expert agreement. Prior to publication, the guidelines were reviewed by independent international reviewers.

Participants/materials, setting, methods: Interventions were characterized in three sections: 1) Diagnosis and diagnostic tests, 2) Laboratory tests and intervention, and 3) Clinical management. Outcomes were efficacy (pregnancy or live birth rate), risk of miscarriage, time to pregnancy, cost, and safety/risk aspects. Some examples of add-ons in the group of laboratory tests and interventions are oocyte and sperm activation, mitochondria load

and transfer, sperm selection, assisted hatching, genetic testing and addition of hyaluronic acid to media.

Main results and the role of chance: The guideline development group formulated more than 30 recommendations. This lecture will focus on the rationale and the recommendations of the laboratory add-ons currently used in fertility clinics. We will discuss the potential beneficial effects on the IVF treatment, its efficacy, and its safety. While most add-ons are not recommended for the general population of ART patients, where specific patient populations might benefit, indications were formulated.

The guideline has been in stakeholder review and is prepared for submission in 2023.

Limitations, reason for caution: Of the recommendations, none are based on high quality evidence and only a few are based on moderate quality evidence. Hence 90% of the recommendations are supported only by low quality RCTs, observational data, professional experience, or consensus of the development group.

Wider implications of the findings: These recommendations aim to provide guidance to health care professionals and laboratory experts in the field of reproductive medicine, to help towards safe and efficient ART treatments for patients where they can participate in informed decision making with realistic expectations and be fully informed about their chances to pregnancy and live birth.

Study funding and competing interest(s): All costs relating to the development process were covered from ESHRE funds. There was no external funding of the development process or manuscript production.

Abstract citation ID: dead093.121
O-102 Add-ons in the ART clinic

A. Pinborg[1], J. Bentzen[2], G. Bozdag[3], T. Ebner[4], J. Harper[5], N. Le Clef[6], A. Moffett[7], S. Norcross[8], Polyzos NP[9], S. Rautakallio-Hokkanen[10], I. Sfontouris[11], K. Sermon[12], N. Vermeulen[6], K. Lundin[13]

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Study question: Which add-ons are effective and safe to use in the ART clinic?

Summary answer: More than thirty recommendations were formulated on the use of add-ons in diagnosis and diagnostic tests, laboratory tests and intervention, and clinical management.

What is known already: Treatment add-ons are not essential for the treatment but optional additional treatments that are sometimes offered on top of routine fertility treatment. There are a wide range of treatment add-ons on offer including tests, drugs, equipment, holistic or alternative therapies, laboratory and surgical interventions, but consistently, they aim to potentiate the effect of treatment or to lessen side effects herein improve pregnancy or live birth rate, reduce the risk of miscarriage or shorten the time to pregnancy. Evidence on whether some treatment add-ons are effective and safe is often missing or absent. Add-ons can be proven or evidence-based (or not) and can be at extra cost (or not), presumable they can be both beneficial and non-beneficial, as there may be absence of evidence to either.

Study design, size, duration: The ESHRE working group for good practice recommendations on add-ons in ART has produced recommendations useful for professionals, patients and policy makers and identified more than 30 add-ons and evaluated each one of them for efficiency and safety and wherever possible also costs. The literature was identified in a systematic search, was reviewed and critically appraised. In the absence of any clear scientific evidence, judgement was based on the professional experience and consensus of the development group. The guidelines are thus based on the best available evidence and expert agreement. Prior to publication, the guidelines were reviewed by independent international reviewers.

Participants/materials, setting, methods: Interventions were characterized in three sections: 1) Diagnosis and diagnostic tests, 2) Laboratory tests and intervention, and 3) Clinical management. Outcomes were efficacy (pregnancy or live birth rate), risk of miscarriage, time to pregnancy, costs, and safety/risk aspects. This lecture will focus on the recommendations relevant for the ART clinic including endometrial receptivity tests, assisted hatching, freeze-all (elective), ICSI (elective), uterine flushing and endometrial scratching.

Main results and the role of chance: The multidisciplinary development group formulated more than 30 recommendations. This lecture provides a summary of the recommendations of the add-ons currently used in fertility clinics. First it is described how an add-on is hypothesized to have beneficial effects on the IVF treatment, its efficacy, and its safety. While most add-ons are not recommended for the general population of ART patients, indications were formulated where specific patient populations might benefit.

The guideline has been in stakeholder review and is prepared for submission in 2023.

Limitations, reason for caution: Of the recommendations, none are based on high quality evidence and only a few are based on moderate quality evidence. Hence 90% of the recommendations are supported only by low quality RCTs, observational data, professional experience, or consensus of the development group.

Wider implications of the findings: These recommendations provide guidance to health care professionals and laboratory experts in the field of reproductive medicine, and will help towards safe and efficient ART treatment, where patients can participate in informed decision making with realistic expectations and are fully informed about their changes to pregnancy and live birth.

Study funding and competing interest(s): All costs relating to the development process were covered from ESHRE funds. There was no external funding of the development process or manuscript production.

The authors have stated their conflicts of interest if any.

POSTER DISCUSSION SESSION
SESSION 33: PSYCHOLOGY & COUNSELLING
Monday 26 June 2023 Hall D2 17:00 - 18:00

Abstract citation ID: dead093.122
P-563 Efficacy of a brief decisional support intervention on the decision conflict and psychosocial aspects among women with IVF failure: a randomized controlled trial

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Study question: Is decisional support intervention (DSI) efficacious in reducing decisional conflict and improving mental health for women experiencing unsuccessful IVF treatment?

Summary answer: Compared to controls, DSI statistically significantly increased informed decision but not depression symptoms.

What is known already: Failure of IVF treatment could be an unavoidable event and a situation of low control for infertile women. The decisional conflict following unsuccessful IVF treatment could be extremely intense considering the critical issues such as the life goal of childbearing, relationships, finance, etc. Meanwhile, with great importance placed on childbearing, many patients are indulged in the treatment cycles while hesitant to consider ending the treatment or the alternatives, leading to a series of psychosocial problems. Therefore, a decisional support intervention should be considered to facilitate the decision-making and adjustment after unsuccessful IVF treatment.

Study design, size, duration: A multi-centers, two-armed, randomized controlled trial, to assess the efficacy of a decisional support intervention on decisional conflict and psychosocial aspects among women who experienced failure of IVF treatment. Between May 2022 to January 2022, a total of 80 participants were randomly allocated to the DSI group or waitlist control group.

Participants/materials, setting, methods: Eligible participants were women who have undergone at least one cycle of IVF treatment and failed to achieve clinical pregnancy into treatment. DSI participants received a one-hour decisional support counseling, which focused on the discussion about the pros and cons in relation to continuation and termination of treatment, family building alternatives as well as their implications. The participants completed questionnaires before treatment (T0), and after the treatment (T1). Analyses included paired sample t-tests and ANOVA.

Main results and the role of chance: 40 subjects in the intervention group and 38 subjects in the control group were included in the intention-to-treat analysis. The between-group effect analysis showed a significant time x group interaction in informed decision ($p < 0.05$), meaning that informed decision increased in the intervention group but decreased in the control group over time. There was also a marginally significant increase in perceived behavioral control ($p = 0.06$), as well as a decrease in decisional conflict of uncertainty ($p = 0.08$) and anxiety symptoms ($p = 0.08$).

Limitations, reasons for caution: Considering the decision might be navigated by the treatment outcome, this study only measured the short-term effect after the intervention. Secondly, this study only enrolled women, who were more vulnerable to the risk of treatment failures and take a more active role in decision-making, compared to men.

Wider implications of the findings: Instead of putting efforts on psychosocial intervention aiming at improving the chance of pregnancy, this study provides an efficacious brief counseling approach, tapping on patients' existing cultural values and beliefs, to help patients 'find the way out' following the IVF treatment failures.

Trial registration number: ChiCTR2200060238

Abstract citation ID: dead093.123

P-577 Understanding how healthcare professionals address the educational and emotional needs of patients seeking fertility care: an assessment of challenges and barriers across eight countries

M. Augustyniak[1], M. Kupka[2], G. Coticchio[3], S. Esteves[4], A. Fincham[5], P. Lazure[1], S. Péloquin[1]

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[3]*9.baby GeneraLife IVF, Department of Science, Bologna, Italy*
[4]*ANDROFERT and University of Campinas UNICAMP, Andrology and Human Reproduction Clinic, Campinas, Brazil*
[5]*Fertility Europe, Office Management Division, Brussels, Belgium*

Study question: What are the current challenges and barriers preventing healthcare professionals (HCPs) from optimally addressing patients' educational and emotional needs in fertility care?

Summary answer: HCPs lack optimal knowledge of patients' needs/preferences for educational and emotional support, have sub-optimal skills addressing them, and underutilize external resources (e.g., psychologists).

What is known already: Couples who have difficulty conceiving a child can face a turmoil of emotions, including anxiety and distress, especially when external social pressures are at play. All patients seeking fertility care deserve their educational and emotional needs to be heard and addressed, as advocated by The European Society of Human Reproduction and Embryology (ESHRE) guidelines. Nevertheless, a 2015 educational and behavioural needs assessment found that HCPs involved in reproductive medicine perceive their skills to identify the needs of patients for psychological and emotional support to be less than optimal, especially when discussing sensitive or distressing issues.

Study design, size, duration: This exploratory mixed-methods study triangulated data from a qualitative phase (76 interviews, June-August 2021) and a quantitative phase (323 surveys, November 2021). Participants included patients (qualitative phase, n = 28), physicians (both phases, n = 249) and laboratory specialists (both phases, n = 122) using assisted reproductive technologies (ART).

Participants/materials, setting, methods: Countries included Brazil, China, France, Germany, Italy, Mexico, Spain, and UK. Interviews were recorded, transcribed and coded. Thematic analyses were performed. Surveys assessed knowledge, skill, agreement, and task performance frequency using 5-point scales. Knowledge and skill ratings of none (1), basic (2) or intermediate (3), as opposed to advanced (4) or expert (5), were considered a gap. Descriptive analysis and crosstabulations with chi-square tests were performed to explore variations by country and professional role/specialty area.

Main results and the role of chance: Patient interviews indicated a need to improve HCPs' ability to set realistic yet encouraging, expectations about conception outcomes. Underlying barriers to fulfilling this need included a lack of optimal knowledge amongst HCPs regarding patients' preferences, skills addressing their needs, and an under-utilization of resources for psychological support. Survey results indicated 31-36% of physicians and laboratory specialists had a knowledge gap of patients' preferences and needs for education and emotional support; 11-39% of physicians had a skill gap for a) educating their patients about factors contributing to couples' infertility and treatments that can optimize chances of conception/pregnancy; b) setting patient expectations about successful fertility outcomes and next steps; and c) providing emotional support to patients/couples experiencing failure to conceive. For laboratory specialists, 23% reported 'never', 'rarely' or 'sometimes' contributing to patient education. When asked how often they "consult references provided by patient organizations regarding the psychological and emotional needs of patients" and "direct patients to support groups or forums (online or in-person)", an average of 47-49% of surveyed HCPs said 'never', 'rarely' or 'sometimes'. Only 49% of surveyed HCPs reported collaborating with psychologists specialized in infertility. The extent to which gaps affected HCPs varied by country and professional role/specialty area.

Limitations, reasons for caution: Although multiple countries were included in this exploratory mixed-methods study, due to small sample sizes, the likelihood of observing a difference in the frequency of reported challenges, barriers and gaps by country due to chance alone could not be excluded.

Wider implications of the findings: Several years after the publication of the ESHRE guidelines for psychosocial care, challenges related to providing adequate educational and emotional support to patients remain. This study identified opportunities and direction for continuing professional development to promote routine psychosocial care in fertility clinics.

Trial registration number: Not applicable for this mixed-methods self-reported educational needs assessment.

Abstract citation ID: dead093.124
P-578 Interactive Effects of Relationship Satisfaction and Social Support on stress: a dyadic study

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[3]Centro de Genética da Reprodução Prof. Alberto Barros, n.a., Porto, Portugal

Study question: Does relationship satisfaction and social support demonstrate actor and partner effects on perceived stress, and is this relationship moderated by reproductive status?

Summary answer: No significant partner effects were found. Relationship satisfaction is related to one's stress. Reproductive status moderates the relationship between one's relationship satisfaction and one's stress.

What is known already: Infertility affects around 15% couples and there seems to be a growing number of couples experiencing infertility. The inability to conceive is experienced by the couple as a stressful situation and should be addressed as a shared problem. Social support seems to be a protective factor for infertility-related stress and correlates directly with individual stress and partner stress among infertile couples. Also, relationship satisfaction has a significant impact on stress and adjustment to infertility, suggesting that low levels of relationship satisfaction predict significant stress levels in infertile women.

Study design, size, duration: This study included 213 heterosexual couples. The participants answered a questionnaire that included self-reported measures for perceived stress, relationship stress and received social support. Data collection was conducted online between October 2016 and October 2018 (detailed information [removed for blind review]).

Participants/materials, setting, methods: This study included 213 heterosexual couples in a relationship, with no children in the current relationship and the women's age was between 20 and 42 years old. Both partners completed the Perceived Stress Scale (PSS), the Relationship Assessment Scale (RAS) and the 2-way Social Support Scale (2-way SSS). Statistical analyses were conducted, and the dyadic analyses were conducted using the Actor-Partner Interdependence Model (APIM) and the Actor-Partner Interdependence Moderation Model (APIMoM).

Main results and the role of chance: A total of 213 couples completed the questionnaire and 45.9% wanted to have children in the future, 8.7% are trying to conceive without an infertility diagnosis and 45.5% received an infertility diagnosis.

None of the partner effects were significant, ($p > .05$), indicating the absence of a significant association between relationship satisfaction and social support from one member and their partner's stress. No significant actor effects were found for neither sex ($p > .05$), regarding social support.

There were significant actor effects for both partners regarding relationship satisfaction, indicating that female and male relationship satisfaction is negatively associated with the stress perceived by one's member (women: $b = -.51$, $SE = .12$, $Z = -4.23$, $p < .001$; men: $b = .45$, $SE = .13$, $Z = -3.60$, $p < .001$).

The reproductive status moderates the relationship between female relationship and female stress ($b = -.31$, $SE = .06$, $Z = -5.41$, $p < .001$, $\beta = -.16$) and also between male relationship satisfaction and male perceived stress ($b = -.26$, $SE = .06$, $Z = 4.30$, $p < .001$, $\beta = -.27$).

Limitations, reasons for caution: The methodological design does not allow inferential causes. The sample may not be representative of the national population, restricting generalizations. Other variables that may have a role in influencing the ones considered, were not included. Only heterosexual couples were considered, which represents a limitation in distinct dynamics.

Wider implications of the findings: Since the romantic relationship can be protective of stress, it becomes relevant that psychological counselling focuses on the quality of marital relationships. It would be ideal for the family planning consultation to inform the importance of both couple members' involvement to screen couples in need of psychological support.

Trial registration number: n.a.

Abstract citation ID: dead093.125
P-581 Family of origin cohesion and intention to pursue fertility preservation in transgender and non-binary people

M. Moura-Ramos[1], A. Pinto[2], J. Reis[2]

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[2]Universidade Lusófona, Escola de Psicologia, Porto, Portugal

Study question: Is the relationship with the family of origins associated with parenthood plans and fertility preservation of transgender and non-binary people?

Summary answer: Family of origin characteristics, namely family cohesion, is associated with the transgender and non-binary people intention to undergo fertility preservation.

What is known already: Few studies have explored parenting intentions and decision to undergo fertility preservation (FP) in transgender and non-binary people. Most studies have focused on the barriers and facilitators of this decision, focusing on the procedures involved in FP, but no studies have assessed the role or psychological (namely relational) variables in the decision making regarding FP. Previous research indicates that family closeness is an important factor in the psychological adjustment of transgender people. Similarly, research indicates that more favorable family relationships were associated with parenting intentions. No studies have examined how relationship with family of origin may affect fertility preservation intentions.

Study design, size, duration: This is a cross-sectional study. Participants were surveyed online from January to April 2022. Dissemination of the study was performed through the social networks of LGBTQIA+ associations.

Participants/materials, setting, methods: Participants were 69 transgender and non-binary people, aged 18-39. The assessment protocol included a questionnaire assessing childbearing intentions, importance of genetic and non-genetic parental connectedness and intention to pursue FP. A validated measure assessing family relationships (Fertility Environment scale, Moos & Moos, 1976) was used, evaluating family cohesion (degree of commitment and support family members provide for one another) and family expression (the extent to which family members are encouraged to express their feelings directly).

Main results and the role of chance: Participants were 40 transgender men (58%), 8 transgender women (11,6%) and 21 non binary (30.4). About 55,1% participants had already initiated hormonal therapy. Almost half of the sample (47,8%) considered having children in the future, while 13 (18.8%) were sure about not wanting children. Genetic connection was not very important (M = 19.51, from 0 -100). A total of 48,1% indicated that that would envision undergo FP in the future.

A mediational model examining indirect effects were tested to examine whether family of origin relationship (cohesion and expression) would be associated with fertility preservation intention.

A significant indirect effect was found, suggesting that family cohesion is associated with the intention to undergo fertility preservation through the effect on the importance of genetic parenting (estimate: 0.56, bootstrap bias-corrected 95% confidence interval 0.052; 1.199) That is, participants with high family cohesion in their family of origin highly valued genetic parenting and this was positively associated with the intention to undergo fertility preservation.

The same indirect effects was tested regarding family expression, but no significant effects were found, that is, the expression of the family of origin is not associated with the intention to undergo PF (estimate: 0.604, bootstrap bias-corrected 95% confidence interval -0.077; 1.366).

Limitations, reasons for caution: The dissemination of the study and participants recruitment was throughout LGBT+ associations, so it is possible that some transgender people that are less prone to be involved in these associations were not reached.

Wider implications of the findings: The importance of the relationships with the family of origin may be an important factor to consider when counselling transgender and non-binary people regarding fertility preservation This may be markedly important when lower family cohesion may be limiting patients' future options.

Trial registration number: Not applicable

SELECTED ORAL COMMUNICATIONS

SESSION 34: IT ALL STARTS (AND MAY END) WITH GAMETES

Tuesday 27 June 2023 Hall A 08:30 - 09:45

Abstract citation ID: dead093.126

O-103 A method for mapping sex-specific meiotic crossovers from PGT-A data elucidates the role of aberrant recombination in the origins of aneuploidy

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Study question: How do the number and location of meiotic crossovers contribute to the formation of aneuploidies observed in preimplantation human embryos?

Summary answer: Normalized across chromosomes, trisomies possess 35% fewer crossovers on average compared to disomies, while the genomic distribution of crossovers is also substantially altered.

What is known already: Meiotic recombination is a crucial source of genetic diversity and is also critical for ensuring the accuracy of chromosome segregation. Understanding the landscape of meiotic recombination, its variation across individuals, and the processes by which it goes awry are long-standing goals in human genetics. Current approaches for inferring the landscape of recombination either rely on population genetic patterns of linkage disequilibrium—capturing a time-averaged view—or direct detection of crossovers in gametes or multi-generation pedigrees, limiting the scale and availability of relevant datasets. Moreover, most of these methods are designed for discovering recombination using data from normal, disomic chromosomes.

Study design, size, duration: We present a method for mapping sex-specific recombination landscapes from low-coverage (<0.1×) data from preimplantation genetic testing for aneuploidy (PGT-A) of embryos with arbitrary ploidy configurations. To overcome the sparsity of these data, our method exploits its inherent relatedness structure, knowledge of haplotypes from external population reference panels, as well as the frequent occurrence of chromosome loss in embryos, whereby the remaining chromosome is phased by default.

Participants/materials, setting, methods: We benchmarked our method by simulating crossovers between known haplotypes. Encouraged by the performance on simulated data we extended our study to retrospective analysis utilizing de-identified PGT-A data obtained between April 2021 and August 2022 at the CReATe Fertility Centre (Toronto, Canada). The data include 20,160 embryos (2,559 IVF patients) with an average depth of coverage of ~0.05×, facilitating the mapping of crossovers at an average resolution of ~150 kbp.

Main results and the role of chance: Our benchmarking results demonstrate high sensitivity and specificity across all ancestries at a coverage of 0.05x per homolog (AUC = 0.989), with AUC declining by 0.014 and 0.053 for when coverage is reduced to 0.025x and 0.013x, respectively. Extending our analysis to real PGT-A data, we observed that our inferred sex-specific landscapes of meiotic crossovers on disomic chromosomes were strongly correlated with published genetic maps from studies based on high-coverage sequencing of parent-offspring trios ($r = 0.86$ for female map; $r = 0.53$ for male map), broadly supporting the accuracy of our method. Notably, the total length of the female genetic map was reduced by 35% for trisomies compared to disomies, consistent with the hypothesized role of reduced crossovers and exchangeless chromosomes in the origins of female meiotic aneuploidy. In addition, the genomic distribution of crossovers is also altered in a chromosome-specific manner. Examples include a reduction in crossovers near the centromere of trisomies versus disomies of chromosome 16, as well as an enrichment of crossovers on the q-arm of trisomies versus disomies of chromosome 22. Together, our results provide a detailed corroboration of the hypothesis that aberrant meiotic recombination contributes to the origins of aneuploidies.

Limitations, reasons for caution: The accuracy of our method is influenced by genomic heterogeneity in depth of coverage, rates of heterozygosity, and mismatches between the ancestry of the reference panel and the tested sequence. Moreover, technical errors such as spurious alignment and genotyping could hinder analysis in repetitive genomic regions.

Wider implications of the findings: Together, our study helps clarify the dual function of meiotic recombination in generating genetic diversity while ensuring meiotic fidelity. Our method for patient-specific mapping of meiotic recombination phenotypes may offer clues about how dysregulation of this process contributes to infertility.

Trial registration number: NIH r R35GM13374

Abstract citation ID: dead093.127

O-104 The Impact of application of CRISPR /dCas9 systems for increasing the expression of FSH receptor in human granulosa cells

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Study question: What are the molecular and physiological effects on human granulosa cells, when **CRISPR/dCas9 epigenome edition technology is efficiently used to increase FSH receptor** (FSHR) activity?

Summary answer: The significant increase in expression of **FSHR maintained by dCAS9 systems has effects on several fundamental cellular events** involving steroidogenic, life-sustaining,cell death related signaling sub-pathways.

What is known already: Manipulation of the expression of FSHR can result in biological events that include fundamental activities of FSH as regulation of folliculogenesis, production of steroidogenic hormones. FSHR can be activated in vitro by FSH analogues, and signaling networks activate ERK1/2 pathway through p38 mitogen-activated protein kinases. CRISPR/dCAS9 can be used to activate or inhibit gene action without silencing the gene. In spite of being used in many systems, interference of FSHR expression by epigenome edition is very **limited** as well as the use of **in vitro tools to increase FSHR expression for investigating IVM, folliculogenesis, PCOS, FSH hyper/hyposensitivity, cancer biology.**

Study design, size, duration: In-vitro cultured HGrC1 (human granulosa cell line) cells were treated by follitropin-alfa (Gonal-f). The experimental groups were designed in triplicates with cells i. without any genome edition and cells that have i. non-targeting gRNA, ii.d**CAS9 activated FSHR**, iii. **dCAS9 activated FSHR and FSH treatment** for 5 min, 15 min, or 1 hr; iv. **only FSH treatment** for 5 min, 15 min or 1 hr. FSHR related gene and protein expressions and estrogen production were evaluated.

Participants/materials, setting, methods: For human FSHR gene, **two separate gRNAs** were designed. Annealing, digestion, ligation,

transformation, colony formation, plasmid isolation, Sanger sequencing analysis, MOI were accomplished for **dCAS9/SAM** system. **HGrC1** (immortalized human granulosa cell line) cells were treated with 75 IU/ml Gonal-f in related groups. After treatment, in triplicates, gene/protein expression for **MTOR, FSHR, AKT, MAPK8, MAPK1, TP 53, CASP 3, ESR1, CYP 19A1** were evaluated by qPCR, WB and IF. **Estradiol** was measured by chemiluminescence immunoassay.

Main results and the role of chance: FSHR has increased significantly in dCas9, dac9 + 5min Gonal-f, dCas9 + 15min Gonal-f, dCas9 + 1h Gonal-f; mostly in 5 min compared to groups without dCas9. The system plasmids did **not show any non-specific effects**.**MAPK1, MAPK8, ESR1, MTOR, AKT, P53** and **CASP3** gene expressions showed significant increase in dcas9 system groups compared to control and NT, whereas in all time-dependent treatments, a significant increase was seen in groups that have dcas9+Gonal-f compared to the ones treated with only Gonal-f. As time dependent manner has been investigated, expressions of **MAPK1, MAPK8 and ESR1 were highest at min 5**, expressions of **CASP and AKT were highest at min 15** and the expression of **MTOR was highest at hour 1**, whereas **FSHR and p53**were strongly expressed at **all time points** ($p < 0.05$ for all mentioned results). Gene expression profiles were also confirmed for P-38 MAPK, AKT, FSHR by WB. IF stainings have revealed higher expression of AKT and MAPK in both dCas9 and dcas9+Gonal-f 5 min compared to the control group, however the intensity of the staining was not satisfactory enough. Interestingly, estrodiol(E2) levels were found to be higher in the 5 minutes only Gonal-f treatment and dcas9 with Gonal-f treatment, while the other groups were similar to control.

Limitations, reasons for caution: -This study has been conducted in **cell line**, rather than primary granulosa cells which are not easy targets for epigenome edition.

-**No testosterone** derivatives were used as substrate for aromatase. This has been reflected in the results as significant increase in FSHR and related genes but non-significant increase in E2 production.

Wider implications of the findings: -dcas9/SAM system can be used efficiently increase **other FSH-receptors**, which is a potent way to reveal molecular sub-pathways in a temporal manner;eg. in **male** reproductive system or in other cells of female RS.

-FSH related conditions as **PCOS** can be examined in vitro.

-**FHSR unresponsive conditions** can be modeled in vitro.

Trial registration number: not applicable

Abstract citation ID: dead093.128

O-105 Chromatin accessibility of oocytes contributes to PCOS transgenerational inheritance

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Study question: What is the underlying mechanism contributing to the transgenerational defects of oocytes and embryos of polycystic ovary syndrome (PCOS)?

Summary answer: The transgenerational inheritance of abnormal chromatin accessibility in the PCOS oocytes is responsible for the defect of oocytes and embryos in transgeneration.

What is known already: Studies have provided evidence of compromised oocyte quality and related adverse impacts. Observed PCOS animal-model oocyte defects include meiotic abnormalities, mitochondrial dysfunction, and increased oxidative stress. Elevated androgen levels are associated with reduced fertilization rates and lower cleavage rates during in vitro fertilization (IVF) treatment. PCOS could be transgenerationally transmitted, and unique altered gene expression has been observed in metaphase II (MII) oocytes from all offspring in PCOS mice.

Study design, size, duration: We profiled the transcriptome and chromatin accessibility of oocytes and preimplantation embryos in F0-F2 offspring of PCOS mice, to explored whether the inheritance of chromatin accessibility defects in PCOS oocytes was responsible for the abnormalities of oocytes and embryos in PCOS offspring.

Besides, oocytes from 13 healthy women and 8 women with PCOS, 24 blood samples from 24 women with/without PCOS were analyzed to validate the molecular mechanisms of PCOS inheritance.

Duration: 2019.5∼2022.12.

Participants/materials, setting, methods: PCOS mice models were established by means of DHEA-exposure. Smart-seq2 and DNase-seq were utilized to investigate the transcriptome and chromatin accessibility of oocytes and preimplantation embryos in F0-F2 offspring of PCOS mice. Bioinformatic analysis was applied to explore the differential gene expression and chromatin accessibility of oocytes and embryos in PCOS mice and offspring comparing to controls.

Common gene signatures in the tissues of PCOS women and PCOS daughters are analyzed compared to control samples.

Main results and the role of chance: In PCOS mice, we observed transcriptional alteration in oocytes and 2-cell embryos compared to normal mice. There are 4229 and 1197 differentially expressed genes (DEGs) in PCOS oocytes and 2-cell embryos. The DEGs includes both maternal factors and zygotic genome activation (ZGA) genes. Interestingly, in PCOS 2-cell embryos, we observed hundreds of pre-activated genes which are silence in normal 2-cell embryo but expressed in PCOS 2-cell embryos. Notably, many DEGs in the oocytes of PCOS mice were also differentially expressed in the oocytes of F2 PCOS mice compared to control mice, supporting the transgenerational inheritance of transcriptional alteration in PCOS oocytes. Mechanismly, the changes of chromatin accessibility were associated with the DEGs in PCOS oocytes and embryos. In particular, chromatin accessibility of the pre-activated gene promoters is elevated in PCOS oocytes compared to that in normal oocytes. Furthermore, many of the abnormal chromatin accessibility signals detected in F0 PCOS oocytes were also observed in F2 PCOS oocytes. Finally, our data showed that some DEGs in the oocytes of PCOS mice were also differentially expressed in human PCOS oocytes compared to the control oocytes. Common genes identified in PCOS offspring may be used as potential predictors of PCOS phenotype.

Limitations, reasons for caution: Which factors contribute to the abnormalities of chromatin accessibility in PCOS oocytes are still unknown.

Wider implications of the findings: The candidate genes may be used as potential predictors of PCOS phenotype and provide the clues for PCOS offspring .

Trial registration number: not applicable

Abstract citation ID: dead093.129

O-106 Bringing Transparency to Oocyte Assessment: the importance of including confounders when building Artificial Intelligence (AI) based support tools to quantify oocyte viability

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Study question: Which confounders (sperm quality, oocyte dysmorphism, culture time, images pre or post-ICSI, age) affect the ability of AI to predict blastulation based on oocyte images?

Summary answer: Sperm quality, oocyte dysmorphism, pre or post-ICSI image should be controlled for when building AI algorithms to predict blastulation based on oocyte images.

What is known already: Previous studies reporting on the use of AI to predict blastulation based on oocyte images have: (i) not accounted for confounders affecting blastulation (i.e. sperm quality, culture time), and (ii) used post-ICSI images; without assessing whether the ICSI procedure affects the oocyte image as assessed by AI. Therefore, there is a risk of mislabeling

viable oocytes as non-viable due to external factors, which could cause uncontrolled bias and failure to generalize when used in clinical practice. The objective was to assess how these confounders affect efficacy of prediction of blastulation from oocyte images by an AI-based oocyte assessment tool: CHLOE-OQ(Fairtility).

Study design, size, duration: Cohort study. Images of 1281 oocytes (February to June 2022) were taken pre and post ICSI using the Embryoscope, and the embryos cultured until day 7. Oocyte donor source and age, oocyte dysmorphias and sperm quality were documented. CHLOE-OQ algorithm was trained, validated and tested in a diverse data set, accounting for pre and post ICSI image datasets, quality of oocytes, quality of sperm and patient age.

Participants/materials, setting, methods: The primary endpoint was blastulation. Sperm quality data was classified into 4 groups: (A)All (n = 1281), (B)donor sperm only (n = 51), (C)donor sperm and normospermic samples from men not diagnosed with male factor infertility (n = 557), (D)abnormal sperm samples and other diagnosed male factor cycles (n = 747). Eggs were classified by source (own/donor), and by dysmorphisms: enlarged perivitelline space, abnormal Zona pellucida, cytoplasmic abnormalities, dark, enlarged oocytes.

Main results and the role of chance: Post-ICSI images had higher mean CHLOE-OQ score than pre ICSI images (0.28 ± 0.1 vs 0.33 ± 0.1, $p < 0.001$, paired t-test). Discrepancies were particularly identified in oocytes that degenerated following ICSI, and scored 0 by CHLOE-OQ despite having higher scores pre-ICSI. Using Post-ICSI images (AUC=0.66, 95% confidence interval, CI: 0.63-0.69, n = 1281) improved the efficacy of prediction of blastulation compared to pre-ICSI images (AUC=0.57: 0.53-0.60, n = 1281, $p < 0.001$), suggesting that ICSI affected the quality morphology of the oocyte, and how an oocyte responds to ICSI, as assessed by AI, contributes to prediction of blastulation.

Efficacy of prediction (AUC) was not affected by the quality of the sperm: (A-OVERALL 0.658 [CI(95%): 0.626-0.687]; B-Donor 0.586 [CI(95%): 0.449-0.728]; C-normospermic 0.645 [CI(95%): 0.600-0.688], D male factor 0.678 [CI(95%): 0.639-0.715]).

Oocyte features associated with low CHLOE-OQ scores were: enlarged perivitelline space, dysmorphic oocytes, abnormal Zona pellucida, cytoplasmic abnormalities and dark and enlarged oocytes. Whilst spherical oocytes with normal zona and perivitelline space were characterized as being more likely to form a blastocyst.

Limitations, reasons for caution: This single-clinic study is retrospective. A multi-center study is underway. External factors affecting blastulation must be accounted for to avoid mislabeling of good oocytes as non-viable. There is also a need to understand oocyte dysmorphias identified by the AI algorithm to ensure biological transparency in clinical decision making.

Wider implications of the findings: Taking into account clinical and gamete confounders when building AI algorithms is a necessary strategy to ensure AI algorithms are generalized when incorporated into clinical practice, whilst reducing bias and promoting transparency in clinical decision making. The risk of not considering confounders leads to mislabeling, bias and inaccurate predictions.

Trial registration number: not applicable

Abstract citation ID: dead093.130
O-107 Sperm chromosome chaos induced by MEIKIN gene mutations leads to blastocyst formation failure

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Study question: What are the effect and corresponding mechanisms of chromosome chaos of sperm caused by mutation of the MEIKIN gene on preimplantation embryo development?

Summary answer: Sperm chromosome chaos induced by MEIKIN gene mutations directly causes blastocyst formation failure by affecting the activation of the embryonic genome and lineage differentiation.

What is known already: In mice, Meikin is a meiosis-specific kinetochore factor that plays a crucial role in mono-orientation and protection of centromeric cohesion in meiosis I and the regulation of chromosome alignment during meiosis II. Both male and female Meikin gene knockout mice are completely infertile. Infertility in male Meikin$^{-/-}$ mice originates from defects in meiotic chromosome segregation and subsequent spermatogenesis. To our knowledge, no mutation of the MEIKIN gene has been reported in humans.

Study design, size, duration: In this study, semen from three patients with MEIKIN mutations and arrested embryos from one of them were analyzed to determine the effect of MEIKIN mutations on sperm and early embryonic development.

Participants/materials, setting, methods: Three male patients with MEIKIN mutations with a specific clinical phenotype were recruited. We assessed mutant sperm morphology using Papanicolaou staining. Sperm FISH and single sperm chromosome aneuploidy analysis(CNV-seq) were applied to identify mutant sperm Chromosomal abnormality. We also investigated the chromosome constitution and gene expression of the arrested embryos from one patient by parallel single-cell genome and transcriptome sequencing.

Main results and the role of chance: Novel mutations in the MEIKIN gene were identified in three infertility males with a specific clinical phenotype(They had good-quality embryos on day 3, but these embryos repeatedly failed to implant or develop to the blastocyst stage). Routine semen assessments were conducted based on the WHO's guidelines, and all these patients were diagnosed with oligoasthenoteratozoospermia. Sperm FISH showed that the frequencies of aneuploidies in spermatozoa were significantly higher than the normal control(89.05%,70.9%,93.24% vs. 5.03%), and the CNV-seq analysis of single spermatozoa confirmed mutant sperm had severe chromosome chaos. Each cell of the arrested embryos from one patient had complicated chromosomal abnormalities, and the abnormal chromosomes were mainly derived from sperm. At the gene expression level, these embryos were arrested at the 8-cell to morula stage transition, and defects activation of a large ZGA and lineage specification genes led to blastocyst formation failure.

Limitations, reasons for caution: In this study, we discovered that mutations in MEIKIN caused male infertility manifesting as sperm chromosome chaos and blastocyst formation failure. However, the molecular mechanism of the sperm chromosome chaos involved in blastocyst formation needs to be further illuminated by using Meikin knockout mouse models.

Wider implications of the findings: We first reported that male with MEIKIN mutations is related to severe sperm chromosome abnormalities and blastocyst formation failure. Therefore, In the process of ICSI, if there are good-quality cleavage embryos that repeatedly fail to implant or develop to the blastocyst stage, it is recommended to examine male factors concurrently

Trial registration number: not applicable

INVITED SESSION

SESSION 35: THE C-SECTION SCAR DEFECT PANDEMIA

Tuesday 27 June 2023 Hall D3 08:30 - 09:30

Abstract citation ID: dead093.131
O-108 C-section scar defect: Hysteroscopic vs laparoscopic repair

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Objective: To present a systematic review of the literature on the hysteroscopic and laparoscopic repair of cesarean scar niche and offer an evidence-based approach to the treatment of cesarean scar niche.
Data sources: A thorough search of the PubMed/Medline, Embase, and Cochrane databases was performed. (PROSPERO registration number CRD42020190668).
Methods of study selection: Studies from the last 20 years that addressed cesarean scar niche repair were collected. Both authors screened for study eligibility and extracted data. All prospective and retrospective studies of more than ten women were included.
Tabulation and integration: The initial search identified 666 articles [PRISMA flow chart]. We excluded duplicates, case reports, reviews, video articles, and technique articles. We also excluded studies describing only laparotomy or vaginal repair as these were not of the scope of this review.

A total of 31 articles met the inclusion criteria, 21 for hysteroscopic resection and 13 for laparoscopic or combined repair (4 articles tested both modalities and appear in both tables).
Results: For abnormal uterine bleeding (AUB), hysteroscopic remodeling relieved symptoms in 60%-100% of cases and laparoscopy in 78%-94%. Secondary infertility was not evaluated in all studies. We found that after hysteroscopic and laparoscopic treatment, 46%-100% and 37.5%-90% of those who wished to conceive became pregnant following the procedure, respectively.

Pain and dysmenorrhea seem to be uncommon. All studies that tested improvement of pain had less than ten women. However, it would appear that between 66% and 100% of women who complain of pain or dysmenorrhea will note a marked improvement to full resolution.
Conclusion: cesarean scar niche or cesarean scar defect is usually asymptomatic. For symptomatic women, a repair is a valid option. For those with RMT (Residual Myometrial thickness) >2-3 mm, hysteroscopic remodeling is the modality of choice with an improvement in AUB, secondary infertility, and pain. For women with a RMT <2-3 mm, laparoscopic repair with simultaneous hysteroscopic guidance shows similar results. Since available data is limited, no cutoff for the correct choice between hysteroscopy or laparoscopy can be concluded. We recommend 2.5 mm as the cutoff value based on common practice and expert opinion, although no significance between hysteroscopic and laparoscopic treatment was shown.

Abstract citation ID: dead093.132
O-109 Avoiding the C-section scar defect

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The cesarean scar defect was first described in 1961 by Poidevin et al. Cesarean delivery is one of the most common surgeries today, and millions of women give birth this way every year. The prevalence of cesarean scar defect is increasing due to increasing cesarean section rates. The cesarean scar defect is called by different names in the literatüre, such as isthmocele, niche, diverticulum, dehiscence of the cesarean scar. It was discovered that the prevalence of isthmocele varied widely. Using contrast-enhanced sonohysterography, the prevalence in a random group of women with a history of CS was between 56% and 84%, whereas the incidence in pelvic ultrasonography fluctuates between 6% and 36%.

The most common gynecological problems that may occur due to isthmocele are menstrual spotting, dysmenorrhea, dyspareunia, chronic pelvic pain, and infertility. The likelihood of scar pregnancy, placentation abnormalities, and uterine rupture increases with subsequent pregnancies. Both hysteroscopic and laparoscopic isthmocele repair techniques are highly effective. Therefore, precautions against isthmocele during cesarean delivery should be made more transparent.

The development of isthmocele is influenced by the timing of the cesarean section (during pre/early or advanced labor), the position of the uterine incision (its distance or proximity to the internal os of the cervix), and the opening and closing (suturation) method of the uterine incision. Once more, both gestational diabetes and being overweight might be viewed as independent risk factors.

Understanding the risk factors for isthmocele development will aid in the development of preventative measures. The two most crucial elements in the formation of isthmocele, according to our knowledge of the literature, are the site of the uterine incision and the suture methods. In this presentation, we will discuss ways to prevent the development of isthmocele.

INVITED SESSION

SESSION 36: DEBATE: MILD VERSUS CONVENTIONAL STIMULATION IN ART

Tuesday 27 June 2023 Hall D1 08:30 - 09:30

Abstract citation ID: dead093.133
O-110 Mild stimulation is the future in ART

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The World Health Organisation has called for a simple, safe, and effective IVF treatment which could be both affordable and widely accessible. Unfortunately, IVF practice in developed countries seems to be trending in the opposite direction, making ovarian stimulation more intense, complex, expensive, and globally inaccessible, under the pretext of maximising the cumulative live birth rate (LBR) or by offering a 'package' of a 'complete family' from a single attempt of IVF.

In reality, data from randomised controlled trials (RCTs) has found no difference in fresh cycle as well as cumulative LBRs whether a mild or high stimulation dose is administered. This is because, higher oocyte yield with high ovarian stimulation does not translate into better-quality embryos and this has been demonstrated in systematic reviews and meta-analyses.

There appears to be confusion between stimulation 'dose' and 'response'. Young women with good ovarian reserve are more likely to have a high response to stimulation despite being treated with a dose that falls within the definition of 'mild stimulation', whereas aggressive stimulation fails to improve the response in poor responders. A rising cumulative LBR with increasing number of oocytes is attributable to these good responders, but this is disconnected with the intensity of ovarian stimulation.

The euploid embryo yield similarly is related to the number of oocytes and cumulative LBR which are influenced by the woman's age and ovarian reserve but not the stimulation dose. Both the numbers of aneuploid and euploid embryos rise with increasing oocyte yield, keeping the proportion unchanged.

Advocates of 'one and done' approach have shown that only 1 in 5 women can produce the requisite number of oocytes to have more than 1 child. This means, the remaining 80% patients would be subjected to intense stimulation, incurring risks of overstimulation, treatment burden and cost without achieving the goal of 'completing' their family. Psychological analysis from a RCT reported a higher incidence of post-treatment depression after Conventional compared to Mild IVF. Even in the era of agonist trigger and 'freeze all

embryos', OHSS has not been totally eliminated- the World Registry reported around 40 cases of severe OHSS in 10 000 cycles. Clinical complications such as haemorrhage and infection associated with overstimulation could be magnified in low-resource settings with lack of facilities and expertise.

Livebirth rates albeit of key importance cannot be the only index of success. Our commitment should be to make IVF accessible globally by adopting effective stimulation protocols that reduce treatment burden and cost for the patient and improve health outcomes for mother and baby. Only mild stimulation IVF can fulfil this global vision and need as an increasing body of evidence has confirmed that mild stimulation is as successful as conventional stimulation, while being safer and less expensive. Individualised mild stimulation protocols are the smartest way forward in a global setting for all patient groups. It is time to reflect on what is best for our patients and society in the long-term.

Abstract citation ID: dead093.134
O-111 Conventional stimulation remains the gold standard

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Ovarian stimulation (OS) remains a crucial step of IVF process since cycle outcomes per cycle after OS are remarkably higher when compared to a natural or modified natural cycle. Within the concept of OS, two approaches are purposed: On one hand, "mild" stimulation claims for the use of low doses of gonadotrophins in order to yield a moderate number of eggs that should be enough to achieve a pregnancy. On the other, the so called "conventional" stimulation aims to maximize ovarian response, with the goal of obtaining the higher number of embryos that is possible and therefore increase the number of attempts per pick up to reach the highest chance of pregnancy for each patient in one cycle.

Defenders of mild stimulation, claim that obtaining oocytes beyond a certain response (classically eight eggs) is useless, since these surplus oocytes won't be able to provide good quality embryos. In other words, this statement is based in a hypothesis according to which a soft stimulation of the ovaries would lead them to mature only the good quality eggs, while those of lower quality would not respond to these low doses and would remain immature in the ovaries. A few numbers of old studies performed with outdated technologies support this this concept.

However, a large body of evidence shows that the contrary is true. Old studies that related ovarian response to cycle outcome considering only the chances of achieving a live birth after just a fresh embryo transfer, showed that live birth rate increased as the ovarian response did up to around 15 oocytes, while it shows a "plateau" beyond this response. But moreover, more recent studies in which the cumulative live birth rate achieved after one live birth is achieved or until all viable embryos that were cryopreserved were transferred, shows that it improves linearly as the ovarian response does, starting to plateau only for ovarian responses larger than 35-40 eggs.

A very recent study that analyses more than 400,000 cycles from the Society for Assisted Reproductive Technology Clinic Outcome Reporting System (SART) confirms these figures. This large sample allows for stratified analysis according to age, BMI, AMH or infertility diagnoses that further confirm the findings. Altogether, these studies show that the concept of some kind of "oocyte quality selection" happens when a mild stimulation is used does not really happen. Large ovarian responses increase the number of good and bad quality oocytes equally.

The idea of ovarian stimulation hampering oocyte quality has been around for more than 30 years. Theorical concepts based on observations in the animal model suggested that this might be the case. However, studies in humans have been unable to prove this hypothesis. In this context, the best model to prove this concept is the intra-patient comparison between the non-stimulated and the conventionally stimulated cycle. Our group has performed two studies following this approach so far. In the first one, we included 46 oocyte donors. PGT-A was performed to the embryos obtained in both cycles, which at time was performed on day 3 embryos with FISH technology. The study showed that there were no significant differences in the aneuploidy rate between the 2 cohorts of embryos (34.8% vs 32.8%), suggesting no harm of ovarian stimulation. These findings have been recently confirmed in 40 infertile patients aged 30-38, in which PGT-A was done with a comprehensive chromosomal analysis using NGS after blastocyst biopsy.

In conclusion, research and clinical evidence show that conventional OS does not harm oocyte quality and increases the cumulative birth rate per oocyte pick-up, making it as the first choice for treating patients with IVF.

Trial registration number: XXXX

INVITED SESSION

SESSION 37: FROM PIONEER TO PRESENT PRACTICE OF FERTILITY CARE PROVIDERS

Tuesday 27 June 2023 Hall D4 08:30 - 09:30

Abstract citation ID: dead093.135
O-112 Jean Purdy - The silent partner

H.J. Kendrew[1]

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The development of a novel technique requires a diverse team whose individuals share the same goal but who each bring a different strength and aspect of their personality to the research/ project.

A "front man" with tenacity drive and vision needs the support of such team. To quote Aristotle "The whole is greater than the sum of it's parts".

This expression could be aptly applied to the pioneers of IVF, Robert Edwards and Patrick Steptoe about whom much has been written. Their drive and determination in the face of societal opposition and competition from across the Atlantic, was supported by a silent group of lesser known individuals.

Jean Purdy (research nurse and laboratory assistant), Muriel Harris (operating theatre superintendent), Lillian Lincoln Howell (philanthropist) and Ruth Fowler (scientist and Robert Edwards wife). Not to mention the 280 unidentified women patients and their partners who played their part. All drawn together in the quest to try and help patients achieve the families they so desired.

In my presentation I am concentrating specifically on the life Jean Purdy and her contribution to the work which sees us all here today in Copenhagen.She had a short but pivotal life, often attributed to being the first embryologist, prematurely dying at the age of 39 from malignant melanoma.

As of March 2021 at least eight million babies worldwide have been born from IVF. I wonder how astounded these pioneers would be to see how far we have come and what they would make of the world wide impact they made in the face of significant criticism, moral outrage and scepticism from their professional peers, the church, media and society?

Abstract citation ID: dead093.136
O-113 Recent research on fertility staff emotions

F. Fedele[1]

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Infertility-related psychological research is traditionally oriented more at analysing the wellbeing of couples undergoing Assisted Reproductive Technologies (ART), than to studying the job-related effects on the healthcare fertility staff.

The unfulfilled desire for a child can cause an emotional crisis for couples: they may experience high levels of stress with physical, emotional, social and financial concerns related to infertility and ART treatment.

Little is known instead about the emotional reaction of healthcare members to infertility and treatment delivery. In fact, it is well documented that patients with infertility problems may challenge usual approaches to care,

leading to potential difficulties in the therapeutic relationship: patients undergoing ART are often dissatisfied with the level of empathy and attention received by the healthcare staff. Besides, patients may be experienced as manipulative, emotionally dependent, self-destructive, non-compliant and with a hostile attitude, thus raising negative feelings of frustration, anxiety, and dislike among healthcare professionals.

This is the aim of the research titled "What about fertility staff emotions? An explorative analysis of healthcare professionals' subjective perspective", aimed at understanding the subjective perspective of the fertility professionals and their emotional dynamics in relationship with patients. An in-depth explorative research study was conducted on 12 healthcare professionals working in the fertility clinic of a public healthcare hospital of Rome. Structured interviews with open-ended questions were conducted and Emotional Text Analysis was carried out to analyse the transcripts of their interviews. Five thematic domains were detected that describe the staff's emotions about their professional experience, as follows: performance anxiety (Cluster 1), ambivalence between omnipotence and powerlessness (Cluster 2), care burden (Cluster 3), feeling of duty (Cluster 4), and sense of interdependence (Cluster 5). This study suggests that the professionals' awareness about the implicitly emotional meanings, beliefs, values, referred to the professional function and therapeutic relationship may facilitate team work and care relationship.

For this reason, the Italian National ART Register of the National Institute of Health and the Department of Dynamic Clinical and Health Psychology of La Sapienza University carried out a research to examine the characteristics of psychological services in Italian ART centres. A questionnaire consisting in 26 questions was sent to the physicians in charge of 341 ART centres active in Italy.

The overall picture was rather disappointing in that only half of the responding centres (47%) have a psychologist permanently on staff and psychological intervention seems to be considered as targeting the inner world of the individual or the couple and not their relationship with the ART context. Moreover, relatively few couples (10-20%) resort to counselling in 70% of the responding centres. Referrals did not seem to be regulated by specific policies and procedures in 60% of the cases and in 70% of centres the fee for psychological counselling was not included in the ART treatment fee. Furthermore, 30% of the responding centres worked with an independent psychologist who is called in upon request.

It is possible to hypothesise instead that health professionals in the field of reproductive medicine could offer their care on an ongoing basis during the course of treatment with an integrated and collaborative approach that also involves:

1. education, training, and support of the fertility clinic staff to help reduce staff stress, prevent burnout, and improve overall patient care.

2. offer support to adopt an emotional accompanying function into the healthcare relationship, through attempts at restitution, clinical supervision, joint listening setting (physician, psychologist, patients), analysis and sharing of the emotional dimensions involved in the Centre's activity, in order to improve the care experience of the patient and of the carers in relation to each other.

This approach may represent a real multidisciplinary model of caretaking.

INVITED SESSION

SESSION 38: FERTILITY PRESERVATION OR GAMETE DONATION FOR CHILDHOOD AND YOUNG ADOLESCENTS WITH HEREDITARY CANCER?

Tuesday 27 June 2023 Auditorium 10-12 08:30 - 09:30

Abstract citation ID: dead093.137

O-114 Fertility preservation in males with hereditary cancer syndromes: balancing the risks and benefits

R. Mitchell[1]

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Abstract citation ID: dead093.138

O-115 Fertility preservation or egg donation for hereditary cancer syndromes?

F. Filippi[1]

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Individuals with hereditary cancer syndromes are at risk of developing multiple tumours and, therefore, undergo life-long surveillance procedures and risk-reducing and/or therapeutic surgeries. Knowledge and testing for hereditary cancer susceptibility genes is rapidly spreading, often performed at the first cancer diagnosis.

Different physical, psychosocial, ethical and economic considerations need to be taken into account when deciding on reproductive options in individuals with a hereditary cancer syndrome.

The psychosocial impact of genetic predisposition to cancer may be particularly problematic and still represents an ongoing challenge for the professional taking care of these patients. In fact, worries about passing on a genetic risk for cancer to offspring are concerns expressed by the 65% of female young adult cancer survivors when specifically interviewed.

At cancer diagnosis, fertility preservation procedures represent an effort to retain the opportunity to have genetically related children. Unfortunately, oocytes, embryo or ovarian cortex cryopreservation do not guarantee live birth. The success rates of these procedures are highly dependent on age, number of oocytes cryopreserved and may be influenced by other medical, lifestyle and (future) male factors. In addition, carriers of pathogenetic gene variants for cancer predisposition should also be thoroughly counselled about preimplantation genetic testing (PGT), a technique allowing the interruption of passing on the gene associated to cancer risk. However, this option inevitably lowers the efficacy of fertility preservation interventions in terms of future pregnancies.

Egg donation is a spreading procedure with higher success rates than fertility preservation techniques. This approach allows individuals to avoid undergoing oocytes retrieval or ovarian tissue cryopreservation surgery, thus avoiding procedural risks (infection, haemorrhage, ovarian hyperstimulation syndrome) and postponement of oncological treatments. On the other side, offspring will not have a genetic link with recipient and the beneficial psychological role of fertility preservation would be missing. In fact, several recent studies have underlined the message of hope that is indirectly perceived by oncological patients when counselled for fertility preservation. Moreover, counselling on the possibility of egg donation is challenging at the time of cancer diagnosis.

Making the right reproductive choice in carriers of hereditary cancer syndromes is a dilemma which can't be solved generalizing. Even patients' attitude could be different according to the time of counselling, whether is at cancer diagnosis or at the genetic predisposition diagnosis in healthy carriers. Egg donation should be considered in the available armamentarium. The complexity of the topic needs a personalized and multidisciplinary counselling.

INVITED SESSION

SESSION 39: RAISING FERTILITY AWARENESS FOR EVERYONE

Tuesday 27 June 2023 Hall D5 08:30 - 09:30

Abstract citation ID: dead093.139

O-116 Benefits

L. Schmidt[1]

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The World Health Organization's Sustainable Development Goal 3.7 covers sexual and reproductive health to ensure universal access to sexual and reproductive health care services, including family planning, information & education and the integration of reproductive health into national strategies.

Globally, infertility is one of the most frequent chronic diseases among men and women of reproductive age. Infertility is associated with a number of different risk factors from medical diseases some being congenital; various infections like tuberculosis, sexually transmitted infections like chlamydia; health-behaviour like smoking, obesity, use of cannabis, advanced age; unsafe abortions; insufficient access to good quality pregnancy care and delivery care; and reproductive risk factors at workplaces and in the environment. In many cases infertility is preventable by reducing risk factors. In order to prevent infertility, national strategies are needed on an individual and societal level.

One of the important strategies is to raise fertility awareness for everyone – this includes increased fertility awareness for young adults, their parents, health care staff, teachers and others who are in contact with young adults, but also politicians and stakeholders who have a huge impact on how to organize our societies including sufficient access to safe reproductive care to support people to have the children they desire (or to stay childfree if they prefer).

On a global level, surveys and interview studies have repeatedly shown that a high proportion of people have insufficient knowledge regarding fertility and risk factors for infertility, and they falsely believe that medically assisted reproduction is nearly always successful. The studies also show that many people are aware of their limited knowledge. Recent studies find that young adults express a need for being better educated in this field and many prefer a course in sexual education including fertility knowledge when they are undergoing their youth education. Study participants prefer to have fertility knowledge in advance in order better to take care of their fertility potential when they desire to have children.

It is also of importance to increase fertility awareness among parents, as a proportion of young adults talk to their parents about when to have children. Parents especially in high-income countries could encourage their children to postpone family building to ages where it could be difficult for their sons and daughters to have sufficient time to have the number of children they desire. Other studies show that also health care professionals like doctors and nurses outside of the fertility field have limited fertility knowledge and hence could give their patients poor advice when it comes to reducing infertility risk factors and when to seek medically assisted reproduction.

There are several benefits of globally raising fertility awareness for everyone: 1) to ensure that people are able to make well-informed decision-making regarding their own family building. 2) To prevent infertility and reduce the proportion of people in need of medically assisted reproduction. 3) To be able to organize societies to become family friendly. 4) To support NGOs and others to fight for universal access to safe sexual and reproductive health care including access to contraception, safe abortions and safe pregnancy and delivery care. 5) To secure sufficient access to high-quality medically assisted reproduction for those in need of treatment to become parents. 6) To stop using harmful substances that impact fertility at workplaces and in the environment.

Abstract citation ID: dead093.140
O-117 Challenges

M. Simopoulou[1]

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Fertility awareness is heavily charged "meaning" different things to different people, from how to avoid pregnancy, to how to have children, but this should not be the only focus. Fertility awareness should employ the life course approach to improve reproductive health and facilitate decision-making in family planning among adolescents, people of reproductive age, primary healthcare, education professionals, and policymakers through development, evaluation, and dissemination of inclusive educational resources. Fertility and reproductive health education are distinct but intertwined. As raising awareness on fertility means that reproductive health should be covered, the International Fertility Education Initiative (IFEI) recently changed our name to the International Reproductive Health Education Collaboration (IRHEC). The work that IRHEC has done with schools, highlights that covering the life course of reproductive health is important for all, irrespective of attitudes.

Finding the right language to communicate harbors risks on unintended negative effects such as dissonance and anxiety. The IRHEC's recent publication focuses on 5 recommendations: 1) to frame fertility awareness messages with (reproductive) autonomy in mind aiming for inclusion of those who do not represent the traditional nuclear family; 2) ensure fertility health messages are empathetic and steer clear of blame; 3) avoid scaremongering and offer a positive angle; 4) address both women and men in fertility health messaging; and 5) tailor messages to particular contexts and audiences and develop resources in close collaboration with the target groups.

The measurable impact of all educational resources should be evaluated. This is challenging owing to lack of standardized education and of specific benchmark for satisfactory knowledge gain, along with the challenge of developing evaluation standards. The IRHEC has identified the need to systematize recommendations and describe the best evidence on effectiveness.

Examining evidence of knowledge, attitudes and behavior in different countries to identify knowledge gaps is key. Research indicates that key ways to educate is in schools, using digital technology, social media and health professionals. For schools, curriculum vary in different countries from teaching nothing, to teaching how to prevent a pregnancy. Reproductive health education in schools will ensure that young adults will be well-equipped towards informed decision-making. The IRHEC has been working towards understanding how to best teach reproductive health, with work done in the UK, and repeated in Belgium and Greece. A teacher's guide in a "train-the-trainers" mentality has been produced to help teachers deliver reproductive health education. The challenge of an effective multidisciplinary collaboration between policymakers and educators is well acknowledged.

Research on education employing social media is valuable to establish who is influencing reproductive health education, what is their impact, what information they are posting, and how effective it is. Data can help to indicate how to best use social media to deliver fertility education.

A "one-size-fits-all" philosophy for reproductive health education is out of the question. Studies have indicated that target audience may perceive the education as guided and feel obligated to comply with societal pressures. This, in turn, may generate misconceptions and backlash resulting in questioning, suspicion and skepticism. Cultural, religious and socioeconomical factors complicate further, as does the role of social media in perpetuating misconceptions. The problem posed by lack of tailored information is showcased considering the paradigm of addressing the LGBTQ+ community, as there is limited data in literature, while the community may be rendered vulnerable because of marginalization, and normative pressure.

To address challenges, multidisciplinary should evolve to interdisciplinary. The future should hold recommendations and guidelines. Data highlights the significance of establishing government-sponsored fertility education programs, across all countries and for all ages, however Governments' approval pauses a conundrum. The way forward features personalized education, whilst aiming for high-quality robust evidence.

POSTER DISCUSSION SESSION

SESSION 40: SQART

Tuesday 27 June 2023 Hall D2 08:30 - 09:30

Abstract citation ID: dead093.141
P-767 Urogenital defects in live births to women dispensed clomiphene citrate: A study within a whole of population birth cohort

M. Davies[1], **A. Rumbold**[2], **L. Moore**[3], **R. Fernandez**[4], **L. Giles**[5], **H. McElroy**[6], **V. Moore**[5]

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Study question: Is the risk of urogenital defects increased in live births where clomiphene citrate is known to have been dispensed by a pharmacist to the individual?

Summary answer: The risk of urogenital defects is elevated in singletons, and approximately doubled in male babies among women dispensed clomiphene citrate, as compared to unexposed women.

What is known already: Clomiphene citrate (CC) has been used for ovulation induction since 1967 & is both inexpensive and largely effective. However, CC is a selective estrogen receptor modulator (SERM), which is a class of drugs with the potential for endocrine disruption during fetal development. The link between urogenital defects and CC has previously been reported by ourselves and others.

Whether the link continues to exist where individual level pharmaceutical prescribing and dispensing patterns are clearly ascertained has not been determined.

Secondly, the interaction of prescribed clomiphene citrate functioning as an endocrine disruptor by fetal sex has not undergone formal statistical testing.

Study design, size, duration: A population-based cohort study of South Australia was assembled by linking all records from the state-wide perinatal registry for births between July 2003 and December 2011, including defects coded to ICD9 and BPA, with Commonwealth government data on pharmaceutical dispensing in the national Pharmaceutical Benefits Scheme (PBS), which includes individual level prescribing and dispensing of drugs for ovulation induction, including clomiphene citrate.

Participants/materials, setting, methods: We analysed de-identified pregnancy outcome data for all births in South Australia, linked to individual level national prescription data, to examine the prevalence of urogenital birth defects (>20 weeks gestation) for births occurring between July 2003 and December 2011following clomiphene citrate dispensing, with 5-years follow-up for defect notifications. Multivariate analysis in STATA within a Secure Unified Research Environment (SURE) was used to calculate odds ratios, adjusted for maternal confounders and socioeconomic circumstance.

Main results and the role of chance: We examined 2,264 singleton live births where women were dispensed clomiphene citrate for infertility. Births among women dispensed clomiphene citrate, compared to those without this exposure, had increased urogenital defects (odds ratio (OR) = **1.73, CI = 1.34 - 2.24).**

This association was slightly reduced after adjustment for maternal characteristics that were potential confounders: age, socio-economic status (based on postcode of residence), Aboriginal ethnicity, born outside Australia in low or middle income country, parity, smoking, pre-existing diabetes or hypertension, pregnancy-induced hypertension and pre-eclampsia (OR = **1.66, CI = 1.28 -, 2.15)**.

There was evidence of an interaction with fetal sex, such that males were at particular risk.

Sex-by-clomiphene interaction	
Male	**1.92 (1.47, 2.52)**
Female	0.88 (0.37, 2.14)
Test for interaction	$\chi^2(1) = 2.72$; $p = 0.09$

The observed main effects are unlikely to be due to chance, and the sex-specific effect is biologically plausible, but limited in power. Further, there were 280 babies from multiple pregnancies to women exposed to clomiphene citrate that were excluded due to statistical limitations.

Limitations, reasons for caution: Our data report pharmacy dispensing, but not observed consumption, although infertile women are highly motivated to take this drug and over 5% of pregnancies were multiples, consistent with extensive use. The analysis for interactions and for multiples is currently restricted by power, which may resolve as the cohort number increases.

Wider implications of the findings: Clomiphene citrate (CC) may be a potent endocrine disruptor inducing urogenital defects, particularly in males. This is important as CC continues to be a WHO essential drug and a first line treatment globally, but with largely unmonitored use and without any industry initiated prospective safety study.

Trial registration number: Not applicable

Abstract citation ID: dead093.142
P-781 Assisted reproductive technology-associated risk factors for retained placenta

S.C. Jwa[1], S. Tamaru[1], M. Takamura[1], A. Namba[1], T. Kajihara[1], O. Ishihara[1], Y. Kamei[1]

[1]Saitama Medical University, Department of Obstetrics and Gynecology, Saitama, Japan

Study question: What are the assisted reproductive technology-associated risk factors for retained products of conception (RPOC) following delivery?

Summary answer: Frozen embryo transfer (FET) with hormone replacement cycles (HRC) constitutes the largest risk factor for RPOC.

What is known already: RPOC is a key cause of secondary postpartum hemorrhage. Previous studies have shown that the use of ART increases the risk of RPOC and subsequent transfusion following delivery. However, the relationships between specific ART-treatment factors and the risk of RPOC have not been elucidated.

Study design, size, duration: We performed a registry-based retrospective cohort study using the Japanese national ART registry, in which all the ART cycles performed in Japan are recorded including the perinatal outcomes.

Participants/materials, setting, methods: Singleton live births between 2007 and 2017 were studied (n = 306,411). Odds ratios (ORs) and 95% confidence intervals (CIs) for the potential risk factors for RPOC associated with fresh and frozen cycles were obtained from multiple logistic regression analysis, incorporating adjustment for maternal age, infertility, and calendar year. Because information regarding assisted hatching (AH) and HRC for endometrial preparation only became available in 2012, these factors were analysed using the data collected between 2012 and 2017.

Main results and the role of chance: RPOC was diagnosed for 132 deliveries (0.04%), of which 122 (92.4%) followed FET cycles and only 10 (0.01%) followed fresh embryo transfer (ET). No significant risk factors for RPOC were identified using the fresh ET data. For FET cycles, the use of HRC as an endometrial preparation method represented the largest risk factor (adjusted OR, 4.9; 95% CI, 2.0 to 12.3), with RPOC occurring in 0.05% of deliveries following HRC (51/97, 958 deliveries) but in only 0.01% following natural cycles (5/47, 79 deliveries). AH was also associated with a significantly higher risk of RPOC (adjusted OR, 1.9; 95% CI, 1.2 to 3.1), although the risk was lower than that associated with HRC). Subgroup analysis showed that these significant associations of HRC and AH with RPOC were present for transvaginal deliveries, but not deliveries by caesarean section.

Limitations, reasons for caution: The registry lacks important information regarding risk factors for RPOC, such as parity, body mass index, and history of previous uterine surgery. Furthermore, the lower prevalence of RPOC in the present study (0.04%) than in other studies (approximately 1%) suggests the possibility of non-differential misclassification for the outcomes of RPOC.

Wider implications of the findings: Although the possibility of residual confounding factors remains, the present findings suggest that additional care should be taken in the management of patients undergoing vaginal deliveries following FET who have HRC or AH.

Trial registration number: Not applicable

Abstract citation ID: dead093.143

P-782 The influence of microfluidic preparation of spermatozoa for ICSI (intracytoplasmic sperm injection) in an unselected IVF population on fertilization rate, embryo-quality and pregnancy rate

C.A. Huniadi[1], I. Zaha[2], S. Liana[2], N. Petronela[3], S. Andrea[2], R. Ioana[4], B. Cosmina[3], R. Miheala[3]

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Study question: Does the microfluidic technique as a new preparation option for sperm in ICSI during IVF have a better outcome in fertilization, blastocysts and pregnancy rate?

Summary answer: The patients that had the sperm preparation for ICSI with the microfluidic sperm selection had similar results as with the gradient centrifugation selection.

What is known already: Semen analysis is a poor predictor of the fertilization potential of spermatozoa and a male factor may contribute to poor outcomes of IVF procedure despite a normal semen analysis. Traditionally, sperm processing is accomplished by density gradient centrifugation or swim-up methods but this have been shown in some studies to increase reactive oxygen species and induce sperm DNA fragmentation. The microfluidic sperm selection (ZyMot- ICSI) is based on the selection of the spermatozoa with the lowest DNA fragmentation rate but studies do not prove better clinical outcomes after this method.

Study design, size, duration: This is a prospective randomized study carried out in a university level fertility clinic in Oradea, Romania from January 2022 to December 2022, including 239 patients in total. The patients that underwent IVF-ICSI procedures for various indications were randomized in two groups: the control group swim-up technique was used for sperm selection for ICSI during IVF and the study group with the ZyMot-ICSI (microfluidic sperm selection technique) was used.

Participants/materials, setting, methods: We conducted a prospective trial comparing 119 couples that were allocated to the classic swim-up technique (control group) and 120 couples that were allocated with the microfluidic technique was used (study group) in our university level clinic to go through IVF. Inclusion criteria were couples with known infertility requiring IVF and willing to participate in the study. Exclusion criteria were: severe oligoasthenospermia (concentration $<$ 5x106 spermatozoa/ml or motility $<$10%), oocyte or sperm donation.

Main results and the role of chance: The statistical analysis showed that there is no significant difference between the fertilization rate (study vs control $p = 0.37$ with absolute difference -1.182, 95% CI (-7.2, 5.4)) or blastocyst rate (study vs control $p = 0.88$ with absolute difference -0.5 95% (-6.5, 6)) nor is there a difference in clinical pregnancy. Even if microfluidic preparation of spermatozoa did not seem to improve the results it may be utilized more broadly for standard IVF, intrauterine insemination and could also improve workflow, decrease intervention by laboratory personal and provide more consistent incubation conditions.

Limitations, reasons for caution: The population studied was inclusive with unselected IVF indications and did not attempt to isolate male factor infertility cases or patients with a history of elevated sperm DNA fragmentation.

Wider implications of the findings: Microfluidic sperm preparation performs similarly to density gradient centrifugation in sperm preparation for IVF in an unselected population.

Trial registration number: Not applicable

Abstract citation ID: dead093.144

P-789 Unexplained subfertility in women aged 38 years and above: could intrauterine insemination be an alternative for in vitro fertilization? A systematic review and meta-analysis

J. Rake[1], M. Mochtar[1], E. Van Putten[1], M. Van Wely[2]

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[2]*Amsterdam UMC, Center for Reproductive Medicine, Center for Reproductive Medicine, The Netherlands*

Study question: Considering current available evidence, how do intrauterine insemination and IVF compare in terms of pregnancy outcomes in women aged $>$ 38 years with unexplained subfertility?

Summary answer: In women $>$ 38 years both IUI and IVF lead to pregnancies and live births, there is insufficient evidence to determine the relative effectiveness

What is known already: Solid evidence has shown that intrauterine insemination (IUI) with ovarian stimulation (OS) is the preferred treatment option for women with unexplained subfertility below the age of 38. In women who are 38 or older IVF is the first choice of treatment, according to the Dutch guideline in order to "not waste any more time" Evidence of IVF being more effective compared to IUI-OS is lacking while IVF compared to IUI-OS is an invasive, costly and burdensome procedure.

Study design, size, duration: We have conducted a systematic review and meta-analysis after a comprehensive literature search which includes articles on IVF, IUI, and both, from May 1985 to May 2022. We have included randomized controlled trials and cohort studies regarding IUI and/or IVF treatment in women aged 38 and above with unexplained subfertility and/or mild subfertility factors.

Participants/materials, setting, methods: The primary outcome was live birth rate and primary safety outcome was miscarriage rate. Data of the comparative IUI versus IVF studies were pooled in a forest plot and expressed in risk ratio with a 95% CI using a random effect model. We calculated prevalence estimates and performed meta-regression to study the effect of age on live birth and miscarriage

Main results and the role of chance: We included 17 studies in our meta-analysis. One RCT compared two IUI cycles with two IVF cycles and found a halving of live birth (RR 0.46, 95% CI 0.25-0.86. $n = 154$), very low-quality evidence especially in view of the unfair comparison in treatment time. Pooling the data of five cohort studies that compared IU with IVF resulted in a comparable risk estimate, though the difference between IUI and IVF was not significantly different (RR 0.56, 95% CI 0.30-1.05, $I^2 = 73\%$, $n = 7288$), low quality evidence in view of the unequal distribution of female age over the groups and the heterogeneity.

Meta-regression on data from IUI studies suggested that IUI above 43 years old resulted in pregnancy chances below 5% (3 studies $n = 397$, only women older than 40). Meta-regression on data from IVF studies (8 studies, $n = 22\,708$) suggested that following IVF, live birth rates declined from about 20% at 38 years to 5% at 43 year and zero above 44 years. At the same time miscarriage rates increased from an estimate of 25% at 38 years old to more than 50% above a female age of 43 years.

Limitations, reasons for caution: Female age, a likely effect modifier, was not equally distributed in the cohort studies, especially in the small IUI cohort studies. The available evidence lacked information on duration of treatment. For a fair comparison the treatment time horizon should be equal.

Wider implications of the findings: It is expected that the average age of women trying to conceive will only further increase within the coming years. Findings of this systematic review underline the importance of a randomized controlled trial to provide more evidence on which treatment should be recommended for women $>$ 38 with unexplained subfertility.

Trial registration number: Not applicable

SELECTED ORAL COMMUNICATIONS

SESSION 41: TIME-LAPSE AND EMBRYO MORPHOKINETICS

Tuesday 27 June 2023 Hall A 10:00 - 11:30

Abstract citation ID: dead093.145

O-118 Morphokinetic embryo behavior in low-prognosis patients according to the POSEIDON criteria: an analysis of 3326 injected oocytes

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[4]*Fertility Medical Group, Clinical department, Sao Paulo, Brazil*

Study question: Do embryo quality and morphokinetic behavior differ in the four groups of low-prognosis women as stratified by the POSEIDON criteria?

Summary answer: Embryo quality and morphokinetic behavior differ across the POSEIDON groups, being more favorable in POSEIDON group 1, as well as were implantation and miscarriage rates.

What is known already: Poor ovarian responders (POR) represent 9-24% of patients undergoing ovarian stimulation, and the clinical management of these patients remains a challenge in everyday practice. In an attempt to standardize the diagnosis of POR, the medical community developed the Bologna Criteria and then shifted to the Patient-Oriented Strategies Encompassing IndividualizeD Oocyte Number (POSEIDON). Embryo monitoring with time-lapse imaging (TLI) may allow the identification of morphokinetic events potentially affected by ovarian response. The aim of this study was to compare embryo quality, and speed and pattern of cell divisions in the four groups of POR as stratified by the POSEIDON criteria.

Study design, size, duration: This cohort study included low-prognosis patients undergoing their first intracytoplasmic sperm injection (ICSI) from Mar/2019 to Apr/2022 at a private-university affiliated IVF center, who met the POSEIDON criteria. A total of 3326 injected oocytes from 846 women were analyzed. Kinetic markers from the point of insemination were recorded. Generalized mixed models followed by Bonferroni post hoc were used to compare morphokinetics among the POSEIDON groups. The post hoc achieved power was > 80%.

Participants/materials, setting, methods: Injected oocytes were cultured in the EmbryoScope$^+$ incubator, which recorded the following kinetic markers: timing to pronuclei appearance and fading (tPNa and tPNf), two (t2), three (t3), four (t4), five (t5), six (t6), seven (t7), and eight cells (t8), morulae (tM), start of blastulation (tSB) and blastulation (tB). Durations of second and third cell cycles (cc2 and cc3) and timing to complete synchronous divisions s1, s2, and s3 were calculated. The KIDScore ranking was recorded.

Main results and the role of chance: The results from the generalized mixed models followed by Bonferroni post hoc for the comparison of means among groups showed that embryos derived from patients in the POSEIDON groups 2, 3 and 4 showed significantly slower divisions compared to those from POSEIDON 1 group (Table 1). The KIDScore rank was significantly lower for embryos derived from POSEIDON groups 2, 3 and 4 (2: 4.4 ± 0.7 vs. 3: 4.2 ± 0.2 vs. 4: 3.0 ± 0.4) compared to those derived from POSEIDON 1 group (4.8 ± 0.1, $p < 0.001$). Group POSEIDON 1 also showed improved implantation (26.9% vs. 2: 22.4% vs. 3: 20.0% vs. 4: 14.0, $p < 0.001$) and miscarriage rates (5.6% vs. 2: 31.2% vs. 4: 50.0%, $p = 0.013$).

Limitations, reasons for caution: The use of historical cohort groups is a drawback. Despite the eligibility criteria for inclusion in the analysis, potential differences in the baseline characteristics cannot be ruled out.

Wider implications of the findings: Embryo development was more favorable in POSEIDON group 1, suggesting a direct relationship between oocyte quantity and quality, and that oocyte quality is determinant of embryo development. These show the reasonability of classifying POR by the POSEIDON criteria, providing information for the appropriate counselling of POR regarding their possible prognosis.

Trial registration number: N/A

Abstract citation ID: dead093.146

O-119 Time-lapse comprehensive portrait of cytoplasmic strings appearing during blastulation: prevalence and implications for embryo assessment.

A. Marconetto[1], F. Innocenti[2], G. Saturno[3], M. Taggi[3], V. Chiappetta[2], L. Albricci[2], R. Maggiulli[2], D. Soscia[2], V. Casciani[2], F. De Falco[4], G. Coticchio[5], A. Ahlström[6], F.M. Ubaldi[2], L. Rienzi[2,7], D. Cimadomo[2],

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O-118 Table 1

Variables (h)	POSEIDON 1	POSEIDON 2	POSEIDON 3	POSEIDON 4	p-value
t2	27.7 ± 0.3[a]	28.2 ± 0.2[ab]	27.0 ± 0.8[a]	28.8 ± 0.4[b]	0.025
t4	39.6 ± 0.3[a]	40.7 ± 0.2[b]	40.7 ± 0.9[ab]	41.2 ± 0.5[b]	0.008
t7	56.3 ± 0.5[a]	57.6 ± 0.3[b]	56.5 ± 1.4[ab]	58.5 ± 0.7[b]	0.017
t8	59.8 ± 0.7[a]	60.9 ± 0.3[ab]	62.5 ± 1.5[b]	62.1 ± 0.8[b]	0.014
tM	85.1 ± 1.55[a]	90.7 ± 0.3[b]	90.1 ± 1.4[b]	90.1 ± 0.8[b]	0.003
tsB	97.7 ± 0.55[a]	101.3 ± 0.3[b]	102.3 ± 1.5[b]	101.4 ± 0.8[b]	0.023
tB	107.6 ± 0.6[a]	110.0 ± 0.4[b]	109.4 ± 1.8[b]	111.4 ± 0.9[b]	0.022
cc2	11.1 ± 0.2[a]	11.0 ± 0.1[a]	11.0 ± 0.2[a]	12.3 ± 0.3[b]	0.018
s2	1.6 ± 0.2[a]	2.4 ± 0.1[b]	2.6 ± 0.5[ab]	2.8 ± 0.3[b]	0.002
s3	9.2 ± 0.4[a]	10.1 ± 0.2[ab]	11.3 ± 1.1[ab]	11.3 ± 0.6[b]	0.012

Study question: What are the prevalence and implications of cytoplasmic strings (Cyt-S) in preimplantation development after blastulation?

Summary answer: Cyt-S are common in human embryos and are associated with faster blastocyst development, larger expansion, and better morphological quality.

What is known already: Cyt-S are dynamic cellular projections connecting inner-cell-mass (ICM) and trophectoderm during blastocyst expansion. Their prevalence in human embryos *in vitro* has been estimated 44-93%. Currently, their role is unknown. Studies in animal models suggested they might be involved in ICM-to-trophectoderm communication or cell fate-related events. Nevertheless, comprehensive descriptions and clear definitions, evidence-based hypotheses about their function, and data about their clinical implications are lacking, limited or controversial. Here we leveraged time-lapse microscopy, artificial intelligence, and chromosomal testing to comprehensively portray these common features of blastulation and expansion processes.

Study design, size, duration: Observational study involving 124 PGT-A cycles in EmbryoScope (Vitrolife) with ≥1 blastocyst (N = 315) between May-2013 and November-2014. Timings from tSB to biopsy (t-biopsy, i.e., blastocyst full-expansion) in hours-post-insemination (hpi) and embryo area (including zona-pellucida in μm^2) were automatically annotated through an AI-based software (CHLOE™, Fairtility). One senior embryologist annotated Cyt-S presence, number, timings, and type (thick cell-to-cell connections and/or threads), and blastocyst collapses (i.e., reduction of embryo area preventing the discrimination of ICM from trophectoderm).

Participants/materials, setting, methods: ICSI, continuous blastocyst culture (day 5-7) in time-lapse incubators, trophectoderm biopsy without zona-pellucida drilling on fully-expanded blastocysts, and qPCR to assess non-mosaic full-chromosome aneuploidies were all conducted. Blastocyst morphological quality was defined according to Gardner's schemes as excellent (AA), good (AB,BA), average (BB,AC,CA) and poor (CC,BC,CB). Only vitrified-warmed euploid single-embryo-transfers were performed. Along with developmental timings, extent of expansion and morphological quality, we also assessed euploidy and live-birth rates in blastocysts with and without Cyt-S.

Main results and the role of chance: 94.4% of the patients (N = 117/124) had ≥1 embryo with Cyt-S (mean:2.2 ± 1.6, range:0-9). 86% of all blastocysts analyzed (N = 271/315) had ≥1 Cyt-S (3.5 ± 2.1, 1-13; duration of the longest Cyt-S: 5.8 ± 3.4 hours, 0.5-20.9). Overall, we analyzed 937 Cyt-S detected at 117.3 ± 11.8 hpi (85.7-160.5) and lasting 3.8 ± 2.7 hours (0.2-20.9). The first Cyt-S of each embryo lasted longer (4.4 ± 3.2 hours) compared to the following (≈3 hours or less). Cyt-S were mostly threads (N = 133/271, 54.2%) or thick cell-to-cell connections becoming threads (N = 382/937, 40.8%) than connections of constant diameter (N = 47/937, 5%). Of the 271 embryos with ≥1 Cyt-S, 71.9% showed these projections only during early expansion processes after tSB (N = 195; 3.0 ± 1.6, 1-10), 7.7% only after collapses (N = 21; 2.6 ± 1.5, 1-6), and 20.3% both in early expansion and after collapses (N = 55; 2.3 ± 1.3 and 3.2 ± 2.4, 1-11 respectively). Poor- versus excellent-quality blastocysts (N = 54/75, 72% versus N = 120/129, 93%, multivariate-OR=0.32, 95%CI 0.11-0.92, adjusted-p=0.03), t-biopsy (multivariate-OR=0.97, 95%CI 0.95-0.99, p = 0.05) and blastocyst area at t-biopsy (multivariate-OR=1.1, 95%CI 1.01-1.18, p = 0.02) were all associated with the prevalence of embryos with Cyt-S. Lastly, while euploidy rates were comparable between blastocysts with and without Cyt-S, an association instead was shown with live-birth-rates per vitrified-warmed euploid transfers (N = 34/77, 44.2% versus N = 0/10, 0%; p < 0.01), although these data are very preliminary.

Limitations, reasons for caution: Some Cyt-S after collapses might correspond to connections already present before these events. Larger datasets are required to confirm putative associations between Cyt-S and clinical outcomes. Only associations could be reported here, but not causations/consequences. Specific basic research studies are required to this end

Wider implications of the findings: Embryos with ≥1 Cyt-S were faster in reaching all blastocyst timings (tSB to t-biopsy) and to expand, achieved a larger area at t-biopsy and had a better morphology. These dynamic features of blastocyst expansion might be physiological and represent biomarkers of embryo quality. Nevertheless, their function is yet unknown

Trial registration number: not applicable

Abstract citation ID: dead093.147

O-120 Predicting Embryo Ploidy Status Using Time-lapse Images

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Study question: Can deep learning models using time-lapse images of embryo development be used to predict embryo ploidy and provide supplemental information to embryologists for clinical decision-making?

Summary answer: We developed a general MDBS-Ploidy model that uses time-lapse images and maternal age to predict embryo quality scores and ploidy status.

What is known already: Ploidy status, or the presence or lack of chromosomal abnormalities, is an important factor in a successful pregnancy. Embryos with abnormalities are classified as aneuploid, whereas those without are euploid. With the advent of artificial intelligence in computer vision and the collection of large IVF-related datasets combining images, videos, and clinical outcomes, a variety of methods have been developed to automatically assess embryo quality and other characteristics using images from time-lapse sequences. To our knowledge, a video classification model validated by heterogeneous data to predict both embryo quality score and ploidy is lacking.

Study design, size, duration: The training dataset for the model consisted of 1,998 time-lapse sequences captured by the Embryoscope®. Time-lapse image sequences consisted of around 360–420 frames captured during 5 days of development. PGT-A results were used as ground truth labels for all ploidy prediction tasks, with embryos classified as euploid or aneuploid. The dataset also included clinical information such as blastocyst score (BS) and maternal age at the time of oocyte retrieval.

Participants/materials, setting, methods: The MDBS-Ploidy consists of two steps. The first step is quality score prediction from day-5 time-lapse video input using a Bidirectional Long Short-Term Memory architecture with added output layers for multitasking. In the second step, the predicted score, in addition to maternal age, is used to predict the ploidy status of the embryo using a logistic regression model. We evaluated the performance of the MDBS-Ploidy using area under the receiver-operating-characteristic (AUROC) on a validation dataset.

Main results and the role of chance: For the MDBS-Ploidy quality score prediction module, the Pearson correlation between scores predicted by the MDBS-Ploidy and ground truth quality scores is 0.70 on the validation dataset, suggesting moderate correlation strength. As for the aneuploidy prediction module, the MDBS-Ploidy can discriminate between euploidy and aneuploidy with an AUROC of 0.76 ± 0.002. The MDBS-Ploidy performs comparably with a model trained on the embryologist-annotated blastocyst score. We replicate these comparative results in external validation datasets as well, namely the Embryoscope+® dataset with 1,000 embryos and the IVI Valencia Spain dataset with 543 embryos. Moreover, the MDBS-Ploidy is completely automated, requiring only time-lapse images from 96 to 112 hpi and maternal age to predict embryo ploidy status. This allows the MDBS-Ploidy to be adapted clinically without interrupting ongoing workflows. The MDBS-Ploidy also provides a certain level of explainability; embryologists can use the intermediate quality score (from the first module) to determine why an embryo is classified as a certain ploidy status. With a recall of 0.84 ± 0.004 for the validation dataset, the MDBS-Ploidy shows promise for successfully selecting euploid embryos. We believe that the MDBS-Ploidy can provide supplemental information for selecting the most viable embryo.

Limitations, reasons for caution: Our model primarily uses data from time-lapse microscopy. Clinics without access to this technology will be unable to use our model.

Wider implications of the findings: This model is clinically relevant for making decisions as to whether an embryo may be chromosomally normal. Automated quality score prediction is instrumental to embryologists who are

currently annotating embryos manually. Future iterations of the model can theoretically be adopted into clinical practice because it is end-to-end and fully automated.

Trial registration number: not applicable

Abstract citation ID: dead093.148

O-121 Temporal development of scores from a time-lapse based artificial intelligence provides no additional benefit compared to the latest score

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Study question: Does the temporal development of scores from an artificial intelligence (AI) based embryo selection model provide additional predictive power compared to the latest score?

Summary answer: Earlier predictions provide no additional predictive power given the latest prediction. Knowing the development of AI scores is thus not beneficial for embryo selection.

What is known already: Traditionally, embryo grading takes into consideration the final morphology score together with the history of embryo development events. Recent publications have shown that time-lapse based AI models trained on clinical outcomes can automatically rank embryos by the likelihood of implantation that equal or surpass traditional methods, without the necessity for manual evaluation. AI-based methods based on time-lapse data may account for the impact of the full temporal development on likelihood of implantation. In this study, we examine if the addition of previous scores adds to the performance of an embryo selection model.

Study design, size, duration: A retrospective multicenter study of transferred embryos (n = 2422) with known implantation data (KID) between 2012-2020 from 21 international clinics. Embryos were cultured in EmbryoScope time-lapse incubators for at least 5 days. Both single and multi-embryo transfers in fresh and warmed cycles were included. Embryos were classified as positive (KIDp) or negative (KIDn) by the presence of fetal heartbeat.

Implantation likelihood was evaluated at cleavage- and blastocyst stages by a 3D CNN model; iDAScore v2.0.

Participants/materials, setting, methods: A kernel-based conditional independence test was used to evaluate if a score at x hours post insemination (hpi) provide extra information compared to a score at y hpi. The test estimates the likelihood that a prediction after x hpi does not provide any additional information with regards to predicting KIDp when a prediction after y hpi is available. These likelihoods (p-values) were Bonferroni corrected to adjust for multiple comparisons.

Main results and the role of chance: KIDp predictions were computed for all embryos at 40, 44, 64, 68, 112, and 116 hpi corresponding to early and median time of transfer for day 2, 3, and 5. For all time points, the conditional independence test showed that there was no added information from knowing predictions at an earlier time point ($p > 0.21$).

Similarly, it was tested if a known later prediction will improve model performance for an earlier prediction. This was always the case ($p < 0.01$) except for predictions close in time (68 vs 64 hpi; $p = 1.0$) and (44 vs 40 hpi; $p = 1.0$). This may reflect that there was little additional embryo development information during a 4-hour period at this development stage.

The above shows that knowledge of the temporal score development does not improve model performance compared to using latest available score. This means that an AI model based on time-lapse videos likely considers earlier embryo development events in the context of the entire development history.

Limitations, reasons for caution: This study is a retrospective study that only looks at AI based on time-lapse videos. There is a potential bias as the embryos were likely of higher quality having been chosen for transfer. It is possible that the results do not generalize to simpler non-3D CNNs.

Wider implications of the findings: Knowing the temporal score development can introduce subjectivity that might lower implantation rates as the earlier scores were shown to not provide any significant prediction improvement.

Trial registration number: not applicable

Abstract citation ID: dead093.149

O-122 The first study to assess the clinical efficacy of CHLOE-EQ on the assessment of embryo viability of embryos cultured in a GERI time-lapse incubator

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Study question: Can an automatic AI scoring system predict ploidy, live birth and utilization? Are there differences in AI scoring between donor and own gametes?

Summary answer: CHLOE-EQ Score is directly associated with oocyte quality, ploidy, utilization, live birth, embryo quality, direct uneven cleavage (DUC), blastulation, utilization and selection for transfer.

What is known already: The integration of AI algorithms, such as CHLOE-EQ, into different Time-lapse systems requires clinical and biological validation. Geri Time-lapse videos have given the embryologists more insight into embryo development. Analyzing this information manually requires time and introduces risk of error. To tackle this issue, AI solutions like CHLOE-EQ (Fairtility) can be used to automatically assess video datapoints. CHLOE-EQ provides an embryo quality score that has been shown to predict embryo viability and ploidy, providing clarity on the underlying biological factors. Before introducing AI tools in clinical practice, it is crucial to confirm their efficacy and validate with clinical data.

Study design, size, duration: A retrospective cohort analysis was conducted at a private clinic in Spain from April 2021 to November 2022, involving the review of 3196 Geri time-lapse videos with a subset of known ploidy and live birth outcomes. The correlation of CHLOE-EQ score with ASEBIR clinic grading was evaluated. As well as with DUCs, oocyte quality, sperm source, blastulation, utilization, selection for transfer, ploidy and live birth.

Participants/materials, setting, methods: Geri time-lapse videos were automatically analyzed by CHLOE-EQ (Fairtility). CHLOE-EQ score was assessed in relation to laboratory (ploidy, clinic ASEBIR embryo scoring, utilization, selection for transfer) and clinical outcomes (live birth), as well as between own vs donor gametes (own eggs >40y vs donor eggs and testicular sperm vs donor sperm) using descriptive statistics and t-test. The accuracy of prediction was measured using binary logistic regression (AUC).

Main results and the role of chance: CHLOE-EQ score was positively correlated with ASEBIR embryo quality (A:8.7 ± 1.9, n = 349 >B:6.8 ± 2.9, n = 470 > C: 5.1 ± 3.0, n = 124 > D:1.3 ± 2.1, n=751; $p < 0.05$). Non-DUCs had higher CHLOE-EQ Score than DUCs [5.3 ± 3.8, n=1798 vs 1.9 ± 0.38, n=643, $p<0.001$]. CHLOE-EQ Score was unaffected by the quality of the sperm sample, with similar CHLOE-EQ scores between donor sperm and testicular derived sperm (4.1 ± 3.9, n=335 vs 3.4 ± 4.2, n=56, respectively, NS).

Embryos that blastulated (yes vs no: 5.4 ± 3.7, n=1996 vs 0.6 ± 2.1, n=309, $p < 0.001$), were utilized (7.4 ± 0.28, n=911, vs 1.0 ± 2.1, n = 1309, $p < 0.001$), selected for transfer (8.7 ± 2.4, n=153 vs 3.3 ± 3.7, n = 2067, $p < 0.001$), were euploid (7.5 ± 2.5, n=72 vs 6.3 ± 3, n=152, $p = 0.001$) and resulted in live births (4.4 ± 4.1, n = 332 vs 3.8 ± 4, n = 499, $p = 0.02$) had a higher CHLOE-EQ score than embryos that did not.

CHLOE-EQ Score is higher in embryos derived from oocytes from donors than own eggs, suggesting that oocyte quality affects CHLOE-EQ score (4.0 ± 4, n = 1189 vs 2.7 ± 3.4, n = 356).

CHLOE-EQ Score is predictive of utilization (AUC=0.95, n=2220, baseline=41%, p<0.001), euploidy (AUC=0.63, n=224, baseline=32.1%, p=0.003), blastulation (AUC=0.94, n = 2305, baseline=86.6%, p < 0.001) and selection for transfer (AUC=0.89, n = 2220, baseline=41%, p<0.001).

Limitations, reasons for caution: This is a retrospective single-center study in which embryos for transfer were selected by human embryologists, and forms part of program to validate the responsible integration of AI into clinical practice in each individual clinic.

Wider implications of the findings: This is the first study presenting the efficacy of prediction of CHLOE-EQ with GERI data. AI tools have the potential to improve consistency, efficiency, and accuracy of embryo assessment and selection. CHLOE-EQ predicts through quantitative and qualitative morphological and morphokinetics information, resulting in more personalized care for each individual embryo.

Trial registration number: not applicable

Abstract citation ID: dead093.150
O-123 Time lapse incubation and morphokinetic embryo evaluation are not enough. Results from a Randomized controlled PILOT study

G. Sacks[1], H. Mozes[1], E.G. Talia[1], O. Schonberger[1], R. Ronn[1], I. Ben-Ami[1], N. Srebnik[1]

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Study question: Does the TLI have an added value in reproductive outcome when separating the system's two components, undisturbed culturing, and morphokinetic embryo grading?

Summary answer: The TLI system itself does not have a significant added value. More advanced algorithms should be applied to aid in embryo selection process.

What is known already: The TLI technology has been widely used for over two decades for embryo culturing. A Cochrane meta-analysis found no significant difference in clinical pregnancy rate using TLI compared to conventional incubators. Another RCT concluded that the addition of TLI morphokinetic data did not significantly improve clinical reproductive outcomes. On the other hand, the benefit of TLI is the identification of abnormal cleavage dynamics and analysis of other morphokinetic parameters although no proven increase in pregnancy rate was shown. Several Artificial intelligence algorithms use TLI data to increase the pregnancy rate and embryo selection process.

Study design, size, duration: A prospective, randomized, double-blinded, single-center, controlled study.

After applying inclusion criteria, 102 women were included in the analysis, 34, 32, and 36 in arms 1,2, and 3 respectively, with a total of 1061 embryos evaluated, 420 in arm 1, 285 in arm 2, and 356 in arm 3. Patients were recruited between November 2017- July 2020.

Participants/materials, setting, methods: University-affiliated Medical Centre in vitro fertilization (IVF) clinic.

Patients were randomized into 3 groups: 1) conventional incubator with morphological evaluation only; 2) TLI incubator with both morphological and morphokinetic evaluation; 3) TLI incubation with morphological evaluation only. All were cultured in MIRI ESCO incubators. The primary outcome was live birth rates. Secondary outcomes were clinical pregnancy rates and cumulative pregnancy rates. Embryo quality and morphokinetic scores.

Main results and the role of chance: No differences were found in the rated day 2 top quality embryos (TQE), day 3 TQE or blastocyst stage TQE, and a similar number of embryos were suitable for either transfer or cryopreservation between the groups. On the other hand, higher rates of multinucleation were found in group 1 (31.9 ± 6 vs. 16.6 ± 5 vs.14.9 ± 5, p = 0.03 for groups 1,2 and 3 respectively).

Regarding primary outcome, we did not find any significant difference in live birth rate between the groups, neither for single embryo transfer cycles (SET) (35% vs. 31.6% vs. 24%, p = 0.708) nor for double embryo transfer cycles (DET) (41.7% vs. 38.5% vs. 36.4%, p = 0.966 for groups 1,2 and 3 respectively). Also, no differences were noted between pregnancy rates and clinical pregnancy rates. After concluding the first part of the study, embryos in arm 3 were retrospectively reevaluated, gaining additional morphokinetic data for the embryo selection process. In more than half of the embryos selected for transfer, the morphokinetic and morphologic evaluations were similar. For the additional 32 embryos from a total of 12 cycles, there was a discrepancy between the grades. In the other cases, the use of morphokinetic scoring would not have changed significantly the end point of pregnancy.

Limitations, reasons for caution: The pandemic affected patient recruitment and smaller numbers than intended were achieved leaving the study sample underpowered for the primary endpoint. Both day 3 and day 5 transfers were performed, and cycles were not always restricted to single embryo transfer, although the study was designed as such.

Wider implications of the findings: This study underlines the maybe unjustified morphkinetic scoring grading system which requires much time and attention from the embryologist to view and annotate and should be reconsidered especially with the rise of substitute software-assisted assessment algorithms showing an additional advantage in embryo selection and implantation rate.

Trial registration number: ClinicalTrials.gov Identifier: NCT02657811.

INVITED SESSION
SESSION 42: LIVE SURGERY SESSION

Tuesday 27 June 2023 Hall D3 10:00 - 13:00

Abstract citation ID: dead093.151
O-124

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Abstract citation ID: dead093.152
O-125

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Abstract citation ID: dead093.153
O-126

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Abstract citation ID: dead093.154
O-127

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Abstract citation ID: dead093.155
O-128

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Abstract citation ID: dead093.156
O-129

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SELECTED ORAL COMMUNICATIONS
SESSION 43: NEW CONCEPTS: OVARIAN STIMULATION
Tuesday 27 June 2023 Hall D1 10:00 - 11:15

Abstract citation ID: dead093.157
O-130 Initiation of ovarian stimulation in the late follicular phase, a randomized controlled trial

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Study question: Does late follicular phase stimulation yield similar outcomes compared with conventional early follicular phase stimulation in a GnRH antagonist protocol in oocyte donors?

Summary answer: Late follicular phase stimulation is not inferior in terms of number of oocytes compared to early follicular phase stimulation in a GnRH antagonist protocol.

What is known already: In patients undergoing fertility preservation for medical reasons, late follicular phase stimulation has been effectively used, resulting in similar numbers of total and mature oocytes obtained, oocyte maturation rate, mature oocyte yield, and fertilization rates compared to conventional early follicular phase ovarian stimulation. Because of LH suppression by endogenous progesterone in the luteal phase, there is less need for the use of a GnRH antagonist.

Study design, size, duration: This is an open label, phase 3, non-inferiority, randomized controlled trial using a two-arm design with 1:1 allocation ratio. The study included 71 oocyte donors between 18 and 36 years, with a regular menstrual cycle length and BMI 19-35 kg/m2, who underwent ovarian stimulation between November 2018 and May 2022. Patients were allocated to either early follicular start (Group A, n = 36), or to late follicular start (Group B, n = 35).

Participants/materials, setting, methods: In Group A, patients followed a fixed GnRH antagonist protocol with r-FSH 225IU daily. In Group B, r-FSH 225IU daily was initiated when a dominant follicle and late follicular hormonal values were observed, a GnRH antagonist was added if serum LH-levels were >10IU/L. The primary outcome was number of cumulus-oocyte-complexes (COCs). Secondary endpoints included number of mature oocytes, consumption of gonadotropins, duration of ovarian stimulation, days of GnRH antagonist used, and cost of the stimulation.

Main results and the role of chance: Using an intention-to-treat analysis, the total number of oocytes did not differ between Group A and Group B (17.7 ± 10.0 vs 17.2 ± 9.1, p = 0.82, difference 0.49 ,95% CI (-4.04 to 5.03)). In the per protocol analysis, after excluding 4 patients, there was no difference between Group A and Group B (18.2 ± 9.7 vs 18.8 ± 7.8, p = 0.62, difference -0.6, 95% CI (-4.9 to 3.7)).The number of mature oocytes did not differ between Group A and Group B (14.1 ± 8.1 vs 12.7 ± 8.5, p = 0.48). In none of the treatment arms OHSS was observed. The duration of stimulation was shorter in Group A than in Group B (10.0 ± 1.6 vs 10.9 ± 1.5 days, p = 0.01). The total amount of r-FSH used was lower in group A than in Group B (2240.7 ± 313.9 IU vs 2453.9 ± 330.1 IU p = 0.008). A GnRH antagonist was used for approximately 6 days in Group A, while in group B, only in one patient a GnRH antagonist was prescribed for 4 days (6.0 ± 1.4 days vs 0.13 ± 0.7 days p < 0.001). There was a significant difference in total medication cost per cycle between both protocols (1147.9 ± 182.8 € in Group A, vs 979.9 ± 129.0 € in group B, p < 0.001), i.e. a cost reduction of 15% for Group B as compared with Group A.

Limitations, reasons for caution: A limitation of the study is the lack of embryology data. Because of adaptations in the oocyte donation program in our center over the years, this study contains a mix of fresh and frozen donation cycles, leading to a very heterogenous group, making correct interpretation of the embryology data difficult.

Wider implications of the findings: Late follicular phase stimulation is as efficient as early follicular phase stimulation in terms of number of oocytes. It is patient-friendly, with reduced cost and reduced number of injections.

Trial registration number: NCT03767218

Abstract citation ID: dead093.158
O-131 BEYOND: a randomised controlled trial comparing efficacy and safety of individualised follitropin delta dosing in GnRH agonist versus antagonist protocols for first ovarian stimulation cycle

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Study question: How does a long GnRH agonist versus a GnRH antagonist protocol affect ovarian response when using individualised fixed daily follitropin delta dose for ovarian stimulation?

Summary answer: BEYOND trial data demonstrates that individualised follitropin delta dosing is safe and effective in a long GnRH agonist protocol in women with AMH ≤35 pmol/L.

What is known already: Efficacy and safety of a fixed daily dose of follitropin delta, individualised based on bodyweight and anti-Müllerian (AMH), have been established in RCTs using a GnRH antagonist protocol. Preliminary study data shows that individualised follitropin delta is efficacious when used with a long GnRH agonist protocol (RAINBOW trial). There is no comparative data for efficacy and safety of individualised follitropin delta in a long GnRH agonist versus an antagonist protocol.

Study design, size, duration: This was a randomised, controlled, open-label, multi-centre trial comparing efficacy and safety of individualised follitropin delta dosing in a long GnRH agonist versus an antagonist protocol in participants undergoing their first ovarian stimulation for IVF/ICSI conducted between May 2019 and February 2022. The primary endpoint was the number of oocytes retrieved. Important secondary endpoints included ongoing pregnancy rates and adverse drug reactions, with a special focus on OHSS. A total of 437 participants were randomised.

Participants/materials, setting, methods: Participants, 18–40 years old with AMH ≤35 pmol/L, were enrolled at specialist reproductive health clinics in Austria, Denmark, Israel, Italy, the Netherlands, Norway and Switzerland. The mean number of oocytes retrieved was compared between the agonist and antagonist protocols using a negative binomial regression model with age and AMH at screening as factors. The analyses were based on all randomised subjects, using a multiple imputation method for randomised subjects withdrawing before start of stimulation.

Main results and the role of chance: Of the 437 randomised subjects, 202 and 204 initiated ovarian stimulation with follitropin delta in the agonist and antagonist groups, respectively. Baseline mean data were: age, 32.3 ± 4.3 years; AMH, 16.6 ± 7.8 pmol/L, respectively. The number of oocytes retrieved was statistically significantly higher in the agonist (11.1 ± 5.9) versus antagonist (9.6 ± 5.5) group, with an estimated mean difference of 1.31 oocytes (95% CI:

0.22; 2.40, p = 0.0185). The difference in number of oocytes retrieved was influenced by the patients' age and ovarian reserve, with a greater difference observed in patients <35 years and patients with high ovarian reserve (AMH >15 pmol/L). Cycle cancellations (2.0% versus 3.4%) and transfer cancellations (13.7% versus 14.7%) were similar in both groups. Ongoing pregnancy rate (36.9% vs 29.1%; estimated difference: 7.74% [95% CI: −1.49; 16.97, p = 0.1002]) and other pregnancy outcomes were higher in the agonist versus antagonist protocol group, but the differences were not statistically significant. The most commonly reported adverse events (≥1% in either group) were similar in both groups (headache, OHSS, nausea, pelvic pain or discomfort, and abdominal pain). Incidence of early moderate/severe OHSS was low (1.5% versus 2.5%). Early pregnancy loss was similar between the groups (20% vs 24% of subjects with positive beta-hCG test, respectively).

Limitations, reasons for caution: Subjects with AMH >35 pmol/L were not enrolled. Clinicians should remain cautious when using a GnRH agonist protocol in patients with AMH >35 pmol/L (greater OHSS risk). OHSS incidence in the GnRH antagonist group may have been lower if a GnRH agonist trigger had been allowed.

Wider implications of the findings: In women with AMH ≤35 pmol/L, a fixed daily dose of follitropin delta (individualised according to bodyweight and AMH) showed similar efficacy when used in a long GnRH agonist protocol with no additional safety signals observed and no additional risk of OHSS versus follitropin delta used in an antagonist protocol.

Trial registration number: ClinicalTrials.gov identifier: NCT03809429; EudraCT number: 2017-002783-40

Abstract citation ID: dead093.159

O-132 Comparison of pregnancy rates in antagonist cycles after luteal support with GnRH-agonist versus progesterone – prospective randomized study

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Study question: Are pregnancy rates in GnRH-antagonist cycles triggered with hCG and luteal phase support (LPS) with intranasal GnRH-agonist or vaginal progesterone comparable?

Summary answer: Nasal GnRH-agonist for LPS is associated with higher pregnancy rates compared with standard progesterone support in antagonist-based cycles triggered with hCG

What is known already: Regardless of its mechanism of action, the use of GnRH-a during the luteal phase in addition to progesterone, either in a single or multiple doses, has become a common practice as a part of ART protocols, based on its association with higher pregnancy and live birth rates . Its use as a sole agent for LPS has not gained the same popularity although studies supporting non-inferiority and even higher live birth rates with GnRH-a as a sole LPS agent have been published.

Study design, size, duration: A single-center, prospective, randomized study. A total of 150 patients underwent 164 GnRH-antagonist-based IVF cycles triggered with hCG and fresh embryo transfer. The study was conducted between June 9th, 2020, and May 5th, 2022.

Participants/materials, setting, methods: The study was conducted in the IVF clinic at University-affiliated tertiary medical center. Patients meeting the inclusion criteria who underwent antagonist-based IVF cycles and hCG triggering were enrolled. Computer-based randomization (1:1 ratio) was performed on the day of oocyte pickup (OPU). In both groups LPS was initiated on the evening of the OPU day with either GnRH-agonist nasal spray (Nafareline, nasal spray 200 mcg twice daily)or vaginal micronized progesterone (Utrogestan 300 mg, 3 times daily).

Main results and the role of chance: A total of 150 patients underwent 164 cycles, 127 cycles of which were included in the study cohort. Of them, 64 (50.4%) and 63 (49.6%) cycles were treated with GnRH-agonist or progesterone respectively as sole luteal phase support. A significantly higher pregnancy rate was found in the GnRH-a group compared with the progesterone group (43.8% versus 25.4% respectively; P = 0.030). After adjustment of several potential confounders such as age, body mass index (BMI), past obstetric history, number of IVF cycles, oocytes retrieved and embryos transferred, GnRH-agonist was still associated with a higher pregnancy rate (odds ratio 3.4, 95% confidence interval 1.4-8.3). Ovarian hyperstimulation syndrome (OHSS) rates were similar between the groups. Significantly higher progesterone levels were measured at β-hCG testing day in the GnRH-a group, both for the cleavage stage embryos (129.1 ± 23.7 nmol/L vs. 97.7 ± 48.9 nmol/L, P = 0.024) and for the blastocysts (127.2 ± 0.1 nmol/L vs. 75.3 ± 49.1 nmol/L, P = 0.034).

Limitations, reasons for caution: This study is not powered for analysis of clinical pregnancy rates, live birth rates and pregnancy outcomes. Larger studies are needed for research of these outcomes.

All patients participating were at low risk for OHSS, therefore the study is underpowered to find significant differences in early or late OHSS.Wider implications of the findings: Our findings suggest that intranasal GnRH-agonist administration as a sole luteal support in antagonist-based IVF cycles triggered with hCG results in higher pregnancy rates in comparison with traditional irritating vaginal preparations while not mandating additional support in the first weeks of pregnancy.

Trial registration number: Clinicaltrials.gov - NCT05484193

Abstract citation ID: dead093.160

O-133 Follicular flushing increases the number of cumulus oocyte complexes retrieved. A Randomized Controlled Trial

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Study question: Does follicular flushing increase the number of cumulus oocyte complexes (COCs) retrieved compared to single aspiration?

Summary answer: Follicular flushing increases the number of COCs retrieved compared to single aspiration.

What is known already: On the basis of published meta-analyses, follicular flushing does not seem to increase the number of COCs retrieved and the probability of pregnancy. However, eligible RCTs in these meta-analyses are characterized by significant heterogeneity regarding the population studied, the needle type and lumen diameter, the aspiration pressure and the number of flushing attempts performed. Moreover, most of the studies do not offer information regarding the flow rate of the aspiration system used. A constant flow rate has been employed in a single RCT, in which a higher number of COCs was retrieved after follicular flushing compared with single aspiration.

Study design, size, duration: This is a single-center, randomized controlled trial, performed between July and December 2022. One hundred and five patients undergoing oocyte retrieval for intracytoplasmic sperm injection, aged <43 years, with BMI 18-35kg/m2 were included in the study. Random allocation of each ovary to flushing or single aspiration was performed by a study nurse on the day of oocyte retrieval, using a computer generated randomization list. Patients could enter the study only once.

Participants/materials, setting, methods: All follicles ≥11 mm were aspirated with the same double-lumen needle, using the same aspiration pressure (190 mmHg) to achieve a flow rate of 0.42 ml/sec. If no COC was found in the initial aspirate, flushing was performed in the corresponding group until a COC was retrieved, or up to a maximum of five times. The primary outcome measure was the number of COCs retrieved per ovary randomized. Values are expressed as median (interquartile range).

Main results and the role of chance: Significantly more COCs were retrieved per ovary randomized in the follicular flushing versus the single aspiration group, in all patients [5 (7) versus 2 (3), p < 0.001, respectively], in patients with high [9 (3) versus 5 (4), p < 0.001, respectively], normal [5 (2) versus 2 (3), p < 0.001, respectively] and low [1 (1) versus 1 (1), p < 0.001, respectively] ovarian response. No COCs were retrieved in 2.9% of the ovaries in the flushing group versus 16.2% of the ovaries in the single aspiration group (p < 0.001).

The oocyte retrieval rate, which was defined as the ratio of COCs retrieved to the number of aspirated follicles ≥11 mm, was significantly higher in the follicular flushing versus the single aspiration group, in all patients [88.9% (25) versus 45.5% (37.5), $p < 0.001$, respectively], as well as in patients with high [81.8% (15.1) versus 45.5% (25.0), $p < 0.001$, respectively], normal [85.7% (28.6) versus 40% (30.0), $p < 0.001$, respectively], and low [100% (0) versus 50% (100), $p < 0.001$, respectively] ovarian response.

No significant differences were observed regarding maturation rate, fertilization rate and the proportion of good quality embryos on day 2 in all patients as well as in patients with high, normal and low ovarian response.

Limitations, reasons for caution: The current study design, by randomizing ovaries instead of patients, eliminates heterogeneity attributed to multiple confounders and allows a more accurate evaluation of the true effect of follicular flushing on the number of COCs retrieved. However, it does not allow the detection of its effect on the probability of pregnancy.

Wider implications of the findings: This is the first RCT to show that follicular flushing increases the number of COCs retrieved compared to single aspiration, independently of ovarian response. The data presented suggests that follicular flushing plays an important role in increasing the efficacy of oocyte retrieval.

Trial registration number: NCT 05473455

Abstract citation ID: dead093.161

O-134 Pretreatment with luteal estradiol for programming antagonist cycles compared to no pretreatment in advanced age women treated with corifollitropin alfa: a randomized controlled trial

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Study question: Does luteal estradiol pretreatment improve the number of oocytes retrieved in women aged 38 to 43 years treated in antagonist protocol with corifollitropin alfa?

Summary answer: Programming antagonist cycles with luteal estradiol did not increase the number of retrieved oocytes in advanced age women, however it was effective for programming.

What is known already: Previous studies showed that programming IVF cycles with estradiol pretreatment is a valuable tool for the organization of IVF centers and patients. However, variable effects of estradiol pretreatment were observed on the number of retrieved oocytes: no difference in normal responders, improved yield in oocyte donors and conflicting results in poor responders but no improvement in those fulfilling the bologna criteria. In advanced age women, recruitable follicles tends to decrease in number and to be more heterogeneous in size but it remains unclear if estradiol pretreatment could yield more oocytes through its negative feed-back effect on FSH intercycle rise.

Study design, size, duration: This non-blinded randomized controlled trial was conducted between 2016 and 2022. Participants were 324 women aged 38 to 43 years who requested IVF treatment. The primary endpoint was the comparison of the total number of retrieved oocytes between pretreated (P) and no pretreated (NP) patients. Secondary endpoints were duration and total dosage of recombinant FSH, cancellation rate, total number of metaphase II oocytes and obtained embryos, fresh transfer live birth rate (LBR) and cumulative LBR.

Participants/materials, setting, methods: This multicentric study (6 centers) enrolled women with regular cycles, weight >50 kg and BMI <32, IVF rank 1-2. According to computerized randomization, micronized estradiol 2mg twice a day was started on day (D) 20-24 continued until Wednesday beyond the onset of menses followed by administration of corifollitropin alfa on Friday i.e. stimulation (S)1 or from D1-3 of a natural cycle in unpretreated patients. GnRH antagonist was started at S6 and additional rFSH at S8.

Main results and the role of chance: Basal characteristics were similar in estradiol P (n = 147; number (mean [SD]) of treatment days 9.8[2.6]) and NP patients (n = 144), both displaying however a wide range of AMH levels (0.3 to 11.6 ng/ml) for advanced age patients. The cancellation rate showed a trend to be lower in P group (11 vs 19 %, $p = 0.11$). The mean number of retrieved oocytes was lower in P compared to NP patients (8.4[6.1] vs 9.1[6], respectively). The treatment difference was -0.7[-2.1 ;0.8] $p = 0.03$ one tailed test . No significant differences were observed in the number of MII oocytes (7[5.5] vs 7.3[5.2], $p = 0.72$) and the number of obtained embryos (5[4.6] vs 5.2[4.2], $p = 0.60$) between the two groups. Oocytes retrieval was more often on working days in P patients (91.9 vs 74.2 %, $p < 0.001$). However, the duration of stimulation was significantly longer (11.7[1.7] vs 10.8[1.8] days, $p < 0.001$) and the extra rFSH dosage in addition to corifollitropin alfa was significantly higher (1040[548] vs 778[504] IU, $p < 0.001$) in P than NP patients. The LBR after fresh transfer in P patients (16.2%[16/99]) was not significantly different from that in NP patients (18.5%[17/92]), $p = 0.82$, nor the cumulative LBR per patients 19.1 % vs 26.4 %, $p = 0.16$ respectively.

Limitations, reasons for caution: Our stimulated women older than 38 years obtained a wide range of collected oocytes (0 to 29) suggesting very different stages of ovarian aging in both groups. Estradiol pretreatment is more susceptible to increase oocyte yield at the stage of ovarian aging characterized by asynchrony of a reduced follicular cohort

Wider implications of the findings: Programming antagonist cycles with luteal estradiol pretreatment is effective in advanced age women to better schedule oocyte retrievals on working days. However, the potential benefit on the number of collected oocytes remains to be demonstrated in a more selected population displaying the characteristics of Poseïdon groups 3 and 4.

Trial registration number: ClinicalTrials.gov NCT02884245

SELECTED ORAL COMMUNICATIONS

SESSION 44: FOCAL OR NOT FOCAL SPERMATOGENESIS : THAT IS THE QUESTION !

Tuesday 27 June 2023 Hall D4 10:00 - 11:30

Abstract citation ID: dead093.162

O-135 Application of AMH determination in preoperative evaluation of micro-TESE in NOA patients

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Study question: To investigate the value of anti-Müllerian hormone(AMH) determination for estimating the sperm retrieval rate(SRR) of microdissection testicular sperm extraction(micro-TESE) in non-obstructive azoospermia(NOA) patients.

Summary answer: NOA with low AMH would have more opportunity to present heterogeneous seminiferous tubules when micro-TESE was performed and had higher SRR, especially in idiopathic cases.

What is known already: For infertile patients with NOA, micro-TESE is considered to have higher SRR than traditional surgery methods. However, serum inhibin B, follicle-stimulating hormone (FSH) and various clinical parameters are not reliable predictors for the presence of focal spermatogenesis and SRR. In male, AMH is a glycoprotein secreted by Sertoli cells and facilitate the regression of Müllerian structures in the developing foetus. It is still controversial that whether AMH level has value to predict the SRR of micro-TESE.

Study design, size, duration: This was a retrospective case-control study. From September 2014 to May 2022, 502 NOA patients treated with micro-TESE were divided into different groups according to their surgery outcome and seminiferous tubules appearance. Age, testis volume, serum AMH, FSH and testosterone level were compared between the different groups. The differences of SRR and AMH level in NOA patients with different etiologies were also compared.

Participants/materials, setting, methods: Micro-TESE was performed at x10 to x20 magnification. An attempt was made to identify seminiferous tubules that were larger and more opaque than others. The procedure was terminated when sperm were retrieved. If all tubules were seen to have an identical appearance, at least three samples (upper, middle, and lower) were extracted. Venous blood samples were drawn from each patient (7–10 AM) after an overnight fast. FSH and AMH were measured by electrochemiluminescence immunoassay.

Main results and the role of chance: Testicular sperms were successfully retrieved in 270 cases (SRR=53.8%). There were no statistical differences in age, testicular volume, FSH and testosterone levels between the patients who succeeded and failed to obtain sperm (all $P>0.05$). The patients who obtained sperms had lower serum AMH level than those without sperm [0.81(0.16, 3.26)μg/L vs.1.37(0.21, 4.84)μg/L, $P<0.05$]. Patients with orchitis or AZFc deletion, cryptorchidism, KS, idiopathic azoospermia would have different AMH level [0.15(0.01, 0.41)μg/L, 5.71(3.57, 8.26)μg/L, 2.29(1.36, 3.81)μg/L, 0.15(0.05, 0.39)μg/L, 2.46(0.75, 5.49)μg/L, $P<0.05$]. Idiopathic azoospermia patients who obtained sperms had higher age but lower testosterone and AMH level than those without sperm [(35.2 ± 8.9) years vs. (32.5 ± 5.5) years, $P<0.05$, (3.1 ± 1.4)μg/L vs. (3.7 ± 2.1)μg/L, $P<0.05$; 1.63(0.35, 3.84)μg/L vs.3.00(1.20, 6.68)μg/L, $P<0.05$], there were no statistical differences in testicular volume and FSH level between the two groups ($P>0.05$). Receiver operating characteristic (ROC) curve showed that cut-off of serum AMH for successful sperm retrieval of idiopathic azoospermia patients was determined to be 2.96, with a sensitivity of 0.710 and specificity of 0.523, area under the curve (AUC) was 0.649. In the cases presenting heterogeneous seminiferous tubules during micro-TESE had lower AMH level and higher SRR than those presenting homogeneous seminiferous tubules [0.55(0.12, 2.05)μg/L vs.2.99(0.76, 6.11)μg/L, 75.9%(236/311)vs. 17.8%(34/191), all $P<0.05$].

Limitations, reasons for caution: Pathology analysis should be involved in the following study. Randomized controlled trial comparing micro-TESE and traditional TESE would demonstrate that whether idiopathic azoospermia patients with higher serum AMH level would have less benefit by microsurgery than patients with lower AMH level.

Wider implications of the findings: Recently, in our pathologic research, NOA patients with extremely lower serum AMH level were observed to have more opportunity to present severe hyalinization in their seminiferous tubules. Tubules with severe hyalinization have less Sertoli cells and seem very thin. Therefore, tubules with spermatogenesis would be easy to identify during micro-TESE.

Trial registration number: not applicable

Abstract citation ID: dead093.163

O-136 Artificial intelligence to assist in surgical sperm detection and isolation

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Abstract under embargo

Abstract citation ID: dead093.164

O-137 Mapping microRNA profiling and dysregulated pathways in non-obstructive azoospermia cases with maturation arrest: A systematic review and in-silico analysis of the affected pathways

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Study question: Could microRNA profiling be a useful tool towards better understanding the pathophysiological mechanisms leading to non-obstructive azoospermia (NOA) due to maturation arrest?

Summary answer: MicroRNA profiling may be an efficient method towards unveiling maturation arrest pathophysiology as microRNAs constitute master posttranscriptional regulators of cell-cycle control during spermatogenesis.

What is known already: Non-obstructive azoospermia constitutes the most severe form of male factor infertility, affecting approximately 10% of infertile men. According to the histological subtype, NOA is classified into three main categories namely hypospermatogenesis, maturation arrest, and Sertoli-cell only syndrome. Maturation arrest is one of the most complex forms of NOA characterized by failure of spermatogenesis. Despite advances, the pathophysiology of maturation arrest remains a conundrum rendering management complex. Recent studies indicate that alterations on microRNA expression patterns could lead to maturation arrest. Mapping microRNA profiling and highlighting dysregulated pathways of maturation arrest cases may lead the way forward.

Study design, size, duration: A systematic review was performed in PubMed/Medline and Embase up to April 2022. Only full-length original studies in humans were included. Strict inclusion-exclusion criteria were applied aiming to isolate studies comparing microRNA profiling between maturation arrest cases versus normal fertile men, and/or men with obstructive azoospermia (OA). Following study selection, data on altered microRNA expression patterns were analyzed to underline differences between the abovementioned groups. Subsequently, in-silico analysis was performed to compare affected gene pathways.

Participants/materials, setting, methods: The studied population consisted of maturation arrest cases. Control groups consisted of men with normal spermatogenesis and/or men with OA. Predicted microRNA–target pairs were retrieved from microT-CDS, while a 0.8 cutoff threshold was applied. The GTEx repository was used to identify microRNA-targeted genes in the testis. Annotations derived from Ensembl and miRbase. Gene-set enrichment analysis was performed employing the KEGG-database. Fisher's exact test was performed in R package limma, setting a 0.01 p-value threshold.

Main results and the role of chance: Ten studies reported altered microRNA expression patterns in maturation arrest versus normal spermatogenesis cases and six studies in maturation arrest versus OA cases. Considering the maturation arrest-normal spermatogenesis arm, analysis revealed that eight microRNAs, which were upregulated in maturation arrest cases, namely hsa-miR-449a, hsa-miR-370-3p, hsa-miR-10b-3p, hsa-miR-539-5p, hsa-miR-22-5p, hsa-miR-605-5p, hsa-miR-491-3p and hsa-miR-302d-3p, affected 106 statistically significant gene-targets in the testis. The three most significantly affected pathways included the "microRNAs in cancer" pathway (55 affected genes, p-value <0.001), the "EGFR tyrosine kinase inhibitor resistance pathway" (29 affected genes, p-value = 0.007), and the "Hedgehog signaling pathway" (22 affected genes, p-value=0.008). Regarding the second arm of maturation arrest-OA, functional analysis indicated that four microRNAs, presenting to be downregulated in maturation arrest cases, namely hsa-miR-181a-5p, hsa-miR-449a, hsa-miR-182-5p and hsa-miR-138-5p, affected 421 statistically significant gene-targets in testis. The three most significantly affected pathways included the "pathways in cancer" pathway (86 affected genes, p-value =0.009), the "microRNAs in cancer" pathway (48 affected genes, p-value < 0.001), and the " cGMP-PKG signaling" pathway (36 affected genes, p-value=0.008). In summary, microRNAs affect the expression of master genes regulating cell-cycle and maturation processes during spermatogenesis, leading to maturation arrest as well as to other associated pathologies, including cancer.

Limitations, reasons for caution: The limited number of the included studies, as well as the small size population characterizing the great majority of them, constitute the main limitations of this study. Another reason for caution is the great heterogeneity observed among the studies regarding the molecular methods employed for microRNA profiling.

Wider implications of the findings: Data presented herein indicate that microRNA functional analysis may be a significant tool towards better understanding the pathophysiological basis of spermatogenesis maturation arrest, indicating future personalized diagnostic and therapeutic targets. Moreover, microRNA analysis may also constitute an efficient method of evaluating predisposition of NOA patients to other pathologies including cancer.

Trial registration number: Not applicable

Abstract citation ID: dead093.165

O-138 Factors influencing sperm retrieval rate of microdissection testicular sperm extraction in nonobstructive azoospermia patients with different etiologies: a retrospective study of 3104 patients

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Study question: What are predictors of successful sperm retrieval (SRR) with microdissection testicular sperm extraction (mTESE) in men with non-obstructive azoospermia (NOA) according to different etiologies?

Summary answer: Predictors for SRR were different in patients with various etiologies and effects of age on SRR in idiopathic NOA and KS patients were opposite.

What is known already: NOA patients due to spermatogenic dysfunction, accounting for about 60 percent of the total azoospermia cases, may have opportunities to obtain sperm by mTESE and overall SRR of mTESE in NOA patients is about 50%. There were some of predictive factors for SRR at mTESE including age, testis volume, serum follicle stimulating hormone (FSH), testosterone (T), inhibin B, anti-Mullerian hormone and testicular histopathology, while the predictive factors of SRR remains underappreciated, especially in accordance with different type of etiology. Most notably, there are some controversies about the relationship between SSR and age at mTESE surgery.

Study design, size, duration: This retrospective study involved 3104 NOA patients with different etiologies treated with the first mTESE at the Reproductive Medical Centre of Peking University Third Hospital from March 2012 to December 2022.

Participants/materials, setting, methods: 3104 NOA patients were classified into seven groups according to etiologies including 1530 males with idiopathic NOA (iNOA), 763 males with Klinefelter syndrome (KS), 345 males with microdeletion of the AZFc, 177 cases with the history of cryptorchidism, 131 with a history of mumps orchitis, 89 males with cryptozoospermia and 69 cases with other causes. The end-point was the presence of one or more sperm. Multi-variable logistic regression was used to analyze sperm retrieval outcome.

Main results and the role of chance: Overall SRR was 43.46%, and there were different SRR in NOA patients with various etiologies with highest SRR of 89.31% in patients with a history of mumps orchitis and lowest SRR of 29.35% in iNOA patients. For patients with a history of mumps orchitis, there was a negative relationship between T and SRR (0.805 [0.654, 0.991] p = 0.041). For cryptozoospermia patients, shorter infertility duration was predictive for successful SRR (0.837 [0.704, 0.995], p = 0.044). Males with bigger testes had more likelihood of positive SRR (1.077 [1.005; 1.153], p = 0.035) in patients with cryptorchidism. BMI was an independent factor of SRR in AZFc-deleted patients (1.084 [1.013, 1.161], p = 0.020). For KS patients, lower male age (0.938 [0.898, 0.979], p = 0.004) and bigger testes (1.214[1.099, 1.342], p<0.001) were predictive for successful SRR. Lower levels of FSH (0.984[0.970, 0.998] p = 0.025) and higher male age(1.079[1.050, 1.110], p<0.001) were predictive for successful sperm retrieval in iNOA patients. So, the optimum age range of iNOA male undergoing mTESE should be 30 to 35 years old with the SRR of 29.13% when considering low SRR of iNOA males aged<30 years (23.96%) and the negative effect of female age on ICSI outcome.

Limitations, reasons for caution: Surgeon performing mTESE maybe another predictor for SRR which is a potential selection bias. Inhibin B and Anti-Müllerian hormone weren't included due to limited information in our database. We couldn't verify the conclusion in more population from multicenter despite the current study with the large sample size so far.

Wider implications of the findings: Our results provide valuable information for NOA patients who want to counsel surgeons about their treatments to help patients and surgeons to perform a shared decision-making for optimal therapy methods, especially about optimal age treated with mTESE in iNOA and KS patients.

Trial registration number: not applicable

Abstract citation ID: dead093.166

O-139 Testicular mosaicism in non-mosaic Klinefelter Syndrome patients with focal spermatogenesis

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Study question: Do testis-specific cells have a normal karyotype in regions with spermatogonia (SPG) and ongoing spermatogenesis in non-mosaic Klinefelter Syndrome (KS) patients?

Summary answer: While SPG and Sertoli cells (SC) have 46,XY karyotype in regions with spermatogenesis, most Leydig cells (LC) and peritubular myoid cells (PTMC) have 47,XXY karyotype.

What is known already: Although the majority of non-mosaic KS patients is azoospermic, some patients with focal spermatogenesis (FS) can have children with artificial reproductive technologies. However, the genetic origin of FS is unknown. Independent of FS, dimorphism in sex chromatin has been reported amongst the seminiferous tubules of KS men. However, the karyotype of testis-specific cells in FS regions and other regions has not been clearly revealed so far.

The histone-3-lysine-27-tri-methylation (H3K27me3) modification is involved in suppression of the extra X chromosome, and its immunohistochemical staining allows the analysis of the X chromosome aneuploidy.

Study design, size, duration: Testicular biopsies were taken from 7 patients in whom spermatozoa were found by testicular sperm extraction (TESE). Muscle cells of female colon samples were used as positive control.

Participants/materials, setting, methods: After tissue processing and paraffine embedding, sections were cut at 5 µm. Immunohistochemical staining was performed with antibodies for H3K27me3 (inactive X chromosome), and MAGE-A4 (SPG), SOX9 (SC) and CYP17A1 (LC). PMTC was evaluated according to their position around the tubules and elongated shape. Inactive X (Xi) positive (Xi^+) and Xi negative (Xi^-) cells were counted and, Xi^+ cells were evaluated as having the 47,XXY karyotype; Xi^- cells were evaluated as having the 46,XY karyotype.

Main results and the role of chance: A total of 25 SPG^+ tubule sections were detected in 4 of 7 samples. Both SPG and SC were Xi^-, i.e. 46,XY karyotype, in all of these tubules. In some of the Sertoli cell only (SCO) tubules, SCs were Xi^+ (i.e. 47,XXY), while in others they were Xi^- (i.e. 46,XY). The rate of Xi^+ SC in SCO tubules containing Xi^+ SCs was 31.6%. Similarly, the Xi^+ ratio in muscle cells in the positive control was 31.0%. PTMCs surrounding tubules with SPG present, or ongoing FS were Xi^+ (i.e. 47,XXY). LCs were Xi^+ (i.e. 47,XXY) around all tubules. However, the percentage of Xi^+ PTMCs (21.4%) and LC (24.6%) was low compared to the female control (31.0%), suggesting mosaicism for these cells as well.

Limitations, reasons for caution: The fact that not all of the cell nuclei coincide with the section plane and the cells are in the mitotic stage limit the detection of Xi with H3K27me3. To overcome this limitation, X chromosome analysis could be performed by different techniques on intact cells isolated from fresh tissue.

Wider implications of the findings: FS occurs only in tubules with 46,XY SPGs and 46,XY SCs. Non-mosaic 47,XXY (diagnosed on blood cells) KS patients may present with testicular mosaicism, increasing their chances for sperm retrieval.

Trial registration number: Not applicable

Abstract citation ID: dead093.167

O-140 studying the effect of mosaicism on the outcome of micro-TESE among Klinefelter syndrome patients

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Study question: Does mosaicism affect sperm retrieval rate in Klinefelter patients?

Summary answer: Patients with mosaic Klinefelter syndrome (KS) have a higher success rate of sperm retrieval than those with non-mosaic KS.

What is known already: Klinefelter syndrome is characterised by the presence of an extra X chromosome in male individuals leading to a 47XXY karyotype and rarely to 46XY/47XXY Mosaicism; it is the most common sex chromosomal aneuploidy and the most common genetic cause of Azoospermia (Kanakis GA et al., 2018 and Gravholt CH et al.,2018). It accounts for 3–4% of male infertility cases and 10–12% of men with Azoospermia (Winters SJ,2018).

Study design, size, duration: After approval of the ethical committee of the faculty of medicine at Cairo University, This prospective study was conducted where 70 men who complained of primary infertility and azoospermia and were diagnosed with Klinefelter Syndrome were recruited for this study between 1/11/2021 and 30/11/2022. Patients with previous testicular disease (torsion, trauma) or who had a history of undescended testes or received chemotherapy or radiotherapy were excluded.

Participants/materials, setting, methods: Seventy patients (n = 70) aged between 20 and 50 who fit the inclusion criteria were recruited and underwent micro_TESE. Semen analysis (Azoospermia), hormonal profile, scrotal colour doppler ultrasonography, Karyotyping and FISH for Y and X chromosomes were performed. Statistical analysis was conducted using SPSS 22nd edition.

Main results and the role of chance: Our study groups comprised 14 patients with mosaic KS and 56 with non-mosaic KS. The clinical characteristics were not significantly different statistically between the two groups. Micro_TESE was performed in all 70 patients; in 26 patients (37.1%), mature sperm were found in the wet preparation.

No statistically significant correlation was found between the presence of spermatozoa in the extraction specimen and age, serum follicle-stimulating hormone, serum testosterone, prolactin or oestradiol. The statistically significant predictor variable for sperm extraction was mosaicism, luteinising hormone, testicular volume and FISH. The sperm retrieval rate of the patients with mosaic and non-mosaic KS was 57.1% and 32.1%, respectively.

Limitations, reasons for caution: 1) Larger (n) number of patients was needed.

2) Despite our valuable findings, further studies should be performed to illuminate the relation between FISH for X and Y chromosomes and sperm retrieval rate success.

Wider implications of the findings: Klinefelter patients with mosaicism should be offered high sperm retrieval rate during IVF counselling.

Trial registration number: n/a

SELECTED ORAL COMMUNICATIONS
SESSION 45: IMPLANTATION AND EARLY PREGNANCY
Tuesday 27 June 2023 Auditorium 10-12 10:00 - 11:30

Abstract citation ID: dead093.168

O-141 Combination of gefitinib and methotrexate to treat tubal ectopic pregnancy (GEM3): a multicentre, randomised, double-blind, placebo-controlled trial

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Study question: Is a combination of parenteral methotrexate and oral gefitinib more effective than methotrexate alone in the treatment of tubal ectopic pregnancy?

Summary answer: In women with a tubal ectopic pregnancy, adding oral gefitinib to parental methotrexate does not offer clinical benefit over methotrexate and increases minor adverse reactions.

What is known already: Current treatment of tubal ectopic pregnancies is with methotrexate or surgery. Methotrexate treatment fails in ~30% of women and they require rescue surgery. At the time of the design of the trial, it had been shown in preclinical studies that tubal implantation sites express high levels of epidermal growth factor receptor (EGFR) and that gefitinib (an EGFR antagonist) augments methotrexate-induced regression of pregnancy-like tissue. There was also evidence from uncontrolled phase I and II trials that raised the possibility that combination methotrexate and gefitinib could be a more effective medical treatment than methotrexate alone to treat stable ectopic pregnancies.

Study design, size, duration: Between 2nd November 2016 and 6th October 2021, we performed a multicentre, randomised, double-blind, placebo-controlled trial across 50 UK hospitals. Eligible participants were women with a tubal ectopic pregnancy deemed suitable for medical management with methotrexate. Inclusion criteria were: aged 18-50 years; pre-treatment serum hCG level of 1000–5000 IU/L; and either a definite diagnosis of tubal ectopic pregnancy or a clinical judgement of 'probable' tubal ectopic pregnancy.

Participants/materials, setting, methods: Participants were administered a single dose of intramuscular methotrexate (50 mg/m^2) and randomised (1:1 ratio) to seven days of additional oral gefitinib (250mg daily) or placebo. The primary outcome, analysed by intention to treat, was surgical intervention to resolve the ectopic pregnancy. Secondary outcomes included time to resolution of ectopic pregnancy and serious adverse events.

Main results and the role of chance: 328 participants were allocated to methotrexate and gefitinib (n = 165) or methotrexate and placebo (n = 163). Three participants in the placebo group withdrew. Surgical intervention occurred in 30% (50/165) of the gefitinib group and in 29% (47/160) of the placebo group (adjusted risk ratio 1.15, 95% confidence interval [CI] 0.85-1.58; adjusted risk difference -0.01, 95% CI -0.10-0.09; p = 0.37). Without surgical intervention, median time to resolution was 28.0 days in the gefitinib group and 28.0 days in the placebo group (subdistribution hazard ratio 1.03, 95% CI 0.75-1.40). Serious adverse events occurred in 3% (5/165) of the gefitinib group and in 4% (6/162) of the placebo group. Diarrhoea and rash were more common in the gefitinib group.

Limitations, reasons for caution: Limitations of the trial include the fact that we only tested one dose regimen. It is possible gefitinib may be effective if a different protocol were used, such as a longer period of administration. Also, we did not carry out pharmacodynamic studies to determine optimal drug bioavailability.

Wider implications of the findings: Our results show that the addition of gefitinib to standard medical management with methotrexate to treat tubal ectopic pregnancy is not clinically effective as it does not reduce subsequent surgical intervention and is associated with higher rates of reported symptoms than placebo.

Trial registration number: ISRCTN67795930

Abstract citation ID: dead093.169

O-142 Surgical evacuation with adjuvant uterine artery embolization: a fertility preserving treatment for advanced live caesarean scar ectopic pregnancies

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Study question: Can advanced caesarean scar ectopic pregnancies be managed safely surgically whilst preserving future fertility?

Summary answer: With the available support of uterine artery embolization, suction and curettage can be an effective treatment for live advanced caesarean scar ectopic pregnancies, avoiding hysterectomy.

What is known already: Caesarean scar ectopic pregnancies (CSEPs) are associated with significant maternal morbidity and termination of pregnancy is often offered to patients to protect their health and fertility. Treatment of early first trimester CSEPs is usually effective and safe, but the management of more advanced cases is more challenging, and hysterectomy has been considered the treatment of choice for second trimester CSEPs.

Study design, size, duration: This was a retrospective cohort study in a tertiary referral centre between 2008-2023. Of 371 women diagnosed with CSEP, 22 (6%) had live advanced CSEPs. 17/22 (77%) patients opted for surgery, whilst the remaining five opted to continue with their pregnancies. CSEP was defined by implantation of the pregnancy into a myometrial defect caused by dehiscence of a lower uterine segment caesarean scar. Advanced CSEP was defined as crown rump length (CRL) of ≥ 40mm.

Participants/materials, setting, methods: A preoperative ultrasound was performed in each patient. All women underwent surgical evacuation under ultrasound guidance and insertion of modified Shirodkar cervical suture as a primary haemostatic measure. Additional haemostatic measures included uterine artery embolization (UAE). Our primary outcome was the rate of blood transfusion. Secondary outcomes were estimated intraoperative blood loss (BL), UAE, and admission to intensive care unit (ICU) and fertility preservation. Descriptive statistics were used to describe these variables.

Main results and the role of chance: For the 17 cases included, median CRL was 54.1 mm (range 40.0 - 85.7) and median gestational sac diameter was 52.7mm (range 41.0 – 82.7). Median gestational age based on CRL was 12 + 2 weeks (range 10 + 6 weeks and 15 + 2 weeks).

Two (12%) women had a recurrent CSEP and 1 woman had a heterotropic pregnancy. On pre-operative ultrasound placental lacunae were recorded in 13 (76%) cases and colour Doppler score was ≥3 in 10 (59%) cases.

A Shirodkar cervical suture was used in all cases, as per our protocol. It was successful in achieving haemostasis by tamponade in 13/17 (76%) cases. In the remaining 4 (24%) tamponade failed to achieve a complete haemostasis and

UAE was required to control persistent arterial bleeding into the uterine cavity. Median BL at the time of surgery was 800 ml (range 250-2500) and 7/17 (41%) patients had a BL of > 1000 ml with 6/17 (35%) requiring blood transfusion. All four women who had UAE required admission to ICU. Three cases had a two-stage procedure with interval UAE to control the bleeding. All patients made a good postoperative recovery and no emergency hysterectomy was required. Three patients fell pregnant again and all pregnancies were normally sited.

Limitations, reasons for caution: Although this is the largest series of advanced CSEPs one limitation is its retrospective design and the relatively small number of cases. A second limitation is no women ≥ 16 weeks were included. Lastly, this study was conducted in a tertiary referral centre and results may not be widely replicable.

Wider implications of the findings: Surgical evacuation with a Shirodkar cervical suture and selective UAE is an effective treatment for advanced CSEPs and should be available to women who want to retain their fertility and avoid a hysterectomy. Pre-surgical planning and collaboration between gynaecologists and interventional radiologists is key in managing these high-risk women.

Trial registration number: not applicable

Abstract citation ID: dead093.170

O-143 Biopsy-free profiling of the uterine immune system in patients with recurrent pregnancy loss and unexplained infertility

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Study question: What are the differences in menstrual blood lymphocytes between controls, patients with recurrent pregnancy loss (RPL) or with unexplained infertility (uINF)?

Summary answer: Compared with controls, RPL- and uINF patients had a different menstrual blood immune profile, including altered NK-subtypes indicating an altered cytotoxicity in these patients.

What is known already: T-cells, particularly regulatory T-cells, and uterine natural killer cells play a vital role as they are the main immunological regulators at the feto-maternal interface. However, an endometrial biopsy (EB) is required for the examination of these cells, necessitating an invasive and uncomfortable procedure. Moreover, the small tissue samples are mainly analyzed by immunohistochemistry, limiting the amount of analysable markers and ultimately results in controversial data. Menstrual blood (MB) has been used in proof-of-concept studies to analyse lymphocyte populations. So far, no study has investigated MB lymphocytes in a well-defined cohort of RPL- and uINF patients in comparison to healthy controls.

Study design, size, duration: In this prospective study, 46 healthy controls, 28 RPL patients and 11 uINF patients were included between 03/2021 and 02/2022. RPL was defined as ≥ 3 consecutive pregnancy losses. For being diagnosed with uINF, all standard-diagnostic procedures for infertility had to provide negative results in couples trying to conceive for >12 months.

Participants/materials, setting, methods: To establish the procedure, lymphocyte compositions in EB in 7 controls were compared with those in MB during 48h. The first 24h and the second 24h were collected separately. In all other RPL- and uINF-patients, as well as controls, peripheral blood (PB) and MB was collected. Density gradient centrifugation was performed to isolate PB and MB mononuclear cells. Cells were analyzed by flow cytometry focusing on the main lymphocyte populations and NK-cell subsets.

Main results and the role of chance: The first 24h of MB closely resembled the uterine lymphocyte composition in EB, with were $CD3^+$ T-cells and $CD56^+$ NK-cells being the predominant lymphocyte populations in EB and MB. We therefore based further analyses on the first 24h of menstrual shedding. In comparison to controls, RPL-patients showed significantly higher MB-$CD56^+$-NK-cell numbers (mean ± SD: 31.13 ± 7.52 vs. 36.73 ± 5.4; p = 0.002). Furthermore, MB-$CD56^{dim}CD16^{bright}$ NK-cells within the $CD56^+$-NK-cell population were decreased in individuals with RPL and uINF compared to controls (mean ± SD: 20.4 ± 11.53;16.34 ± 14.65;15.7 ± 5.91; Ctrl vs. RPL p = 0.011; Ctrl vs. uINF p = 0.02). Further, compared to controls and RPL patients, uINF patients had lower $CD3^+$ T-cell counts (mean ± SD Ctrl. vs. uINF: 47.93 ± 11.11 vs. 38.81 ± 5.04, p = 0.013), with decreased $CD4^+$ (mean ± SD Ctrl. vs. uINF: 25.78 ± 7.91 vs. 15.66 ± 4.88, p = 0.001) and $CD8^+$ T-cells (mean ± SD Ctrl. vs. uINF: 15.32 ± 4.14 vs. 8.43 ± 2.85, p < 0.001). Interestingly, uINF-patients showed higher $CD25^{high}CD127^{dim/neg}$ regulatory T-cell counts than controls (mean ± SD Ctrl. vs. uINF: 1.34 ± 0.49 vs. 1.84 ± 0.51, p = 0.009). The cytotoxicity receptors NKp46 and NKG2D were more present in uINF and RPL patients. Furthermore, RPL and uINF patients had significantly higher peripheral $CD56^+$-NK-cell counts as compared to controls (mean ± SD: 8.4 ± 3.5;11.42 ± 4.05;12.86 ± 4.29; Ctrl vs. RPL p = 0.021; Ctrl vs. uINF p = 0.009).

Limitations, reasons for caution: The small sample size of our study limits our ability to extrapolate the findings to other populations, as well as perform further subgroup analyses in controls, RPL and uINF. Future research must concentrate on NK cell subpopulations in larger cohorts, potentially in a multi-center setting.

Wider implications of the findings: Strengths of this study include well phenotyped participants and that the immunological milieu of the endometrium is directly compared for EB and MB. In future studies, this non-invasive analysis might enable to identify and monitor patients who could profit from (immunomodulatory) medications, and thereby improve live birth rates.

Trial registration number: not applicable

Abstract citation ID: dead093.171

O-144 Critical assessment of possible clinical benefits of culturing human embryos until day 7

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Study question: Is there any benefit of extending culture until day (D) 7 to increase euploid blastocysts yield and clinical outcomes in a PGT-Aneuploidy analysis (PGT-A) program?

Summary answer: Extended culture until D7 increases euploid blastocysts yield and cumulative live birth. Highest benefit was observed in patients aged ≤41 with ≤2 D5/D6 blastocysts biopsied.

What is known already: It is well-known that female age is the strongest predictive factor of embryo aneuploidy and has been associated with delayed blastocyst development in vitro. Culturing embryos until D7 might increase biopsied blastocyst yields and euploidy outcomes among women undergoing ART as already been published. However, extended culture until D7 increases IVF workload and implies extra cost. It is unclear whether the practice is justified when a certain number of blastocysts have already been biopsied on D5/D6. Therefore, the additive value of extended culture until D7 needs further evaluation. Studies published in the literature have small sample sizes and are controversial.

Study design, size, duration: This single center observational study included 30,941 blastocysts from 6,976 consecutive IVF/ICSI PGT-A fresh autologous cycles performed between 2017 and 2022. Patients who underwent PGT-A by Next Generation Sequencing (NGS) were considered for the analysis. Only blastocysts graded ≥BL3CC (Gardner) that underwent TE biopsy on D5, D6 or D7 were included. Data were stratified by age categories (<37, 37-41 and >41) and on number of biopsied blastocysts performed on D5 and/or D6 of embryo culture.

Participants/materials, setting, methods: The additive value of extended embryo culture was assessed in terms of absolute numbers (percentage of D7 euploid blastocysts obtained) and theoretical benefit to cumulative live birth rate (having at least 1 live birth by using all available euploid blastocysts) as calculated by binomial density function. Results was assessed between female age strata (<37, 37-41, >41) and number of embryos biopsied in D5/D6 (No biopsy, ≤2, 3-4, 5-6 or ≥ 6 biopsied).

Main results and the role of chance: A total of 18,478 blastocysts were biopsied on D5 (38.5%), D6 (55.2%) or D7(6.3%). Euploidy rate decreased significantly for blastocysts biopsied on D5, D6 and D7 (55.6%, 39.7% and 27.1%, $P < 0.001$, respectively). When stratifying patients by age, euploidy rates were consistently higher for D5 biopsied blastocysts compared to D6 and D7 (<37: 61.1%, 50.0% and 38.5%, $P < 0.001$; 37-41: 39.0%, 27.8% and 20.1%, $P < 0.001$; >41: 18.5%, 9.0% and 3.9%, $P < 0.001$, for D5, D6 and D7, respectively). The chances of obtaining at least one euploid D7 blastocyst was higher among patients <37 and 37–41 years compared to > 41 years (7.2% and 4.4% vs. 0.6%, $P < 0.001$). Among women <37, a > 25% increase in cumulative live birth rate (theoretical) was detected in 10.1% with no biopsy on D5/D6, 2.5% with 1-2 blastocysts biopsied on D5/D6 and only 0.3% with ≥3 blastocysts biopsied on D5/D6 ($P < 0.001$). In older women (37-41), a > 25% increase in cumulative live birth rate (theoretical) was found in 5.6% with no biopsy on D5/D6, 2.5% with 1-2 blastocysts biopsied on D5/D6, and only 0.4% with ≥3 blastocysts biopsied on D5/D6 ($P < 0.001$). Women >41 years rarely benefited from extended culture util D7 (<1%) regardless of the number of blastocysts biopsied on D5/D6.

Limitations, reasons for caution: The study was based on a retrospective analysis data and results were calculated on a theoretical cumulative live birth. While this study provides one of the largest number of blastocysts analyzed in PGT-A and "freeze all" strategy, results should not be extrapolated to other populations or different ART routine practices.

Wider implications of the findings: Usable blastocysts (6.3%) and euploidy rates (27.1%) are significantly lower with D7 blastocysts compared to D5/D6 blastocysts. Patients aged >41 do not benefit from extended embryo culture to D7 (<1%). Culturing blastocysts for 7 days should be mainly decided based on the number of blastocysts biopsied on D5 or D6.

Trial registration number: not applicable

Abstract citation ID: dead093.172

O-145 From mathematical modeling to real-life clinical data: live birth rates up until the tenth blastocyst transfer without PGT-A

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Study question: What is the cumulative live birth rate (CLBR) from the first until the tenth consecutive blastocyst transfer, across all age-groups, if no PGT-A is applied?

Summary answer: Our clinical data confirm existing mathematical models: without PGT-A, a CLBR of 70.0% after three, 88.9% after seven and 95.9% after ten transfers was observed.

What is known already: The rate of 'true' repeated implantation failure (RIF) from endometrial origin is an important topic of debate in our scientific community. Its existence was challenged with the reporting of CLBRs >90% after three consecutive euploid blastocyst transfers. These findings are not directly applicable when PGT-A is not routinely performed. Mathematical models have been developed to tackle this issue by taking into account (1) the anticipated euploidy rate based on the oocyte's age and (2) the probability of an euploid embryo to implant. So far, no actual patient data have confirmed the validity of this model.

Study design, size, duration: This is a single-center, retrospective, observational cohort study including 4320 unique women who underwent up to ten consecutive blastocyst transfers between 2010 and 2020. All age categories were included, as well as fresh/frozen and single/double embryo transfers. Patients using donor oocytes, PGT or cleavage stage embryo transfers were excluded or led to drop-out in the study.

Participants/materials, setting, methods: Patient characteristics were retrieved and cumulative outcomes were analyzed. A Kaplan-Meier curve was plotted for CLBR. For each transfer cycle the live birth rate (LBR), double embryo transfer rate (DET) and multiple pregnancy rate were calculated.

Main results and the role of chance: The mean age of the patients included in the study was 31.8 (± 4.5) years at the time of the first ovarian stimulation. The mean body mass index was 23.7 (± 4.3) kg/m^2 and mean basal FSH 6.8 (± 2.3) IU/L. The mean number of stimulations per patient was 1.21 (± 0.5) and a total mean number of 4.6 (± 3.2) blastocysts were obtained per patient. The mean time to the pregnancy leading to live birth was less than one year (0.3 ± 0.7).

We observed the highest LBR (46.3%, 2002/4320) after the first blastocyst transfer followed by 35.4% (593/1672), 32.3% (275/851), 32.2% (147/456), 32.8% (78/238), 21.3% (26/122), 23.9% (17/71), 25.6% (10/39), 31.8% (7/22), 18.2% (2/11) for the second until the 10th transfer, respectively.

The Kaplan-Meier curve showed CLBRs to mount from 70.0% after the third, up to 88.9% after the seventh and 95.9% after the tenth blastocyst transfer.

We noticed a steady increase in DET rate from only 6.4% in the first up to > 27% as of the fourth and >44% as of the seventh transfer with acccompanied multiple pregnancy rates of 1.3% over 10.9% and 11.8%, respectively.

Limitations, reasons for caution: The main limitation is the study's retrospective nature. As our center performs cleavage stage and blastocyst transfer, the included population is a good prognosis one as embryology allowed to perform extended culture. Nevertheless, to investigate RIF due to endometrial causes, this could also be seen as an asset.

Wider implications of the findings: These patient data confirm mathematical models and further question the prevalence of insurmountable, endometrial origin RIF. Preserved LBRs (even in higher cycle ranks) and a CLBR >95% provide hope and reassurance to couples with failed embryo transfers and encourage them to continue treatment if blastocysts are available.

Trial registration number: NA

Abstract citation ID: dead093.173

O-146 Oocyte donation does not increase live birth rates in young women suffering from recurrent pregnancy loss

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Study question: Does oocyte donation improve reproductive outcomes in recurrent pregnancy loss (RPL) patients?

Summary answer: Oocyte donation increases live birth and reduces miscarriage rates in RPL women older than 35, but it does not improve reproductive outcomes in younger patients.

What is known already: Recurrent pregnancy loss (RPL) is defined as 2 or more pregnancy losses prior to 20-24 weeks of gestation and has an incidence of approximately 1 to 2% in couples trying to conceive. Known risk factors for RPL are embryo aneuploidy, advanced maternal age (AMA), previous miscarriages, uterine abnormalities, parental chromosomal abnormalities, antiphospholipid syndrome, endocrine factors, such as thyroid function or obesity, and thrombophilia. However, 50-70% of patients suffering from RPL do not present any known risk factor, making the management of idiopathic RPL patients particularly challenging for ART clinicians.

Study design, size, duration: This is a cohort retrospective study involving 2 centers. Charts from 18,273 women undergoing IVF treatment between

2011 and 2020 were reviewed. A total of 912 patients (5%) met the definition of RPL and were classified into study groups based on their age and oocyte origin: ≤35 years old receiving oocyte donation (n = 39) or using their own oocytes (n = 35), and AMA women using donor eggs (n = 716) or their own (n = 122).

Participants/materials, setting, methods: Demographic variables analysed included known risk factors, number of transferred embryos, day of embryo transfer (3 vs 5) and sperm origin (partner/donor). Differences in biochemical, clinical, ongoing pregnancy, miscarriage and live birth rates were assessed by Pearson's Chi-squared or Fisher's exact test. P-values <0.05 were considered significant.

Main results and the role of chance: RPL patients had 2.77 ± 1.27 pregnancy losses overall (2.76 ± 1.37 in women ≤35 and 2.77 ± 1.26 in patients >35). Most RPL patients (91.9%) were of AMA. Other RPL-associated risk factors, including uterine malformations, previous pregnancy losses, chromosomal abnormalities and thrombophilia were identified in 207/912 patients (22.7%). Interestingly, these were more frequent in patients <35 (37.8% vs 21%, p = 0.001). Young RPL women presented higher rates of karyotype abnormalities than older patients (17.6% vs 3.8%, $p < 1x10^{-04}$), while showing a similar incidence of uterine abnormalities (14.9% vs 12.9%, p > 0.63) and thrombophilia (4.1% vs 4.7%, p > 0.81).

RPL patients >35 preferentially underwent oocyte donation (85.4% vs 52.7%, $p < 1x10^{-04}$), which led to significantly higher biochemical (52.5% vs 17.1%, $p < 1x10^{-04}$), clinical (42.4% vs. 8.5% $p < 1x10^{-04}$), ongoing pregnancy (37.9% vs 5.1%, $p < 1x10^{-04}$), live birth (32% vs 4%, $p < 1x10^{-04}$) and lower miscarriage rates (10.5% vs 40%, p = 0.0186) than in autologous cycles. In contrast, RPL patients <35 had similar reproductive outcomes: biochemical (41% vs. 52.9), clinical (28.2% vs. 37.1%), ongoing pregnancy (25.6% vs 29.4%), live birth (25.6% vs 26.4%) and miscarriage rates (9% vs 23%), regardless of oocyte origin (donated vs own, p > 0.05 for all cases). Importantly, this was also true for a small subgroup of idiopathic young RPL patients (n = 13).

Limitations, reasons for caution: The main limitation of this study is its retrospective nature, which does not allow for full elucidation of all potential confounders. The number of young RPL patients undergoing oocyte donation may be too low to draw significant conclusions.

Wider implications of the findings: RPL can be resolved in AMA patients by using donor oocytes, which points to key roles of oocyte quality and aneuploidy underlying RPL aetiology. However, RPL is not ameliorated by oocyte donation in young women, suggesting an endometrial/systemic origin and highlighting the need to further study mechanisms driving this disorder.

Trial registration number: Not applicable

SELECTED ORAL COMMUNICATIONS

SESSION 46: CLINICAL AND LABORATORY PROGNOSTIC FACTORS IN ART

Tuesday 27 June 2023 Hall D5 10:00 - 11:30

Abstract citation ID: dead093.174
O-147 Factors influencing ICSI outcome after sperm retrieval in nonobstructive azoospermia patients with different types of etiologies: a retrospective study of 1157 patients

L. Zhang[1], T. Wenhao[2], L. Ping[1], H. Kai[2]

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[2]*Peking University Third Hospital, Department of Urology, Beijing, China*

Study question: What are predictors of fertilization, clinical pregnancy, miscarriage and livebirth delivery outcomes after microdissection testicular sperm extraction (mTESE)-ICSI in NOA patients with different etiologies?

Summary answer: Predictors for ICSI outcome were different in patients with various etiologies and type of NOA are associated with ICSI outcome in these patients.

What is known already: NOA patients due to spermatogenic dysfunction, accounting for about 60 percent of the total azoospermia cases, may have opportunities to be fathers with their own biological children by mTESE combined with ICSI. For them, there were some of predictive factors for ICSI outcome including sperm motility, sperm morphology, testis volume and levels of reproductive hormone including follicle stimulating hormone (FSH), luteinizing hormone (LH) and testosterone (T), while the predictive factors of ICSI outcome remains underappreciated, especially about the relationship between ICSI outcome and male factors after reviewing the large scale clinical data.

Study design, size, duration: This retrospective study involved 1157 NOA patients with treated 1534 complete ICSI cycles at the Reproductive Medical Centre of Peking University Third Hospital from March 2012 to October 2021.

Participants/materials, setting, methods: 1534 ICSI cycles included 558, 359, 243, 155, 128, 56 and 35 cycles performed in males with idiopathic NOA (iNOA), Klinefelter syndrome (KS), AZFc microdeletions, cryptorchidism, a history of mumps orchitis, cryptozoospermia and with other causes separately. Primary outcome was livebirth delivery per ICSI cycle (LBR) and secondary outcomes were clinical pregnancy rate per ICSI cycle (PR) and fertilization rate (FR). Multi-variable logistic and linear regression was used to analyze ICSI outcome.

Main results and the role of chance: Overall LBR, PR and FR were 37.35%, 44.98%, 49.34% separately. Patients with mumps orchitis showed the highest LBR, PR and FR of 53.13%, 60.94% and 59.52% respectively, and AZFc-deleted patients displayed the lowest LBR, PR and FR of 25.93%, 30.04% and 32.87% separately. Lower female age (0.947 [0.904;0.993], p = 0.024), more transferred embryos (1.345 [1.225;1.477], p<0.001), higher AFC (1.033[1.011;1.056], p = 0.003) were related with higher LBR, and higher male BMI (0.974[0.950; 0.999], p = 0.046) and lower AFC (1.057[1.034; 1.080], p<0.001) were related with lower PR. Comparing outcome of mumps orchitis group, patients with cryptorchidism were associate with lower LBR(0.467[0.281; 0.778], p = 0.003), PR (0.440[0.268;0.721], p = 0.001) and FR(-0.169 [-0.226; -0.11], p<0.001) separately. AZFc-deleted patients had a relationship with lower LBR (0.426[0.260; 0.697], p = 0.001), (0.273[0.170; 0.437], p<0.001) and FR (-0.282 [-0.336; -0.228], p<0.001) respectively. KS patients were related with lower LBR (0.455[0.288; 0.720], p = 0.001), PR (0.477 [0.306; 0.745], p = 0.001) and FR (-0.073 [-0.126; -0.020], p = 0.007) separately. iNOA patients were associated with lower PR (0.616 [0.409; 0.928], p = 0.020) and FR (-0.076 [-0.124; -0.029], p = 0.002) respectively. Furthermore, patients with cryptozoospermia were related with lower FR (-0.141 [-0.220; -0.061], p = 0.001).

Limitations, reasons for caution: Sperm characteristics maybe predictors for ICSI outcome which is a potential selection bias. Pituitary prolactin and Anti-Müllerian hormone weren't included as covariates due to limited information in our database. We couldn't verify the conclusion in more population from multicenter despite the current study with the large sample size so far.

Wider implications of the findings: Our results provide valuable information for NOA patients who want to counsel surgeons about their ICSI outcome to help patients and surgeons to perform a shared decision-making for optimal therapy methods, especially for AZFc-deleted patients with worse ICSI outcome who may consider donor semen to get better ICSI outcome.

Trial registration number: not applicable

Abstract citation ID: dead093.175

O-148 Cumulative embryotoxic effect of IVF plastic devices used sequentially in routine clinical practice could slow the embryo development and delay blastulation

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Study question: Is there an embryotoxic cumulative effect resulting from IVF plastic consumables (PC) when used sequentially under clinical routine practice?

Summary answer: Reduced blastulation rates and slow development were detected in some associations of PC evaluated by the Mouse Embryo Assay (MEA) suggesting possible cumulative embryotoxicity.

What is known already: Recommendations from European (ESHRE) and American (ASRM) societies regarding the use of IVF PC suggest that they should undergo appropriate quality control tests to detect the possible presence of embryotoxins. The main test used by manufacturers to exclude any embryotoxicity is the MEA. This assay is currently performed individually on each PC so that a certificate of conformity is delivered for each product/lot. However, several PC are used during a single IVF cycle, potentially creating an embryotoxic cumulative effect. To our knowledge, the cumulative toxicity of several PC used sequentially on gametes and embryos has not been studied so far.

Study design, size, duration: The objective of this prospective study was to determine if there is cumulative embryotoxicity when several PC are used together under clinical routine conditions. Ten associations, each containing 13 to 31 PC, were designed, and assessed for embryotoxicity using a MEA methodology. The tested combinations replicated the different steps used in an IVF cycle, including sperm collection and selection, oocyte pick-up, fertilization, embryo culture, embryo transfer, vitrification and thawing and sperm freezing.

Participants/materials, setting, methods: A defined volume of culture media was used to extract the toxicity of several PC used in every association in triplicate. The MEA tests were performed on each medium extraction with 21 one-cell stage fresh mouse embryos. Blastocyst formation rates after 96 and 120h of culture (Day 5/6), blastocyst good quality rates and hatched blastocyst rates were compared between the tested and control groups. The total cell number per blastocyst was also evaluated.

Main results and the role of chance: No toxicity was detected in the first two associations comprised of 22 and 23 PC, which mimicked the collection (either in sperm cups or in spermicide-free condoms) and processing of sperm samples.

However, the third association comprised of 32 plastic devices replicating the sperm collection in sperm cups, processing, and freezing in high security straws, showed toxicity as the mean blastulation rate at day 5 was reduced to 20.6% compared to 95.6% in the control group ($p < 0.05$). Surprisingly, the mean blastulation rates observed at day 6 was 55.6% versus 100.0% in the control group ($p < 0.05$). The blastocysts obtained at day 6 showed a mean number of cells ($n = 112.5$) significantly reduced in comparison with the control group ($n = 167.9$) ($p < 0.05$), suggesting a sublethal presence of toxins which slowed embryo development down and delayed blastulation.

More experiments involving toxicity assessment in other associations of PC are currently undergoing and will be presented at the conference.

Limitations, reasons for caution: Sensitivity could vary depending on the methodologies used for toxicity extraction and MEA. The main cause, if any, of the detected toxicity will need to be pinpointed by analyzing each element of the affected associations individually.

Wider implications of the findings: Also, professionals should rationalize and minimize the number of plastic consumables used during the IVF procedures to reduce the potential accumulation of toxicity in the embryo culture system.

Trial registration number: Not applicable

Abstract citation ID: dead093.176

O-149 Endometrial thickness as a predictor of live birth and perinatal outcomes following oocyte donation

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Study question: Does endometrial thickness (EMT) predict the occurrence of live birth following oocyte donation?

Summary answer: Our study found that EMT is predictive of live birth in oocyte donation.

What is known already: EMT is a widely used surrogate marker of endometrial receptivity and is believed to be associated with in vitro fertilization pregnancy outcomes. However, the evidence thus far is mixed, with many studies (and even meta-analyses) suggesting that EMT may have little/no predictive value. One potential reason for the heterogeneity in results may be that most studies assessed mostly fresh autologous embryo transfers, without accounting for embryo quality nor the effect exogenous ovarian stimulation may have on endometrial receptivity and subsequent pregnancy/perinatal outcomes. Moreover, most subcategorized EMT to facilitate interpretation, which may have inadvertently introduced bias to the analyses.

Study design, size, duration: This retrospective, multicentre, cohort study analyzed all single blastocyst embryo transfers following oocyte donation from 2010-2019 subdivided into regular intervals of EMT (<7.0 mm, 7.0-8.9 mm, 9.0-10.9 mm, ≥11.0 mm). EMT was also evaluated as a continuous variable, comparing the best-fitting fractional polynomial against the linear function, to minimize bias due to residual confounding that may occur following the categorization of continuous variables. Confounder adjustment was performed using multivariable generalized estimating equations regression analysis.

Participants/materials, setting, methods: The oocyte donation model was chosen to minimize the influence of potential confounding factors such as poor embryo quality and the effect of ovarian stimulation on endometrial receptivity. The main objective of the study was to compare live birth rates (LBR). Our secondary outcomes included other surrogate pregnancy (hCG-positive pregnancy, clinical pregnancy, and miscarriage rates) and perinatal (gestational age at birth, preterm birth under 37 weeks, birthweight z-score, small and large for gestational age) outcomes.

Main results and the role of chance: In total, 33915 embryo transfers were analyzed. Confounder variables included were female donor and recipient age, recipient BMI, female factor infertility, male factor infertility, number of mature oocytes donated, oocyte status (fresh versus vitrified), sperm source (partner or donor), embryo status (fresh versus vitrified), embryo quality, endometrial preparation (natural or artificial cycle) and year of transfer. When compared to the reference EMT of 9.0-11.0 mm, EMT <7.0 mm was associated with lower hCG positive pregnancy rates (51.3% vs 57.6%; aOR 0.82 CI 0.75-0.89), lower clinical pregnancy (41.9% vs 49.1%; aOR 0.79 CI 0.73-0.86), higher miscarriage rates per hCG positive pregnancy (37.5% vs 31.4%; aOR 1.31 CI 1.16-1.47) and lower LBRs per transfer (32.1% vs 39.5%; aOR 0.76 CI 0.69-0.83). Regarding perinatal outcomes, we found significantly higher preterm delivery rates following an EMT <7.0 mm (14.1% vs 9.9%; aOR 1.54 CI 1.21-1.96) and higher large for gestational age rates associated with EMT ≥11.0 mm (16.4% vs 12.2%; aOR 1.40 CI 1.07-1.83). The fractional polynomial analysis revealed that the subcategorization of EMT caused clinically-relevant residual confounding. Specifically, LBRs assumed an approximate bell-shaped relationship with EMT, with LBR rates decreasing for both thinner EMTs (<7 mm subgroup) and thicker EMTs (≥11.0 mm subgroup).

Limitations, reasons for caution: The retrospective nature of the study and the inherent risk of bias related to unmeasured confounding may have impacted the results. Another potential limitation is the lack of perinatal outcome data due to loss of follow-up, which should be considered when interpreting these results.

Wider implications of the findings: Our results support the use of EMT as a predictor of live birth and as a marker of endometrial receptivity and competence. Moreover, it stresses the potential danger of subcategorizing all EMT <7.0 mm and >11.0 mm as equivalent, as the study found that these subgroups have varying LBR.

Trial registration number: not applicable

Abstract citation ID: dead093.177

O-150 Birth defects reporting and the use of oral dydrogesterone in assisted reproductive technology : a global pharmacovigilance study

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Study question: Is dydrogesterone exposure during early pregnancy associated with the reporting of birth defects ?

Summary answer: We observed an increased reporting of birth defects, such as congenital heart defects and hypospadias, with dydrogesterone, especially compared to progesterone use.

What is known already: In assisted reproductive technology (ART), intravaginal administration of progesterone is the standard of care to overcome luteal phase progesterone deficiency induced by ovarian stimulation. In the recent years, the Lotus I and Lotus II randomized controlled clinical trials, demonstrated that oral dydrogesterone, a synthetic progesterone derivative, was non-inferior for pregnancy rate at 12 weeks of gestation and could be an alternative to micronized vaginal progesterone (MVP). Safety profiles in both mother and child were similar. However, concerns have been raised recently regarding an association between dydrogesterone usage during early pregnancy and congenital heart disease in the offspring.

Study design, size, duration: We performed a case-non case study, similar in the concept to case-control study, using the international pharmacovigilance database, VigiBase. Study cohort consisted in spontaneous reports regarding pregnant women identified using the ad-hoc standardized query (SMQ "Pregnancy and neonatal topics"). Birth defect cases were reports containing a term related to "congenital, familial and genetic disorders" System Organ Classes (SOCs) (excluding genetic, infectious and metabolic abnormalities). Non-cases were reports of any other adverse drug reaction.

Participants/materials, setting, methods: Through a case–non case study conducted since database inception to 12/31/2021, we first compared the reporting of birth defects with dydrogesterone to that of any other drug, then to any other drug used for ART. Secondly, we performed a comparison on the reporting of birth defects for dydrogesterone with progesterone. Results are presented as reporting odds ratio (ROR) and their 95% confidence interval (95%CI). For each comparison, two sensitivity analyses were performed.

Main results and the role of chance: Among 29,120,563 individual case safety reports, 50,653 were related to the use of drugs for ART. Of these, 375 were cases of birth defects, including 60 (16%) with dydrogesterone. Dydrogesterone cases were mostly reported from Europe (73%) by physicians (82%). No other teratogenic drug was suspected in the onset of birth defect for dydrogesterone. 44 cases out of 60 (73.3%) were compatible with major birth defect (MBD) cases according to EUROCAT classification. These cases contained a total of 55 MBD, consisting mainly in genital defects such as hypospadias (n = 18, 32.7%), congenital heart defects (n = 15, 27.3%) limb defects (n = 10, 18.2%) and digestive system defects (n = 6, 10.9%). In the primary analysis, a significant disproportionate reporting of birth defects was found with dydrogesterone when compared to any other drug (ROR 5.4, 95%CI [3.9-7.6]) and to any other ART agent (ROR 5.9, 95%CI [4.2-8.4]). In the head-to-head comparison to progesterone, we found an increased reporting of birth defect with dydrogesterone (ROR 5.4, 95%CI [3.7-7.9]).These results were confirmed in both sensitivity analyses.

Limitations, reasons for caution: First, under-reporting, being inherent to pharmacovigilance systems, impedes the measurement of the incidence of adverse drug reaction and can limit the sensitivity of signal detection. Second, drug causality, not being the same for all cases, is challenging for such events and requires further assessment. However, sensitivity analyses showed consistent results.

Wider implications of the findings: Physicians should be aware of this potential risk and caution should be used when prescribing dydrogesterone for luteal phase support. Further data are needed to confirm that safety signal.

Trial registration number: Not applicable

Abstract citation ID: dead093.178

O-151 Intrahepatic cholestasis of pregnancy and relevant neonatal outcomes in IVF versus spontaneous conception: a prediction nomogram-based study

J. Yang[1], X. Zhang[1], G. Huang[1], H. Ye[1]

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Study question: Does the incidence of intrahepatic cholestasis of pregnancy (ICP) differ in spontaneous conception(SC) versus in vitro fertilization (IVF) conception?

Summary answer: We observed that the ICP rate was higher in IVF than in SC, and first built a prediction nomogram to find the predictors of ICP.

What is known already: There are limited clinical studies comparing intrahepatic cholestasis of pregnancy(ICP) and neonatal outcomes in puerperae who spontaneously conceived and those who conceived via assisted reproductive technology. Several studies comparing the maternal, laboratory, and perinatal characteristics of in-vitro fertilization(IVF) and spontaneous pregnancies concerning ICP show that IVF treatment has an increased risk of ICP in singleton and multiple deliveries. However, given that it is difficult to collect big data, most research on IVF patients with ICP involve small sample size or case reports.

Study design, size, duration: This was a retrospective real-world data study that linked the information from puerperae and neonates. We included 4,467 puerperae who conceived via ART, and 28,336 puerperae who conceived spontaneously.

Participants/materials, setting, methods: Cochran–Mantel–Haenszel (CMH) analysis and a general linear model (GLM) were used to control bias. We compared the related serum-derived indicators and neonatal outcomes of ICP patients with in vitro fertilization (IVF) and SC. Multivariate logistic regression analysis, a forest plot, and nomogram were used to assess impact factors and risk prediction.

Main results and the role of chance: Logistic analysis adjusted for confounders revealed significant differences in the ICP rate of singleton delivery (4.24% vs. 3.41%, adjusted OR = 1.26 [95% confidence interval (CI) 1.03–1.53], *P*=0.025) and in groups with total bile acids(TBA) ≥40 and <100 μmol/L (14.77% vs. 10.39%, aOR=1.31[95% CI 1.06–1.63], *P*=0.023) between IVF and SC. When we divided newborns into singleton and twins delivery, the GLM revealed a higher rate with Apgar score <7 (13.44% vs. 3.87%; aOR=3.85 [95% CI: 2.07–7.17], *P* <0.001) and fetal distress for IVF in comparison with SC (19.32% vs. 5.55%; OR = 3.48 [95%CI: 2.39–6.95], *P* <0.001) in the singleton group. In multivariate logistic regression analysis, five factors were independent predictors of ICP: body mass index (BMI) (aOR=1.75 [95% CI 1.03–2.13], *P*=0.036), number of embryo transferred (ET) (single ET vs. double ET: aOR=4.82 [95% CI 3.83–6.05], *P*<0.001), E_2 level on the ET day (aOR=2.79 [95% CI 1.79–4.05], *P*=0.011),

fresh ET which compared with frozen ET (FET) (aOR=1.40 [95% CI 1.09–1.80], P=0.008), and severe ovarian hyperstimulation syndrome which compared with non-OHSS (aOR=3.97 [95% CI 1.79–8.80], P<0.001). These predictive factors in the logistic regression model were integrated into the nomogram (C-index=0.735 [95% CI, 0.702–0.764]); for each patient, higher total points indicated a higher risk of ICP.

Limitations, reasons for caution: The limitations of this study include its retrospective nature, and the balance in baseline characteristics between IVF and SC measured using statistical methods.

Wider implications of the findings: Our results provide evidence for the incidence of ICP between spontaneously conceived and those who conceived via IVF, and found predictors of ICP in IVF treatment. It could assist physicians in making clinical decisions avoiding risk factors during IVF, and taking preventive countermeasures for patients.

Trial registration number: not applicable

Abstract citation ID: dead093.179

O-152 Benign epithelial ovarian tumors following use of fertility drugs: a register-based cohort study from Denmark

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Study question: Is use of fertility drugs associated with an increased risk of benign epithelial ovarian tumors among women with infertility?

Summary answer: Among women with infertility, use of fertility drugs is not associated with an increased risk of benign epithelial ovarian tumors.

What is known already: Benign epithelial ovarian tumors are the most common neoplasms in the ovaries. These benign tumors share some of the same risk factors, which are associated with ovarian cancer. The general understanding of the pathogenesis and etiology of ovarian cancer is sparse; therefore, knowledge on factors influencing development of benign epithelial ovarian tumors—and hence likely ovarian cancer—is warranted. Previous studies on the association between fertility treatment and ovarian cancer are conflicting, whereas no previous study has examined the association between use of fertility drugs and benign epithelial ovarian tumors.

Study design, size, duration: Retrospective register-based cohort study of nearly 145,000 women included in the Danish Infertility Cohort during 1995–2017 at age 20–45 years. Women were followed from one year after infertility diagnosis until first benign epithelial ovarian tumor or one of the censoring criteria: borderline ovarian tumor, ovarian cancer, oophorectomy, emigration, death or end of follow-up (December 31, 2018).

Participants/materials, setting, methods: Using the Aalen-Johansen estimator, we determined 10-year cumulative risks of benign epithelial ovarian tumors among approximately 25,000 women who used fertility drugs (defined within one year after infertility diagnosis: clomiphene citrate, gonadotropins, human chorionic gonadotropin, gonadotropin-releasing hormone [GnRH] receptor modulators, and progesterone) compared with women diagnosed with infertility and no drug use. Additionally, we used Cox proportional hazards regression models to calculate hazard ratios (HRs) and 95% confidence intervals (CIs).

Main results and the role of chance: Our results showed that the crude 10-year cumulative risk of benign epithelial ovarian tumors were 0.32% (95% CI: 0.29%–0.36%) for women who used any fertility drug compared with 0.49% (95% CI: 0.40%–0.60%) in women with no use of fertility drugs (risk difference: -0.17% [95% CI: -0.28–(-0.07)]). When adjusted for age, calendar period of infertility diagnosis, educational level, parity, obesity, polycystic ovary syndrome, and origin of infertility information, the HR for use of any fertility drug was 0.73 (95% CI 0.55–0.96). In general, the 10-year risk differences as well as the HRs for each drug evaluated separately were similar to the results for use of any drug. Lastly, we tested the robustness of our results by various pre-defined secondary analyses, and this did not change our results.

Limitations, reasons for caution: We did not include information on number of cycles and treatment regimens as these are only partly available in the registers.

Wider implications of the findings: Among women with infertility, we observe a lower relative risk of benign epithelial ovarian tumors for women treated with fertility drugs compared with women who did not use fertility drugs. However, this finding translates into a small decrease in absolute risk, which does not appear to have any clinical relevance.

Trial registration number: Not applicable

POSTER DISCUSSION SESSION
SESSION 47: EMBRYOLOGY

Tuesday 27 June 2023 Hall D2 10:00 - 11:30

Abstract citation ID: dead093.180

P-172 Effect of anti-centromere antibodies on multi pronuclear formation

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[2]*Asada Institute for Reproductive Medicine, Laboratory, Kasugai, Japan*

Study question: Do the anti-centromere antibodies (ACA) affect the multi pronuclear formation (MPF) rate?

Summary answer: MPF rate was higher in patients with ACA. The MPF rate of ACA patients with antibody titers ≥160-fold was significantly higher than in titers 40-fold.

What is known already: ACA is an anti-nuclear antibody (ANA), and specifically recognizes the centromere. Recently, several studies have reported that the rate of oocyte maturation is lower in patients with ACA. Moreover, in MI oocytes collected from ACA patients, female chromosomes were frequently dispersed in the cytoplasm.

Study design, size, duration: A total of 4756 patients from our clinic were tested for ANA before oocyte retrieval from January 2014 to December 2021. After retrospective ANA testing, patients were classified according to 3 groups; 62 ACA patients (with only ACA), 1134 non-ACA patients (with ANA but not with included ACA) and 3560 non-ANA patients (without ACA and any ANA).We considered ACA-positive levels at titers ≥40. ACA patients were further classified into 6 groups by antibody titer.

Participants/materials, setting, methods: The MPF rate after ICSI was compared in the 3 groups (ACA patients, non-ACA patients, non-ANA patients) and at 6 ACA titers in each group (titer of ACA; 40-fold, 80-fold, 160-fold, 320-fold, 640-fold, ≥1280-fold).MPF rate was calculated by dividing the number of embryos that formed three or more pronuclei by the number of embryos inseminated by ICSI.Ryan's method was used for multiple comparisons of ratios.

Main results and the role of chance: MPF rate was 3.8% (1997/53240) in non-ANA patients, 4.3% (733/17003) in non-ACA patients, and 32.1% (351/1092) in ACA patients, being significantly higher in ACA vs other groups (P<.01). In comparisons between ACA titers, MPN rate was 8.7% (4/46) at 40-fold , 13.0% (3/23) at 80-fold , 36.1% (56/155) at 160-fold , 32.4% (48/148) at 320-fold , 36.0% (111/308) at 640-fold , and 31.3% (129/412) at ≥ 1280-fold, respectively. MPN rate of patients with titers ≥160-fold was significantly higher than with 40-fold.

Limitations, reasons for caution: A potential limitation of the present study is the small sample size. This is because ACA patients account for only 1% of patients who underwent ART treatments.

Wider implications of the findings: MPF rate in ACA patients was significantly higher than in non-ANA and non-ACA patients and was also significantly higher in ACA patients with titers of ≥ 160-fold vs 40-fold. The dispersion of the female chromosome in the cytoplasm of MI oocytes may be a cause of MPN formation.

Trial registration number: None

Abstract citation ID: dead093.181
P-171 Identification of aneuploid embryos using cell free DNA in embryo culture medium

F. Kondo[1,2], H. Kitasaka[1,2], H. Watanabe[1,2], Y. Kida[1,2], S. Takeda[1,2], N. Fukunaga[1,2], Y. Asada[1,2]

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Study question: Can noninvasive preimplantation genetic testing (PGT) using cell free DNA from embryo culture medium discriminate embryonic aneuploidy status?

Summary answer: Aneuploid embryos in older patients can be identified by cell free DNA in embryo culture medium.

What is known already: There have been reports of non-invasive PGT by analysing cell free DNA (cfDNA) released from embryos into culture medium, and found to be highly correlated with the analysis results of Trophectoderm (TE)-biopsy (Carmen.Rubio, 2020).We have initiated in 2021, a preliminary research program for the clinical application of niPGT. In aneuploid embryos, we have found a tendency for a high concordance rate between cfDNA analysis and blastocyst analysis results, suggesting the usefulness of cfDNA analysis in terms of identifying aneuploid embryos.Subsequently, we examined the usefulness of cfDNA analysis by comparing the concordance rate with cfDNA analysis and TE biopsy of blastocysts.

Study design, size, duration: After thawing pronuclear-stage frozen embryos for which consent was obtained for research use, they were co-cultured until Day 4, and then individually cultured on in new dishes.40 embryos that reached morula on day 4 or day 5 and blastocyst on day 6 or day 7 were included.

Participants/materials, setting, methods: The biopsied TE, the biopsied blastocyst, and the cfDNA obtained after individual culture were all sent to an external provider (Igenomix: Valencia, Spain) for chromosomal aneuploidy analysis by NGS. The concordance rate of the analysis for blastocysts in cfDNA and TE was calculated by dividing them into euploidy and aneuploidy, and by age. Concordance between the results of the blastocyst analysis and the culture medium were assessed according to the standards of the inspection company.

Main results and the role of chance: For the blastocyst analysis results, the concordance rate with cfDNA was 76.5% (26/34) and the concordance rate with TE was 85.3% (29/34), showing no difference. For the euploid blastocysts analyzed, the matching rate with cfDNA was 61.1% (11/18) and with TE was 77.8% (14/18), showing no difference. Similarly, for aneuploid blastocysts, the concordance rate with cfDNA was 93.8% (15/16) and with TE was 93.8% (15/16), showing no difference. Detailed analysis of aneuploid blastocysts revealed that the concordance rate with cfDNA was 88.9% (8/9) and the concordance rate with TE was 88.9% (8/9) in women under 39 years of age. In addition, the concordance rate with cfDNA was 100.0% (7/7) with TE was 100.0% (7/7) in those aged 40 and over. (Fisher's exact test)

Limitations, reasons for caution: Concordance between cfDNA and blastocyst analyzes in euploid embryos was low, continued follow-up is considered necessary.

Wider implications of the findings: In a detailed analysis of aneuploid blastocysts aged 40 years or older, Similar to TE, cfDNA showed a high concordance rate with the results of blastocyst analysis, demonstrating that cfDNA provides sufficient accuracy for identifying aneuploid embryos in women over 40 years of age.

Trial registration number: not applicable

Abstract citation ID: dead093.182
P-152 Velocity of cytoplasmic movement in trophectodermal cells depends on the keratin cytoskeleton

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Study question: Can dysfunction of the keratin cytoskeleton, known to severely hinder postimplantation development of mammalian embryos, be detected by analysis of cytoplasmic movement in trophectodermal cells?

Summary answer: Depletion of keratins increases cytoplasmic movement in trophectodermal cells in extent depending on the developmental stage and on the location of the trophectodermal cells (mural/polar).

What is known already: Recent literature data indicate that keratins, intermediate filament proteins, are key regulators of trophectoderm function in mouse and human embryos. Keratin knockouts display trophoblast fragility, placental bleeding, and lethality after implantation. However, depletion of keratin 8 (Krt8) and keratin 18 (Krt18), which are most abundant in preimplantation embryos, does not lead to severe phenotype up to the blastocyst stage at least in mice and cattle, so can easily remain unnoticed. However, it affects the biomechanical properties of the trophectoderm. Analysis of cytoplasmic movement allows for biomechanical assessment of the trophectoderm in a non-invasive way.

Study design, size, duration: We analyzed 188 mouse embryos (66 control, 60 with Krt8 depletion, and 62 with Krt18 depletion) to quantitatively assess changes in the velocity of cytoplasmic movement caused by a knockdown of Krt8 and Krt18. Additionally, we correlated the velocity of cytoplasmic movement in trophectodermal cells ($n = 70$ embryos) with the ability to form outgrowths. Appropriate statistical tests were used to determine the statistical significance of the obtained results. A value of $p < 0.05$ was considered statistically significant.

Participants/materials, setting, methods: Zygotes were injected with Krt8 and Krt18 siRNAs or with scrambled siRNAs used as a negative control. The efficiency of the knockdown was tested on mRNA and protein levels. The embryos were cultured for 3 or 4 days and the velocity of cytoplasmic movement in polar and mural trophectoderm cells was measured. Additionally, we correlated the cytoplasmic movement velocity in trophectodermal cells in E3.5 embryos with their ability to implant in vitro (i.e., form outgrowths).

Main results and the role of chance: Our research showed that the velocity of cytoplasmic movement of trophectodermal cells varies depending on the embryo's developmental stage. Namely, the velocity of cytoplasmic movement was higher in E3.5 blastocysts than in E4.5 blastocysts. In addition, the velocity of cytoplasmic movement was higher in polar (8.45 ± 2.64 nm/s in E3.5 and 6.79 ± 1.96 nm/s in E4.5 control embryos) than in mural (6.30 ± 2.30 nm/s in E3.5 and 4.98 ± 1.89 nm/s in E4.5 control embryos) trophectodermal cells. Noteworthy, the depletion of Krt8 and Krt18 increased the velocity of cytoplasmic movement both in the mural and polar trophectodermal cells. However, it appears that changes caused by the depletion of these keratins depend on the embryo's developmental stage. For example, the depletion of Krt18 increased the velocity of cytoplasmic movement in mural trophectodermal cells in E3.5 embryos (9.61 ± 3.80 nm/s), while the depletion of Krt8 - in E4.5 embryos (6.65 ± 2.35 nm/s). In addition, data from our preliminary experiments with in vitro implantation suggested that embryos characterized by the higher cytoplasmic movement velocity were unable to form an outgrowth. The above data suggest that the velocity of cytoplasmic movement depends on the keratin cytoskeleton and probably can be used to predict an embryo's ability to implant.

Limitations, reasons for caution: We used mouse embryos, a well-established model of early mammalian embryo development. However, analogous experiments should be repeated on other mammalian species, including humans, if we wish to pursue the possibility that cytoplasmic velocity in trophectodermal cells is an embryo quality marker.

Wider implications of the findings: Our data indicate that cytoplasmic velocity in trophectodermal cells may be a valuable tool in embryo quality assessment, as it reflects the functionality of the keratin cytoskeleton in embryos as well as correlates with the embryos' ability to implant.

Trial registration number: not applicable

Abstract citation ID: dead093.183
P-170 A sibling oocyte study using a real-time image analysis system to identify optimal puncture positions in Piezo-ICSI

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Study question: Is the newly developed image analysis system capable of visualizing the shape features of the oolemma in real-time, useful for reducing oocyte degeneration during Piezo-ICSI?

Summary answer: Unintentional membrane rupture (UMR) can be reduced by this real-time image analysis, which lowers the risk of oocyte degeneration following Piezo-ICSI.

What is known already: UMR in the puncturing process of ICSI often predisposes oocyte degeneration. Identifying the appropriate puncturing positions may decrease the likelihood of UMR and thus degeneration, but this cannot be visualized via a microscope. We have reported that visualization of the shape features of the oolemma using moving image analysis during Piezo-ICSI can locate areas on the oolemma where UMR is likely to occur. It is feasible to assess the incidence of UMR and oocyte degeneration by performing ICSI while selecting appropriate puncturing positions with the aid of the newly developed imaging system.

Study design, size, duration: Our team have developed an image analysis system called ICSI POSITION DETECTOR (IPD), which can identify areas where rupture is likely to occur and visualize it in real-time using a video monitor. We prospectively evaluated the usefulness of IPD in a sibling oocyte study. From January 2020 to August 2021, a total of 1268 oocytes obtained in 225 oocyte retrieval cycles (average maternal age: 38.3 ± 4.7 years old) was included.

Participants/materials, setting, methods: The oocytes were randomly assigned to two groups: IPD-using vs. non-IPD-using. In the IPD-using group, Piezo-ICSI was performed at the "appropriate" position with a low chance of UMR indicated by IPD. In the non-IPD-using group, Piezo-ICSI was performed blindly. The rates of UMR, oocyte degeneration, fertilization and embryonic development were compared between the two groups. In addition, in the non-IPD-using group, moving images were recorded during Piezo-ICSI and analyzed retrospectively using IPD.

Main results and the role of chance: The rates of UMR and degeneration were significantly lower in the IPD-using group compared to the non-IPD-using group (6.0% vs. 11.9%, $P < 0.001$ and 1.6% vs. 4.6%, $P < 0.01$, respectively). The rates of fertilization (83.7% vs. 79.7%), blastocyst formation (51.9% vs. 51.0%), and good-quality blastocyst (24.7% vs. 24.3%) were not significantly different. Retrospective analysis of moving images using IPD on the non-IPD-using group showed that in 45.3% of oocytes (286 out of 632) ICSI was performed at a position with a high chance of UMR (inappropriate position). When ICSI was performed at the appropriate positions using IPD, the rates of UMR (6.0% vs. 18.2%, $P < 0.001$) and degeneration (1.6% vs. 7.3%, $P < 0.001$) were significantly lower, while the rates of fertilization (83.7% vs. 74.5%, $P < 0.01$) and blastocyst formation (51.9% vs. 43.0%, $P < 0.05$) were significantly higher than those when ICSI was performed at an inappropriate position. The rate of good-quality blastocyst (24.7% vs. 19.7%) was also higher, but not statistically significant. These results indicate that IPD can reduce the risk of UMR, thereby lower the degeneration rate. Furthermore, the embryonic development was better when using IPD to identify the appropriate position to perform ICSI, suggesting that UMR seems to be associated with poor ICSI outcome.

Limitations, reasons for caution: In this study, Piezo-ICSI was performed by only two designated embryologists in a single center. It did not assess clinical outcomes. Further research involving more embryologists is needed. Moreover, it would be feasible to investigate whether the usage of IPD is similarly effective on conventional-ICSI outcome.

Wider implications of the findings: The application of IPD to perform ICSI at the appropriate position can significantly avoid UMR, and thereby reduce oocyte degeneration and provide better embryonic development. We therefore, consider IPD to be a highly clinically useful tool, which contributes to the production of more embryos that can be used for treatment.

Trial registration number: not applicable

Abstract citation ID: dead093.184
P-201 Developmental analysis of blastocysts with blastomere exclusion during compaction and its relationship to pregnancy outcomes and preimplantation genetic testing for aneuploidy results

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Study question: What effects do blastocysts with blastomere exclusion during compaction have on embryo morphokinetics, morphology grade, genetic testing for aneuploidy (PGT-A) results, and pregnancy outcomes?

Summary answer: Blastocysts with blastomere exclusion leads to delay blastulation and poor morphology grade, but does not affect pregnancy outcomes and euploidy in PGT-A.

What is known already: It has been reported that aneuploid blastomeres are excluded during compaction, which is a mechanism for chromosome repair in blastocysts through the exclusion of aneuploid cells.Furthermore, in this phenomenon, it is known that the first cleavage of embryos is often abnormal, in which three or more cells originate from a single cell division event.However, this phenomenon has not been reported in the evaluation of embryo morphokinetics, artificial intelligence (AI) scores, or PGT-A results.

Study design, size, duration: In this retrospective study, we analyzed clinical medical reports at the Takahashi Women's Clinic in Japan. We included 934 blastocysts cultured in a time-lapse incubator (EmbryoScope) after intracytoplasmic sperm injection (ICSI) from January 2018 to June 2021.

Participants/materials, setting, methods: Blastocysts cultured using a time-lapse incubator (EmbryoScope) after ICSI were classified into a control group ($N = 515$) with blastomere compaction or a BE group ($N = 419$) with blastomere exclusion during compaction. The first type of cleavage, AI score (IDAScore), embryo morphokinetics, and single frozen-thawed blastocyst transfer outcome were evaluated.Logistic regression analysis was performed to compare the two groups, considering patient age, blastocyst grade (Gardner classification), culture days, body mass index, and basal anti-Müllerian hormone level.

Main results and the role of chance: Comparing the control and BE groups, the first cleavage abnormality rate was 16.9% vs. 67.3% ($P < 0.001$), the percentage with good embryo grade (Gardner criteria ≧BB) was 93.4% vs. 61.0% ($P < 0.001$), and the mean score by AI analysis was 8.3 ± 1.3 vs. 6.4 ± 1.9 ($P < 0.001$), respectively. The BE group had a higher rate of abnormal cleavage and a significantly worse embryo grade and AI score than the control group.In the evaluation of embryo morphokinetics, the time to morula was 84.2 ± 7.0 h vs. 89.0 ± 8.5 h ($P < 0.05$) and time to blastocyst was 105.9 ± 9.4 h vs. 114.7 ± 11.4 h ($P < 0.05$). The BE group significantly delayed blastulation compared to the control group.The euploidy, mosaicism, and aneuploidy rates of PGT-A were 13.6% (9/66), 9.1% (6/66), and 77.3% (51/66) in the control group vs. 13.2% (5/38), 13.2% (5/38), and 73.7% (28/38) in the BE group, respectively. There was no significant difference in the PGT-A results between the two groups. Furthermore, the single-blastocyst transfer pregnancy rates (gestational sac with heart activity) in the control and BE groups were 47.1% (147/312) and 32.6% (60/184), respectively. Pregnancy rate was lower in the BE group, but was not significantly different from that of the control group.

Limitations, reasons for caution: The study was conducted at a single in vitro fertilization center. Embryo transfer results were based on ongoing pregnancies, while live birth data for all pregnancies are not yet available.

Wider implications of the findings: Although the BE group was graded worse, statistical analysis considering the grade showed no significant difference in PGT-A and pregnancy rates between the two groups.These results suggest that blastocysts with blastomere exclusion can be useful as transferable embryos if they grow to a transferable grade.

Trial registration number: not applicable

Abstract citation ID: dead093.185
P-217 Artificial intelligence (AI)-supported MAGENTA oocyte assessments shown to prospectively correlate with utilizable blastocyst development in patients, and for the first time in oocyte donors

J. Pons Ballester[1], M. Alavés[1], J. Teruel[1], J. Fjeldstad[2], N. Mercuri[2], A. Krivoi[2]

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Study question: Can MAGENTA, an AI oocyte assessment tool, prospectively assess the quality of oocytes retrieved from patients and donors as it relates to reproductive outcomes?

Summary answer: MAGENTA AI oocyte assessments provide insights on the quality of mature oocytes retrieved from both patients and young donors that correlate with utilizable blastocyst development.

What is known already: Oocyte donation has increasingly become a treatment path for many patients struggling with infertility. With specific criteria, oocyte donation aims to provide higher quality oocytes to increase the chances of success for recipients. However, not all oocytes from even a young donor will be of the same quality or have the same chances of reproductive success. MAGENTA is a non-invasive oocyte AI image analysis tool that provides an assessment of oocyte quality through a score on a scale of 0-10. MAGENTA scores in patient oocytes have previously been shown to correlate with subsequent development of a blastocyst, and its quality.

Study design, size, duration: A prospective study was conducted from April-November 2022 by Equipo Médico Crespo (Valencia, Spain) utilizing MAGENTA to assess the oocyte quality of patient and donor oocytes by assigning a score on a scale of 0-10. MAGENTA scores of denuded metaphase II (MII) oocytes were assessed for correlation to reproductive outcomes (fertilization and blastocyst development). 1,313 fresh MII oocytes retrieved from 126 subjects (68 patients, 59 donors) between the ages of 19-47 were included for analysis.

Participants/materials, setting, methods: Prior to ICSI, a non-invasive light microscopic image was taken of denuded MII oocytes utilizing specialized image capture software. Images were uploaded and analyzed by MAGENTA to provide a score, which remained blinded for the duration of the study. Reproductive outcomes regarding each mature oocyte were collected over the fertility cycle. Blastocyst development was defined by a Gardner grade by Day 5/6 post-ICSI. A utilizable blastocyst was considered a Gardner grade of 2CC or greater.

Main results and the role of chance: The following comparisons were assessed by Welch's Two Sample t-tests.

Overall, successfully fertilized oocytes had higher mean MAGENTA scores (4.5) than those that did not (3.9) ($p < 0.01$); furthermore, those oocytes that successfully developed into a blastocyst had higher mean MAGENTA scores (4.8) than those that did not (4.0) ($p < 0.01$).

Patient oocytes were found significantly older (mean age: 38.5) compared to donated oocytes (mean age: 25) ($p < 0.01$).

Patient oocytes that successfully developed into a blastocyst had a higher mean MAGENTA score (4.7) than those that did not (4.0) ($p < 0.01$); similarly, donated oocytes that successfully developed into a blastocyst had a higher mean MAGENTA score (4.9) than those that did not (4.1) ($p < 0.01$).

The MAGENTA scale was divided into 4 groups: A: 0-2.5 (346 oocytes); B: 2.6-5 (454 oocytes); C: 5.1-7.5 (358 oocytes); D: 7.6-10 (155 oocytes), over the whole dataset.

There was an overall increasing proportion of oocytes that developed into not only blastocysts, but more impressively, utilizable blastocysts from the lowest to highest MAGENTA score groups with significant differences between the patient oocytes scored in group B(38%) and C(50%) ($p < 0.05$), and the donated oocytes scored in group A(39%) and B(52%), and group C(54%) and D(66%) ($p < 0.05$) by Two Proportions Z-tests.

Limitations, reasons for caution: This study was conducted in fresh oocyte ICSI cycles. The use of frozen patient and donor oocytes should be assessed by VIOLET prior to vitrification, which takes into consideration freezing and thawing statistics. Further data is required to determine any correlation of MAGENTA with embryo ploidy or implantation status.

Wider implications of the findings: MAGENTA's a valuable tool for oocyte quality assessments correlating with utilizable blastocyst development amongst both autologous and donated oocytes. Although young donors are assumed to have high quality oocytes, this is not always true. MAGENTA assessments for donor oocytes could be essential to provide insights when these cycles unexpectedly fail.

Trial registration number: not applicable

INVITED SESSION
SESSION 48: EUROPEAN AND GLOBAL ART MONITORING

Tuesday 27 June 2023 Hall D1 11:45 - 12:45

Abstract citation ID: dead093.186
O-153 Assisted Reproductive Technology (ART) in Europe 2020 and development of a strategy of vigilance: Preliminary results generated from European registers by the ESHRE EIM Consortium

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Study question: What are the reported data on cycles in ART, IUI and fertility preservation interventions in 2020 as compared to previous years and what are the main trends over the years?

Summary answer: author: The 24rd ESHRE report on ART and IUI shows a progressive increase in reported treatment cycle numbers in Europe, a small decrease in the number of transfers (IVF + ICSI) with more than one embryo with a trend to decreasing multiple delivery rates, outcomes for IUI cycles are similar to previous years. WHAT IS KNOWN ALREADY: Since 1997, ART

aggregated data generated by national registries, clinics or professional societies have been collected, analyzed by the European IVF-monitoring Consortium (EIM) and reported in 23 manuscripts published in Human Reproduction and Human Reproduction Open.

Study design, size, duration: Yearly collection of European medically assisted reproduction (MAR) data by EIM for ESHRE. The data on treatments performed between January 1 and December 31 2020 in 35 European countries were provided by either National Registries or registries based on personal initiatives of medical associations and scientific organizations.

Participants/materials, setting, methods: In all, 1157 clinics offering ART services in 35 countries reported a total of 713 708 treatment cycles, involving 110 097 with IVF, 253 095 with ICSI, 239 759 with frozen embryo replacement (FET), 46 610 with preimplantation genetic testing (PGT), 59 906 with egg donation (ED), 344 with IVM of oocytes and 3897 cycles with frozen oocyte replacement (FOR). European data on IUI using husband/partner's semen (IUI-H) and donor semen (IUI-D) were reported from 1176 institutions offering IUI in 28 and 18 countries, respectively. A total of 102 702 treatments with IUI-H and 36 476 treatments with IUI-D were included. A total of 18 270 fertility preservation (FP) interventions from 15 countries including oocyte, ovarian tissue, semen and testicular tissue banking in pre-and post-pubertal patients were reported.

Main results and the role of chance: In total, 1157 IVF clinics participated (88.5% of registered clinics in the participating countries). Next to these also 1176 IUI units reported their data. In the 35 reporting countries, after IVF the clinical pregnancy rates (PR) per aspiration and per transfer in 2020 were similar to those observed in 2019 (27.9% and 32.9% versus 28.5% and 34.6%, respectively). After ICSI the corresponding rates were also similar to those achieved in 2019 (24.3% en 32.2% versus 26.2% and 33.5%). After FET with own embryos, the PR per thawing is stabilizing, 35.1% in 2019 and 34.6% in 2020. After ED the PR per fresh embryo transfer was 50.4% (50.5% in 2019) and per FOR 45.3% (44.8% in 2019). In IVF and ICSI together, the trend towards the transfer of fewer embryos continues with the transfer of 1, 2, 3 and $\geq$4 embryos in 62.1%, 32.3%, 2.2% and 0.3% of all treatments, respectively (corresponding to 55.4%, 39.9%, 2.6% and 0.2% in 2019). This resulted in a proportion of singleton, twin and triplet DRs of 90.5%, 9.3% and 0.2%, respectively (compared to 87.7%, 12.0% and 0.3%, respectively in 2019). Treatments with FER in 2020 resulted in twin and triplet DR of 7.0% and 0.1%, respectively (versus 9.3% and 0.1% in 2019). After IUI, the DRs remained similar at 8.9% after IUI-H (9.2% in 2019) and at 12.4% after IUI-D (12.1% in 2019). Twin and triplet DRs after IUI-H were 8.3% and 0.4%, respectively (in 2019: 8.7% and 0.3%) and 5.8% and 0.2% after IUI-D (in 2019: 6.2% and 0.2%). The majority of FP interventions included the cryopreservation of oocytes (n = 5 365 from 14 countries) and of ejaculated sperm (n = 11 571 from 14 countries).

Limitations, reasons for caution: As the methods of data collection and levels of completeness of reported data vary among European countries, the results should be interpreted with caution. For this abstract, some countries were not able to provide adequate data about the number of centers and initiated cycles and deliveries. WIDER IMPLICATIONS OF THE FINDINGS: The 24rd ESHRE report on ART and IUI shows a continuous increase of reported treatment numbers and MAR-derived live births in Europe. Being already the largest data collection on MAR in Europe, continuous efforts to stimulate data collection and reporting strive for future quality control and completeness of the data and offer higher transparency and vigilance in the field of reproductive medicine.

Trial registration number: XXXX

Abstract citation ID: dead093.187

O-154 ICMART preliminary world report 2019

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Abstract title: International Committee for Monitoring Assisted Reproductive Technologies (ICMART) Preliminary World Report on ART, 2019

Study question: In 2019 what was the global utilization, effectiveness and safety of ART?

Summary answer: Globally, ART utilization and data collection continue to increase but with wide variations in utilization, effectiveness and safety.

What is known already: ICMART began ART global data collection in 1991. Utilization, effectiveness and safety have continuously improved with more cycles, higher pregnancy rates and lower multiple birth rates, the latter due to the transfer of fewer embryos. Frozen embryo transfer (FET) and donor egg cycles continue to increase. However, wide variations in practice and outcomes exist globally. Approximately 10 million ART babies have been born. ICMART has helped develop registries internationally. A new electronic data collection platform has been developed with the University of New South Wales (UNSW) in Sydney, Australia; nevertheless, data collection and quality remain challenging.

Study design, size, duration: Countries and regions annually collect ART data, some prospectively and others retrospectively. ICMART retrospectively requested data from all known global sources for 2019 and reviewed them for missing or incorrect data. The dataset was reviewed and corrected by ICMART, in partnership with UNSW for validation and analysis, then ICMART finalized the results tables. Standardized definitions from The International Glossary on Infertility and Fertility Care, 2017, and previously developed methods were used. Preliminary results are presented.

Participants/materials, setting, methods: The European IVF Monitoring Consortium (EIM), Latin American Network of Assisted Reproduction (REDLARA), Australian/ New Zealand Registry and African Network and Registry for ART (ANARA) submitted regional data, and other countries contributed national data, through standardized formats, to ICMART. A few individual clinics with no registry access also contributed. Data received were reviewed, corrected, and validated to the extent possible, analyzed and summarized by ICMART using descriptive statistics.

Main results and the role of chance: Data collection and analysis are ongoing, so the presented results are preliminary. The number of ART cycles continues to increase, but utilization is still highly variable among countries and regions. Regional and country differences persist in the age of women treated, number of embryos transferred, live birth rates, rate of multiple births, use of ICSI, cryopreservation cycles and other factors.

The role of chance is minimal. Actual global ART results are limited to reporting countries and clinics representing 90 to 95% of global cycles. However, this is a very large sample size from which imputation of total global results is performed.

Limitations, reasons for caution: Most, but not all, countries report to ICMART. Some countries have limited data and many countries have limited data validation. ICMART can perform only minimal verification of submitted data. Widespread adherence to consensus definitions provided in the Glossary takes time and requires translation into multiple languages. Standardization of validation and reporting is an ongoing process because of missing data and continuing changes in clinical practice.

Wider implications of the findings: ICMART World Reports standardize data, track trends, enable comparisons, stimulate questions and improve ART

quality. Better understanding of ART increases societal acceptance and support for equitable access and ART research.

Abstract citation ID: dead093.188
O-155 Building bridges to harmonize different data collection systems worldwide - need and challenges

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In order to transfer data from individual IVF centers and a national register into a regional register and then merge this data into a world register, certain basic definitions must be established.

The treatment strategies of each country are influenced by the legislative and insurance framework.

The expectations of patient couples also vary greatly from region to region.

All of this can only work if there are definitions of what data is to be collected and in what way.

Above all, there are difficulties in creating statistics when the raw data first has to be adjusted and merged.

This article is intended to point out some difficulties, but also to show solution concepts.

INVITED SESSION
SESSION 51: COMPARING THE REAL AND THE FAKE
Tuesday 27 June 2023 Hall D5 11:45 - 12:45

Abstract citation ID: dead093.189
O-156 Embryo-like models: Using 3D gastruloids to understand human development

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The mammalian embryo develops through coordinated cell fate decisions that enable emerging spatiotemporal and morphogenetic complexity. While much insight into this process has been gained through studying model organisms, including mouse embryos, equivalent experiments are technically and ethically challenging in the human embryonic context. Instead, we use gastruloids: 3-dimensional aggregates of pluripotent stem cells that undergo multilineage differentiation to all three germ layers, polarise their gene expression and exhibit axial organisation that recapitulates many of the features of gastrulation and early organogenesis. By closely examining the dynamics and coordination of cell fate decisions and spatial gene expression organisation, we hope to better understand the regulatory logic behind early developmental events, particularly those that are human-specific. Likewise, by carefully manipulating the environment in which these gastruloids are grown, we can bias the structures towards particular lineages and morphological structures of interest. Doing so allows us to explore many of the principles of early development, and provides an opportunity to probe the mechanisms of biomedically-relevant conditions, such as congenital abnormalities.

Abstract citation ID: dead093.190
O-157 Modelling human early development

L. David[1]

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Understanding human peri-implantation development is necessary to improve in vitro fertilisation. Technical progress, such as scRNAseq and integrated stem cell models have dramatically improved our ability to understand human peri-implantation development. However, in-depth analysis and systematic validation in human embryos remains necessary to build a reliable reference. Here, we started by integrating pre- and post-implantation scRNAseq embryo datasets to generate a peri-implantation reference, highlighting progression of fates during early human development. Second, we identified the dynamics of genes modules, groups of genes with similar expression pattern. In particular, this highlighted the hierarchy of transcription factor potentially driving cell fate progression. Importantly, we performed prolonged embryo culture to validate by immunofluorescence progression of EPI and TE fates. Moreover, we compared the expression profile of the identified markers in stem cell models. Finally, we used the combination of gene modules to benchmark blastoid models. Altogether, our study paves the way for modelling human peri-implantation development with stem cell models.

Trial registration number: NA
Study funding:
Funding source:

SELECTED ORAL COMMUNICATIONS
SESSION 49: CRYOPRESERVATION AND CRYOTRANSFER
Tuesday 27 June 2023 Hall D4 11:45 - 13:15

Abstract citation ID: dead093.191
O-158 Prediction of implantation of vitrified-warmed blastocysts using a deep learning algorithm on a single post-warming image of each embryo

L. Conversa Martínez[1], **M.Á. Valera Cerdá**[1], **L. Bori Arnal**[1], **L. Murria Aguilar**[1], **A. Cobo Cabal**[2], **M. Meseguer Escrivá**[2]

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Study question: Can a deep learning algorithm determine the implantation likelihood of vitrified blastocysts from single time-point post-warming time-lapse images obtained from EmbryoScope?

Summary answer: A significant positive correlation was found between the score given by the deep learning algorithm and the implantation rate of the frozen blastocysts analyzed.

What is known already: Despite the safety and success of the vitrification technique, many of the best morphologically classified embryos prior to vitrification do not maintain their characteristics when returned to room temperature. Since artificial intelligence (AI) has been shown to be useful in assessing the quality of fresh embryos at different stages, it could also be used to predict the outcome of frozen embryo transfers. This is the first attempt to distinguish vitrified blastocysts that will implant from those that will not by applying deep learning on a single image taken in the period between warming and transfer.

Study design, size, duration: This retrospective single-centre study included 689 blastocysts with known implantation data. All of them were vitrified and warmed using the Cryotop method (Kitazato, Biopharma, Japan). Warmed embryos were assessed by experienced embryologists according to the ASEBIR morphological scoring system and the degree of expansion. Immediately after warming, blastocysts were placed in EmbryoScope (Vitrolife, Denmark) time-lapse incubators for 2-5 hours to let them re-expand until embryo transfer.

Participants/materials, setting, methods: A pre-transfer embryo image was provided to the algorithm (Life Whisperer Viability), which returned scores from 0 to 10. Data were analyzed by chi-square test or one-way ANOVA. Multivariate logistic regression analysis was performed including embryo score, oocyte origin (donated vs. autologous), oocyte age, patient age, oocyte handling (fresh vs. vitrified eggs), and day of vitrification (5 vs. 6). The area under the receiver operating characteristic (ROC) curve (AUC) was used to calculate evaluation performance.

Main results and the role of chance: There was a significant difference between the scores given to vitrified implanted and non-implanted embryos. The mean value was 4.98 [95% CI 4.70-5.26]* for non-implanted embryos and 6.16 [95% CI 5.84-6.48]* for implanted embryos. When dividing the embryo score by quartiles, the implantation rate for each quartile was 27.7% for Q_1, 33.7% for Q_2 (Odds ratio (OR)=1.31 [0.82–2.09]), 44.8% for Q_3 (OR = 2.05 [1.30–3.22]*) and 48.3% for Q_4 (OR = 2.46 [1.56–3.87]*). The contribution of each increased unit of the algorithm score to the implantation (OR = 1.16 [95% CI: 1.09–1.23]*) was statistically significant. The deep learning algorithm score successfully predicted implantation with an AUC of 0.65 [95% CI: 0.60–0.69]*. Moreover, the algorithm performed better than the ASEBIR morphological classification, which achieved an AUC of 0,62 [95% CI: 0.58–0.66] in predicting implantation. *$P < 0.001$.

Limitations, reasons for caution: Our clinic was not involved in the development of the algorithm. Furthermore, it was designed to assess fresh embryos prior to vitrification but not thawed ones, so this study should be considered an external trial. A post-warming full video analysis by AI could possibly provide additional information.

Wider implications of the findings: The use of predictive models on vitrified cycles may help to select the best frozen embryo for transfer and predict which embryos will result in implantation failure early enough to thaw another one with a better chance of success. It also indicates that AI can generalize to thawed embryo assessment.

Trial registration number: -

Abstract citation ID: dead093.192

O-159 Impact of open and closed methods of vitrification on oocyte mitochondria function and RNAseq transcriptome profile: a preclinical screening study

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Study question: Do current methods of open vitrification (OV) and closed vitrification (CV) impact on oocyte mitochondria function and transcriptome?

Summary answer: Oocyte vitrification confers unique mitochondria distribution patterns and alters the expression of stress response genes and mitochondria function relative to control (fresh) oocytes.

What is known already: Mitochondria are essential for maintenance of metabolic function and developmental competence at physiological conditions. Conversely, open and closed methods of oocyte vitrification are associated with physical, chemical and non-physiological changes, and exposures to cryoprotecting agents which could be harmful at extremely high and low temperatures.

Study design, size, duration: Ninety-four unfertilised Metaphase II (MII) oocytes were donated to research between May 2018 and August 2021. Fifty-seven oocytes were randomly allocated to the different study groups. Similarly, thirty-seven oocytes were allocated to the different study control groups.

Participants/materials, setting, methods: Fourteen women underdoing IVF/ICSI treatment cycles were involved in the study. cDNA was obtained for qPCR and the Smart-Seq2 protocol and single-cell RNA-Seq was performed (illumina NextSeq2000). Mitochondrial staining on live-oocytes was performed using Mito-tracker Deep Red stain, and images evaluated using fluorescence microscopy. Differentially expressed genes were identified using the R package edgeR and functional gene ontology was performed using an over-representation analysis via WebGestalt. Correction for multiple testing was calculated using Benjamini-Hochberg method

Main results and the role of chance: Mean mitochondrial intensity/density showed similarities between open (OV) and closed vitrification (CV) and control (fresh oocytes FO): 25.8 (12.7-44.9, standard deviation [SD] 10.2) for FO oocytes (n = 15), 30.3 (16.2-44.8, SD 10.1) for OV oocytes (n = 11), 26.6 (13.3-64.6, SD 15.3) for CV oocytes (n = 12) (p = 0.617). Distinct mitochondria distribution patterns (cytoplasmic and peripheral) were associated with OV and CV oocytes. Twice as many peripheral to cytoplasmic patterns were observed in FO and OV oocytes in contrast to CV oocytes (p = 037). Mean expression of the gene SDHB, which is associated with the TCA cycle and the mitochondrial electron transport chain was reduced in vitrified oocytes (n = 13) (0.0879 ± 0.0216 vs 0.0593 ± 0.0479), as was the anti-apoptotic gene BCL2 (p = 0.0061), relative to control oocytes (n = 13), (p = 0.01). Conversely, genes associated with apoptosis and stress response (BAX (p = 0.002) and BCL2L1 (p = 0.016)) were significantly enriched relative to control oocytes. RNAseq transcriptome analysis revealed a total of 71 differentially expressed genes following vitrification relative to control oocytes. Genes associated with critical metabolic processes (death domain binding, BCL-2 homology 3 (BH3) domains, and exopolyphosphatatse activities) were enriched in vitrified oocytes relative to the control group (FDR>0.05).

Limitations, reasons for caution: The use of unfertilised metaphase II (MII) oocytes on day 1 as a preclinical application screening model for vitrification may not reflect the molecular status and integrity of clinical grade MII oocytes on day 0.

Wider implications of the findings: This study emphasizes the inherent impact of vitrification on oocyte mitochondria function and transcriptome relative to control oocytes. Importantly, unintended perturbations may be detrimental to oocyte health and developmental competence, suggesting the need for a continuous safety screening and monitoring of ART methods of cryopreservation and offspring long-term health.

Trial registration number: National Institute for Health Research (NIHR). Grant Reference: ICA-CDRF-2015-01-068

Abstract citation ID: dead093.193

O-160 Do day of vitrification, grade of expansion and blastocyst quality affect embryo survival rate? An observational study in oocyte recipient patients

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Study question: Which parameters are the most predictive of embryo survival rate in oocyte recipient cryotransfer cycles?

Summary answer: We have identified that only day of vitrification and grade of expansion have an impact on embryo survival rate

What is known already: Embryo vitrification is a well stablished technique that has led to survival rates around ± 96%. Recent studies, based on day of vitrification, grade of expansion and blastocyst quality, are trying to identify which vitrified blastocyst has the highest potential to survive and to implant. However, controversial results are present in the literature, improvements in laboratory quality parameters and embryo culture could unleash into no significant differences regardless of morphological parameters. Nevertheless, having an acceptable survival rate does not mean to have a good implantation rate, biomarkers to identify the best potential blastocyst to survive and to implant are needed

Study design, size, duration: An unicenter-based retrospective observational clinical study was performed at IVI Madrid, approved by the local institutional review board (IRB). The study included all the warmings at blastocyst stage from oocyte recipient patients from January 2019 to April 2022. A total of 4.196 of warmed blastocysts were assessed, out of which 4.006 blastocysts were transferred

Participants/materials, setting, methods: All warmed blastocysts were allocated in groups according to the day of vitrification, grade of expansion and quality. The primary outcome analysed was embryo survival rate that was calculated as the number of viable embryos divided by the total number of embryos warmed. Vitrification and warming were performed following

Kitazato method (Cryotop, Kitazato, Biopharma). Embryo classification was assessed previous vitrification according to IVIRMA criteria. Statistical analyses were conducted using univariate and multivariate regression model

Main results and the role of chance: The mean maternal oocyte recipient age was 42.27 ± 3.89 years old and body mass index 23 ± 3.74 kg/m2). In a multivariate logistic regression analysis, a statistically significant association between embryo survival rate and the day of vitrification and grade of expansion was found. Chance of survival was significantly reduced for blastocyst vitrified on day 6 (n = 737) compared to day 5 (n = 3459) (93,2 % versus 96,0% [OR = 0.976 (0.960-0.993); p=0.05]). Regarding grade of expansion, cavitated blastocyst (n = 840) survival rate was 97,7% [OR = 1;p<0.001], expanded blastocyst (n = 1866) was 96,2% [OR = 0.095 (0.978-1.013)], hatching blastocysts (n = 1465) was 94% [OR = 0.978 (0.960-0.997); p=0.02) and fully hatched blastocysts (n = 25) was 60% [OR = 0.704 (0.648-0.765);p<0.001)]. However, no association was found neither between lower inner cell mass quality [OR = 0.964 (0.842-1.104); p=0.596) nor trophoectoderm [OR = 1.004 (0.981-1.028); p=0.746)] and survival rate. Analysing the combination of these three variables will give us the possibility to choose the best embryo to warm. The impact of day of vitrification, grade of expansion and blastocyst quality on implantation, clinical pregnancy, miscarriage, and live birth rates will be included in further studies

Limitations, reasons for caution: A weakness of this study is that it is retrospective in nature. The single-group design employed in retrospective studies limits the researchers' ability to determine cause and effect. Further studies are needed to determine the impact of these variables on the survival rate

Wider implications of the findings: Based on our findings, blastocyst survival rate is influenced mostly by the day of vitrification and degree of expansion. To be able to select the best blastocyst to warm, a comparison between different day of vitrification when we have a similar morphology and embryo quality cohort is needed

Trial registration number: Not applicable

Abstract citation ID: dead093.194

O-161 Performance of IDAScore prediction models on clinical, obstetric and neonatal outcomes of single vitrified-thawed blastocyst transfer

B. Huang[1], M. Bingxin[1], J. Lei[1]

[1]Huazhong University of Science and Technology, Tongji Hospital- Tongji Medicine College, Wuhan- Hubei, China

Study question: Does a free-annotation embryo scoring system (IDAScore) prediction model effective in clinical assisted embryo selection?

Summary answer: iDAScore could be used to predict the clinical pregnancy and live birth.

What is known already: iDAScore model, as a free-annotation and deep learning-based embryo scoring system, was created according to deep learning model by training 115,832 embryos and 14,644 known implantation embryos from 18 reproductive medical center. Then, every blastocyst was scored without any manipulation and intervention by embryologists. Although iDAScore's performance was validated by a few reproductive centers, it has not yet been verified in a sizable cohort study. As a result, the effectiveness of iDAScore should be assessed in more reproductive clinics.

Study design, size, duration: A total of 6,291 vitrified-thawed single blastocyst transfer (SBT) cycles from 2018 to 2021 at the Reproductive Medicine Center, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China, were analyzed a free-annotation and deep learning-based embryo scoring system (IDAScore) (EmbryoScope Plus, Vitrolife, Sweden).

Participants/materials, setting, methods: All blastocysts undergoing SBT were scored from 1 to 9.9 with ida system. Blastocyst scores were grouped according to quartile.The effectiveness of ida system was evaluated according to clinical outcomes, perinatal and neonatal outcomes of different groups (1.0-8.0, 8.1-8.9, 9.0-9.3, 9.4-9.9).

Main results and the role of chance: The iDA scores decreased with increasing maternal age.

Clinical pregnancy rate and live birth rate increased with iDAScores, and there was a significant correlation ($p < 0.001$). The abortion rate decreased slightly with the increase of iDAScores, but there was no significant difference ($p > 0.05$). Ectopic pregnancy rate did not change significantly with iDAScores. No statistically significant difference were observed between single and twin live birth among different iDAScore groups ($p > 0.05$).

For perinatal and neonatal outcomes, no significant difference was shown in four iDAScore group ($p > 0.05$). Among them, gestational age and premature birth rate were similar ($p > 0.05$). Besides, no significant difference was shown in types and rate of pregnancy complication ($p > 0.05$). For newborn babies, the birth weight and sex were statistically similar across all groups ($p > 0.05$). The birth defect outcome was also evaluated. No significant difference were shown on different types of birth defect in all groups ($p > 0.05$).

The uni-variate and multi-variate logistic regression for live birth was also analyzed. iDAScore were significantly correlated with a positive live birth probability (OR: 1.200, 95% CI: 1.148-1.253, $p < 0.05$).

Limitations, reasons for caution: The experimental samples in this study are all blastocyst transfer cycles. There is no guiding significance for other centers using the cleavage embryo transfer cycle.

Wider implications of the findings: iDAScore could be used to predict the clinical pregnancy and live birth. For the blastocyst that has obtained live birth, different iDAScore did not cause significant differences in obstetric and neonatal outcomes. Therefore, iDAScore determined blastocyst implantation more than fetal development.

Trial registration number: not applicable

Abstract citation ID: dead093.195

O-162 Fresh or frozen Day 6 blastocyst transfer : Is there still a question?

L. Ferreux[1], M. Bourdon[2], A. Chargui[1], C. Maignien[2], C. Chapron[2], P. Santulli[2], C. Patrat[1], K. Pocate-Cheriet[1]

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Study question: Whether slow developing expanded day 6 (D6) blastocysts should be either transferred during fresh cycle or systematically vitrified at D6

Summary answer: Fresh D6 blastocyst transfer is independently associated with reduced live birth rate compared to frozen thawed D6 transfer

What is known already: Live birth rate (LBR) after D5 blastocyst transfers are significantly higher when compared with D6 embryos in both fresh and frozen-vitrified embryo transfer cycles according to the last published meta-analyses. Therefore, for women obtaining only D6 blastocysts, chances of pregnancy may be lower but still persist and these embryos should be transferred. The question of the best strategy for transfer (i.e. in fresh or frozen cycles) remains unclear while data on this subject are scarce.

Study design, size, duration: Retrospective observational cohort study. Patients having only embryos reaching blastocyst stage at D6 were included between January 2018 and May 2021. Two groups were compared : patients having a fresh D6 transfer (D6 fresh transfer group) and patients having a frozen thawed D6 transfer (D6 frozen transfer group).

Participants/materials, setting, methods: 830 D6 single blastocyst transfers (736 frozen blastocysts and 94 fresh blastocysts) were analyzed. LBR and neonatal outcome were compared between groups, as well as the influence of clinical characteristics and data related to COS protocols. Correlation between D6 blastocyst morphology according to Gardner's classification and livebirth occurrence was also evaluated. Statistical analysis of the data was carried out using univariate and multivariate logistic regression models.

Main results and the role of chance: LBR was significantly lower after D6 fresh blastocyst transfer compared to D6 frozen thawed blastocyst transfer

[5.3% (5/96) vs 12.2% (90/736), $p < 0.05$]. Moreover, when comparing a subgroup of 68 first D6 frozen embryo transfers (1st D6 frozen transfer group) to the 94 fresh D6 blastocyst transfers (D6 fresh elective group), the superiority of D6 frozen blastocyst transfers regarding LBR was also confirmed (17.6% vs 5.3% $p < 0.001$, respectively). Concerning neonatal outcomes, the mean birth weight was comparable between the two groups. Univariate logistic regression analysis taking into account blastocyst morphology parameters showed that TE grade was the only parameter significantly associated with LBR after D6 embryo transfer ($p < 0.001$).

Multiple logistic regression revealed that D6 embryo fresh transfer was independently associated with reduced LBR compared to frozen thawed D6 transfer (OR 0.367; 95%IC 0.143-0.945; $p = 0.038$). Moreover, our results showed that transferring a good or top quality D6 blastocyst increases 3 fold chances of live birth.

Limitations, reasons for caution: The number of fresh cycles seems relatively small, but the consistency of our results was guaranteed by the homogeneity of the 2 subgroups, standardization of all biological and clinical procedures and the robust statistical analysis methodology used in this study.

Wider implications of the findings: Despite their lower potential, D6 blastocysts have to be transferred with the objective to improve LBR, especially in women obtaining only these type of embryos. Our results recommend to transfer D6 blastocysts in frozen cycles. However, these findings have to be confirmed on larger series in prospective randomized trials.

Trial registration number: not applicable

Abstract citation ID: dead093.196
O-163 The influence of aquaporin 3 on dynamics of cavitation, implantation and re-expansion after vitrification/warming

E. Kosyl[1], **A. Ajduk**[1]

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Study question: We wished to investigate whether aquaporin 3 (AQP3) is involved in trophectoderm-related events: cavity formation and maintenance, implantation and re-expansion after the vitrification/warming process.

Summary answer: AQP3 regulates blastocoel formation and expansion. It is also required for implantation (as assessed by in vitro implantation assay) and maintaining developmental potential after vitrification.

What is known already: During blastocoel expansion blastocyst pulsates, i.e. contracts and re-expands in an oscillatory way. Recent studies utilizing a mouse model have shown, that the dynamics of cavitation and biomechanical properties of the trophectoderm are intertwined, and our results suggest, that it may, to a certain extent, reflect the developmental capabilities of blastocysts. Moreover, the dynamics of blastocyst re-expansion after vitrification/warming is also suggested to be a promising biomarker of embryo quality. As AQP3 is involved in water influx and transportation of small molecules (such as cryoprotectants) through the epithelial layer, it is a good candidate for a regulator of blastocoel expansion.

Study design, size, duration: We analyzed 142 mouse blastocysts to correlate the dynamics of their cavitation with their ability to implant in vitro (an outgrowth assay). We investigated the expression of *Aqp3* throughout the preimplantation development. We tested how inhibition of AQP3 affects the dynamics of cavitation ($n = 37$; control $n = 118$) and formation of outgrowths ($n = 77$; control $n = 81$). We vitrified blastocysts with a limited activity of AQP3 ($n = 41$; control $n = 38$) and investigated their ability to re-expand and implant in vitro.

Participants/materials, setting, methods: The dynamics of cavitation was assessed by time-lapse imaging. After filming, mouse blastocysts were cultured for additional 4 days to test their ability to form outgrowths. Inhibition of AQP3 was performed by adding 100μM DFP00173 (a selective AQP3 inhibitor) to the culture medium. AQP3-inhibited blastocysts were analyzed for the dynamics of cavitation and outgrowth formation. Additionally, embryos with a limited activity of AQP3 were vitrified, warmed and then the outgrowth assay was performed.

Main results and the role of chance: Blastocysts unable to implant in vitro properly (i.e. forming too small outgrowths or not forming outgrowths at all) contract longer and more frequently than embryos that form correct outgrowths. Moreover, in such blastocysts, we observed shorter and weaker re-expansion phases. As AQP3 may be one of the regulators of cavitation, we followed its expression in embryos and showed that *Aqp3* mRNA level increases from the 2-cell to the morula stage. AQP3 protein can be found in the cytoplasm during cleavage and translocates to basolateral membranes in trophectodermal cells in blastocysts. We revealed that inhibition of AQP3 resulted in delayed cavity formation. Moreover, inhibition of AQP3 significantly weakened the blastocyst's ability to (re-)expand and also extended the duration and decreased frequency of the blastocoel contractions. Importantly, changes in the duration of contractions and amplitude of re-expansions were similar to those observed in blastocysts not able to implant in vitro properly. This accords with our observation that blastocysts with a limited activity of AQP3 are mostly unable to create outgrowths. Finally, we proved that inhibition of AQP3 strongly weakened re-expansion of the cavity after vitrification/warming. Furthermore, such blastocysts had a significantly lower ability to implant in vitro after vitrification.

Limitations, reasons for caution: We examined the role of AQP3 in blastocoel (re)-expansion and the relationship between cavitation dynamics and embryo's ability to implant only in mice, so additional studies on other mammalian species, including humans, are needed. Moreover, our in vitro implantation assay does not fully reflect the complexity of implantation in vivo.

Wider implications of the findings: Our data indicate, that AQP3 regulates the dynamics of blastocoel expansion in mouse embryos. Impaired dynamics of cavitation, unsuccessful implantation or vitrification failures might be a result of decreased AQP3 activity, possibly not only in a mouse but also in other mammalian species, including humans.

Trial registration number: not applicable

INVITED SESSION

SESSION 50: IS IT BENEFICIAL TO PREPARE PATIENTS FOR THE POSSIBILITY THEIR FERTILITY TREATMENT MIGHT BE UNSUCCESSFUL?

Tuesday 27 June 2023 Auditorium 10-12 11:45 - 12:45

Abstract citation ID: dead093.197
O-164 The emotional and ethical complexity of end of treatment in ART

D. Leone[1]

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In the context of Assisted Reproductive Technology (ART), the decision to end the treatment is very complex and involves emotional and ethical dimensions. It should occur after a series of failed attempts and when "the chances for a successful outcome are so low that it is in the patient's best interest to stop any further attempt at it"[1]. The lack of a clearly defined biological endpoint makes the decision to end treatment extremely challenging and is one of the most complex bad news within the doctor-patient relationship. The decision to end treatment can be experienced by patients as an existential failure; they are overwhelmed by all sorts of emotions related to the acknowledgment that the pregnancy they hoped for might eventually never happen. When treatment is interrupted, hope could be lost with feelings of intense sorrow, and failing to become a parent requires rethinking a new identity. Patients who end ART treatment may experience a crisis characterized by shock, denial, anger, frustration, and loss of control. Many patients approach ART treatment thinking that repeated attempts will sooner or later lead to the long-awaited pregnancy. This attitude could turn into "over-persistence", the condition in which patients insist on continuing ART treatment with very low or any chance of success. According to the literature, doctors may

mitigate patients' emotional distress related to the end of treatment (EoT) through effective communication, which has the potential to facilitate the process of acceptance and reduce psychological suffering.

Likewise, physicians may experience intense emotions (e.g., frustration, denial). Clinicians reported feelings ranging from the idealization of the process to impotence, and these emotions intertwined with a sense of duty could influence decisions about ART treatment and lead to compulsive use of ART procedures. Clinicians remain fully aware of the probability of success and perceive patients' expectations as a burden, resulting in performance anxiety.

The decision to end unsuccessful ART treatment also involves ethical aspects. In particular, the decision to end treatment raises the dilemma between beneficence and patient autonomy. The request for (over)persisting ART treatment may come from patients with a strong desire for parenthood, who do not accept the possibility of giving up on biological pregnancy. On one hand, any choice about EoT must be in accordance with the patient's self-determination; on the other hand, doctors should consider the patient's beneficence and favorable risk/benefit ratio. EoT should be considered as a matter of proportionality of care, recognizing that benefits may be less than the medical, economic, but especially psychophysical burdens. The value of distributive justice and equity must also be addressed. Owing to rising healthcare costs, health professionals have an ethical duty to manage medical services, eliminating all those non-essential procedures requiring a waste of resources and/or time with few or no significant results.

1. Boivin J, Takefman J, Braverman A. Giving bad news: 'It's time to stop'. In Macklon N. IVF in the medically complicated patient: A guide to management. Milton Park, UK: Taylor & Francis Group, 2005. p. 233-240.

Abstract citation ID: dead093.198
O-165 Pros and cons of preparing patients for the possibility their treatment may be unsuccessful

S. Gameiro[1]

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POSTER DISCUSSION SESSION
SESSION 52: FERTILITY PRESERVATION
Tuesday 27 June 2023 Hall D2 11:45 - 12:45

Abstract citation ID: dead093.199
P-391 Importance of oxygen tension in human ovarian tissue in vitro culture

F. Vitale[1], **L. Cacciottola**[1], **F. Yu**[1], **M. Barretta**[1], **C. Hossay**[1], **J. Donnez**[2], **M.M. Dolmans**[3]

[1]*Université Catholique de Louvain - Institut de Recherche Expérimentale et Clinique, Gynecology Research Unit, Bruxelles, Belgium*
[2]*Société de Recherche pour l'Infertilité SRI, Society for Research into Infertility, Bruxelles, Belgium*
[3]*Université Catholique de Louvain - Cliniques Universitaires Saint-Luc, Gynecology Research Unit - Gynecology Department, Bruxelles, Belgium*

Study question: Is there any difference between 20% and 5% oxygen (O_2) tension on the viability and quality of human follicles contained in cultured ovarian cortex?

Summary answer: 5% O_2 yields higher follicle viability and quality than does 20% O_2 tension after 6 days of in vitro culture (IVC).

What is known already: The primordial follicle (PMF) pool resides within the ovarian cortex, where in vivo O_2 tension ranges between 2% and 8%. However, IVC is commonly performed at atmospheric O_2 tension (20% O_2), yielding low follicle survival rates. Elevated O_2 tension or insufficient antioxidant protection can cause excessive reactive oxygen species (ROS) accumulation, leading to DNA lesions, such as oxidative stress damage and double-strand breaks (DSB).

Study design, size, duration: This prospective experimental study included frozen-thawed ovarian cortex from 6 adult patients (mean age: 28.5 years; age range: 26–31 years) undergoing laparoscopic surgery for non-ovarian diseases. Ovarian cortical fragments were cultured for 6 days at (i) 20% O_2 with 5% CO_2, and (ii) 5% O_2 with 5% CO_2. Non-cultured fragments served as controls.

Participants/materials, setting, methods: Cortical fragments were analyzed according to: hematoxylin and eosin (H&E) staining for follicle count and classification; cleaved caspase-3 immunostaining to identify follicle apoptosis; 8-hydroxy-2-deoxyguanosine (8-OHdG) and gamma-H2AX (γH2AX) immunolabeling to detect oxidative stress damage and DNA DSBs; and β-galactosidase staining to assess follicle senescence. Droplet digital PCR was also performed to further explore gene expression of superoxide dismutase 2 (SOD2) from the antioxidant defense system, and cyclin-dependent kinase inhibitors (p21 and p16) as senescence-related genes.

Main results and the role of chance: Apoptosis ($p = 0.002$) and follicle senescence ($p = 0.03$) rates were significantly lower in the 5% O_2 than the 20% O_2 group. Moreover, granulosa cells (GCs) in follicles in the 20% O_2 group exhibited significantly ($p < 0.001$) higher oxidative stress damage rates than in the 5% O_2 group. DNA DSB damage rates in GCs of follicles were also significantly higher ($p = 0.001$) in the 20% O_2 than the 5% O_2 group. SOD2 values were significantly greater in the 5% O_2 group compared to the 20% O_2 group ($p = 0.04$) and the non-cultured group ($p = 0.002$). Expression of p21 was significantly increased in 20% O_2 ($p = 0.03$) and 5% O_2 ($p = 0.008$) compared to no culture. Moreover, the 20% O_2 group showed significantly greater p16 expression ($p = 0.04$) than the non-cultured group, while no significant variation was observed between 5% O_2 and no culture.

Limitations, reasons for caution: This study focuses on improving follicle outcomes during the first step of ovarian tissue IVC, where follicles remain in situ within the tissue. The impact of O_2 tension in further steps, such as secondary follicle isolation and maturation, was not investigated here.

Wider implications of the findings: Our findings suggest that 5% O_2 tension culture is a promising step towards potentially solving the problem of poor follicle viability after IVC.

Trial registration number: N/A

Abstract citation ID: dead093.200
P-394 Neurotrophin-4 supplementation during human secondary follicle in-vitro-culture supports morphologically normal blastocyst formation

Y.C. Guo[1], **L. Jia**[1], **P. Sun**[1], **W. Su**[1], **T. Li**[1], **C. Fang**[1]

[1]*The Sixth Affiliated Hospital- Sun Yat-sen University, Reproductive Medicine Research Center, Guangzhou, China*

Study question: Can matrix-free culture system supplemented with Neurotrophic factor 4 (NT4) improve human follicular development and achieve mature and fertile oocytes *in vitro*?

Summary answer: NT4 promotes the growth, steroid hormone production of human secondary follicles cultured *in vitro* (including post- and pre-pubertal patients), thereby yielding mature and fertile oocytes.

What is known already: Reconstituting folliculogenesis *in vitro* play vital roles in reproductive biology research, fertility preservation and reproductive toxicity testing. However, fertile oocyte from *in-vitro*-grown (IVG) human follicle remains unachieved. It has been demonstrated that NT4 promotes *in-vitro* survival, growth, and maturation of mice secondary follicles. NT4 and its receptors have been detected in human ovaries. However, if the advance proved in mice model is effective to replicate in human has not been reported.

Study design, size, duration: Discarded ovarian tissues after ovarian tissue cryopreservation (OTC) were collected from 6 patients aged from 11 to 21 years old who underwent unilateral oophorectomy for fertility preservation, after obtaining written informed consent. Isolated secondary follicles were

cultured *in vitro* with or without NT4 in a matrix-free system for 4-6 weeks individually.

Participants/materials, setting, methods: Secondary follicles isolated from each patient were randomly assigned to control or NT4 group, followed by incubation on the ultra-low attachment microplate individually. Follicle growth and survival were assessed by microscopy. Anti-Müllerian hormone (AMH), estradiol and progesterone levels were quantified in the medium. Oocyte marker expression was evaluated by DEAD box polypeptide 4 (Ddx4) staining. Oocyte mature and fertile potential were assessed by *in-vitro* maturation and intracytoplasmic sperm injection (ICSI) with donated sperm, separately.

Main results and the role of chance: In control condition, isolated follicles survived for 4-6 weeks with increased diameters over time ($P < 0.05$), reaching a terminal diameter of 967.00 ± 41.1 µm with the confirmed induction of steroidogenesis and expression of widely accepted oocyte markers (DDX4). Three out of twelve (25%) alive follicles were matured successfully *in vitro*, most of which produced morphologically normal MII oocytes with sizes of 120 ± 2.1 µm. The diameter and steroid hormone production were significantly higher in the group cultured with NT4 than in the control group ($P < 0.05$). An increased efficiency of MII oocyte production in the NT4 group was also observed, while the difference was not statistically significant. MII oocyte obtained from the control group showed abnormal fertilization after ICSI. In contrast, MII oocyte acquired from the NT4 group progressed to available blastocyst.

Limitations, reasons for caution: The population included were all patients with thalassemia. Whether this culture system is effective for patients with other diseases is unknown. Limited by the small sample, the effect of NT4 on maturation competence needs further confirmation. Oocytes obtained have not been quantified with ploidy status and epigenetic signatures.

Wider implications of the findings: Surplus ovarian tissue after OTC, which is otherwise discarded, may serve as an additional precious source of fertile oocytes for fertility preservation, even for pre-pubertal girls, without a threat of tumor reintroduction. The system described here will provide a powerful research tool for reproductive biology research and reproductive toxicity testing.

Trial registration number: not applicable

Abstract citation ID: dead093.201

P-412 Long-term in vitro dynamic culture of bovine ovarian cortical tissue (BOCT) increases follicle growth, viability and AMH secretion

V. Barbato[1], V. Genovese[1], V. De Gregorio[1], A. Travaglione[1], A. Candela[1], G. Catapano[2], A. Amoresano[3], S. Serpico[3], G. Pinto[3], G. Mondrone[4], T. D'Hooghe[5], S. Longobardi[5], W. Zheng[5], R. Gualtieri[1], R. Talevi[1]

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[5]Merck KGaA, Global Medical Affairs Fertility, Darmstadt, Germany

Study question: Does improved oxygen availability and biomechanical stimulation during long-term dynamic in vitro culture of BOCT enhance secondary follicles growth and health?

Summary answer: Long-term dynamic versus static in vitro culture of BOCT increases the proportion, viability and AMH secretion of growing follicles.

What is known already: A limiting factor in multistep in vitro folliculogenesis is the low yeld of healthy secondary follicles after ovarian cortical tissue culture. We previously reported that dynamic in vitro culture of ovarian cortical tissue for 7 days in a newly designed perifusion bioreactor (patent 102020000027290) enhances follicle growth and health compared to conventional static culture. The bovine represents a valuable animal model to study early folliculogenesis in vitro.

Study design, size, duration: Bovine ovaries (age 8-24 months) were collected from slaughterhouse. BOCT strips from the same ovary were cultured for 14 days in perifusion bioreactors (PB, dynamic culture) and conventional dishes (CD, static culture). BOCT cultured in both PB and CD was monitored by collecting spent media every 2 days and through histology, live-dead confocal analysis at the end of culture.

Participants/materials, setting, methods: BOCT slices (0.5mm thick) were obtained by a custom tissue slicer, chopped into 1x1mm strips and cultured in group of 10 under in PB and CD. Follicle stages and quality in fresh (D0) and cultured tissues were evaluated by histology (hematoxylin-eosin staining), follicle viability was estimated by labelling with live-dead far-red and propidium iodide at the confocal microscope. Whole tissue viability (spectrophotometric LDH assays) and AMH secretion (mass spectrometry) were evaluated on spent media.

Main results and the role of chance: Overall, 939 follicles were analyzed (histology, 338; viability, 601). Day 0-Follicle stages: primordial, 82.4%; primary, 13.2%; secondary, 4.4%; follicle quality: grade 1-2, 37,6%; grade 3, 62,4%; live follicles: 94.3%. Day 14: PB vs CD - Follicle stages: primordial, 3.8 vs 5.7%,NS; primary, 75 vs 86.1%, NS; secondary, 21.2 vs 8.2%, $P < 0.05$; follicle quality: grade I/II, 70 vs 18.9%, $P < 0.01$; grade III, 30 vs 81.1%, $P < 0.01$; live follicles 62.6 vs 35.3%, $P < 0.01$. LDH activity in PB was markedly lower than in CD at all culture times (PB_{14d} 150.5 nmol vs CD_{14d} 233.1 nmol). During 14 days culture, AMH secretion continuously increases in PB (1700 picomol/Vtot) whereas in CD it markedly decreases during culture, falling down at very low values at the end of culture (67 picomol/Vtot). Findings indicate that the increased transport of solutes and dissolved oxygen, and the biomechanical stimulation in our novel dynamic bioreactor are key factors during ovarian tissue culture. Analysis of LDH and AMH levels in spent media confirm that dynamic culture better mantains general tissue health and promote a better functionality of growing follicles.

Limitations, reasons for caution: Although the bovine is considered a reliable model for human folliculogenesis, the study should be validated on human ovarian tissue.

Wider implications of the findings: Poor yield of secondary follicles during organ culture is a limiting step in the in vitro production of mature oocytes from primordial follicles. The use of a newly developed dynamic bioreactor for long-term culture could represent a valuable tool for in vitro multistep folliculogenesis.

Trial registration number: not applicable

Abstract citation ID: dead093.202

P-432 Gonadal function and fertility preservation in girls with Hodgkin lymphoma treated according to the EURONET-PHL-C2 Protocol: the fertility add-on study

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[3]Princess Maxima Centre and Erasmus MC- Sophia Children's hospiral, Department of Haematology/Oncology, Utrecht and Rotterdam, The Netherlands
[4]Royal Hospital for Sick Children, Department of Haematology/Oncology, Edinburgh, United Kingdom
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[6]Princess Maxima Center and 1.Emma Children's Hospital- Amsterdam UMC- Vrije Universiteit Amsterdam-, Pediatric Oncology, Utrecht and Amsterdam, The Netherlands

Study question: What is the gonadotoxic potential of the EURONET-PHL-C2 treatment protocol for female childhood Hodgkin lymphoma patients, and how frequently are co-treatments to preserve fertility applied?

Summary answer: Treatment-induced amenorrhea (72%) persisted in > 10% of cases. Results on AMH are available by April 2023. Fertility preserving (co-)treatments were applied in 27% of patients.

What is known already: Current treatment for childhood Hodgkin lymphoma (HL) is highly effective with survival rates exceeding 90%. However, HL treatment affects gonadal function and HL survivors were proven to be at risk of premature ovarian insufficiency. In effort to reduce late effects, treatment protocols were adapted and toxic procarbazine was successfully omitted. The current EuroNET-PHL-C2 protocol aims to reduce use of radiotherapy by intensifying chemotherapy. The cumulative dose of cyclophosphamide is increased by 25% in the intensified treatment-arm, the impact of this change in therapeutic protocol on gonadal function is currently unknown. Therefore, a fertility study was incorporated in the EuroNET-PHL-C2 study.

Study design, size, duration: This international, prospective, multicenter cohort study is embedded in the EuroNET-PHL-C2 study, an European phase-3 treatment study evaluating the effectivity of HL treatment with OEPA-COPDAC (OEPA: vincristine sulfate (oncovin), etoposide, prednisolone and doxorubicin (adriamycin); COPDAC: cyclophosphamide, vincristine sulfate (oncovin), prednisone and dacarbazine) versus OEPA-DECOPDAC (DECOPDAC: COPDAC with additional doxorubicin and etoposide) in a randomized setting. In the present fertility add-on study, 205 (104 girls, 101 boys) patients were included between January 2017 and September 2021.

Participants/materials, setting, methods: Female patients, aged <18 years, treated according to the EuroNet-PHL-C2 protocol for classical HL, were recruited across 18 sites (the Netherlands, Belgium, Germany, Austria, Czech Republic). All parents and patients (aged ≥12 years old) provided written informed consent.

Serum AMH levels and menstrual cycle were evaluated over time (at diagnosis, during- and directly after treatment, 2 years post-diagnosis) and compared between OEPA-COPDAC and OEPA-DECOPDAC treatment groups. Moreover, use of available fertility preservation treatments was evaluated.

Main results and the role of chance: In the present analysis, 100 girls were included of whom 88 completed 2 years of follow up. Median age at diagnosis was 15 years (7-18), 8 girls were prepubertal and 92 post pubertal (87% postmenarchal). 17 girls were diagnosed at an early stage of HL (TL1) and 83 in more advanced stages (TL2/TL3, 66% received COPDAC and 33% DECOPDAC). 5 patients (5%) were irradiated in the pelvic area. Of the 46 postmenarchal girls who did not receive hormonal contraceptives during treatment, 33 (72%) experienced treatment-induced amenorrhea, at least 4 (12%) had persisting amenorrhea at 2 years post-diagnosis. 4 girls (22%) who stopped taking hormonal contraceptives after treatment had no (return of) spontaneous cycle. 2 girls (2%) underwent ovariopexy. 4 girls cryopreserved oocytes before treatment and 12 cryopreserved ovarian tissue (OTC). 15 (15%) received GnRH analogues as a co-treatment.

Data on AMH-levels up to 2 years post-diagnosis will be available by April 2023. Change in AMH will be evaluated, comparing number of received chemotherapy-cycles (3/4/6), DECOPDAC versus COPDAC and pubertal stage at time of diagnosis. Additional analyses will be performed to assess the impact of applied fertility preservation methods on gonadal function (i.e. AMH levels after OTC or receiving GnRH agonist).

Limitations, reasons for caution: The current analysis included data up to 2 years post-diagnosis. Potential recovery or late emerging effects of treatment remain unknown. The studied population comprises young girls with diagnosis of HL often concurring with pubertal transition, during which AMH levels naturally rise. SD z-scored will be used to analyze AMH results.

Wider implications of the findings: The fertility add-on study is the first study to prospectively evaluate reproductive markers in children treated for HL. Study results are highly valuable to determine effect of the proposed new treatment regimen for childhood HL on fertility .

Trial registration number: Clinicaltrials NCT02684708; EudraCT number 2012-004053-88

INVITED SESSION

SESSION 53: MHR SYMPOSIUM: SPERMATOZOA AND OOCYTES: DISTINCT PREPARATIONS FOR A ZYGOTIC FUTURE

Tuesday 27 June 2023 Hall D3 14:00 - 15:00

Abstract citation ID: dead093.203
O-166 Distinct molecular signatures of successful sperm in situ

J.J. Chung[1]

[1]*Yale University School of Medicine, Dept. of Cellular & Molecular Physiology, New Haven, U.S.A.*

Out of millions of ejaculated sperm, a few reach the fertilization site in mammals. Flagellar Ca2+ signaling nanodomains, organized by multi-subunit CatSper calcium channel complexes, are pivotal for sperm migration in the female tract, implicating CatSper-dependent mechanisms in sperm selection. Here using biochemical and pharmacological studies, we demonstrate that CatSper1 is an O-linked glycosylated protein, undergoing capacitation-induced processing dependent on Ca2+ and phosphorylation cascades. CatSper1 processing correlates with protein tyrosine phosphorylation (pY) development in sperm cells capacitated in vitro and in vivo. Using 3D in situ molecular imaging and ANN-based automatic detection of sperm distributed along the cleared female tract, we demonstrate that spermatozoa past the utero-tubal junction possess the intact CatSper1 signals. Together, we reveal that fertilizing mouse spermatozoa in situ are characterized by intact CatSper channel, lack of pY, and reacted acrosomes. These findings provide molecular insight into sperm selection for successful fertilization in the female reproductive tract.

Abstract citation ID: dead093.204
O-167 The good egg: on the onset of transcription in mouse and human embryos

A. Perry[1]

[1]*University of Bath, Department of Life Sciences, Bath, United Kingdom*

At the moment of union in fertilisation, sperm and oocyte are transcriptionally silent, but little is understood about how transcription initiates in the newly-formed embryo. In this talk, I shall suggest why this fundamental process has remained arcane for so long, and how my lab has sought to unlock its secrets in mouse and human embryos. By combining micromanipulation and single-cell RNA-sequencing, we have revealed a programme of embryonic transcription initiation beginning within four hours of fertilisation in the mouse. The talk will outline our efforts to characterise this initiating transcription, and its conservation in human one-cell embryos. Transcribed genes predict regulation by oncogenic factors, including c-Myc, whose features will be detailed, together with a model suggesting predictive parallels between the onset of embryogenesis and cancer.

Trial registration number: XXXX

INVITED SESSION

SESSION 54: SHOULD WE STEER CLEAR OF EUGENICS IN REPRODUCTIVE MEDICINE?

Tuesday 27 June 2023 Hall D1 14:00 - 15:00

Abstract citation ID: dead093.205
O-168 The dark shadow of historical eugenics on reproductive technologies and practices

G. Cavaliere[1]

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Eugenics is often referred to in discussions on the ethics of reproductive technologies. References to the word "eugenics" are often used to draw distinctions between ethically permissible and impermissible technologies, and permissible and impermissible uses of these technologies. While historians have long argued that 20th century eugenics cannot be reduced to a uniform set of practices, references to eugenics in discussions on the ethics of reproductive technologies have proven difficult to eradicate. Some authors stress the similarities between past eugenics and present reproductive technologies (what I define here as "the continuity view") to condemn these technologies. Others focus instead on the differences between past and present (what I define here as "the discontinuity view") to defend reproductive technologies.

In this talk, I canvass the meanings and uses of the word "eugenics" in relation to the permissibility of reproductive technologies and argue that disagreement concerning the value and ethical standing of these technologies originates in divergent views of condemnable and justifiable features of the past. Moreover, I suggest focusing on the reproductive preferences of those accessing fertility treatment as a starting point for any analysis concerning the permissibility of novel reproductive technologies.

Trial registration number: XXXX

Abstract citation ID: dead093.206
O-169 Reproductive ethics beyond designer babies

K. Hens[1]

[1]*Universiteit Antwerpen, Antwerp, Belgium*

INVITED SESSION

SESSION 55.: PATIENT SESSION: INFERTILITY HURTS, DON'T LEAVE US WITHOUT SUPPORT

Tuesday 27 June 2023 Hall D4 14:00 - 15:00

Abstract citation ID: dead093.207
O-170 The true impact of fertility problems: insights from the Fertility Network UK and Middlesex University survey

E. Rees[1]

[1]*Fertility Network UK, Wales Co-Ordinator, London, United Kingdom*

Fertility Network UK's survey of approaching 1,300 fertility patients in the UK reveals the devastating toll infertility wreaks on people's mental health, relationships, finances and career. Released at the start of National Fertility Awareness Week 2022, the findings highlight the emotional and financial impact, as well as the lack of information provided by GPs and the limited support options, such as counselling services.

Background: Fertility challenges and the use of assisted conception are increasing. Yet access to funded treatment and associated supports, such as counselling, is often limited, so the financial and emotional impacts of treatment are problematic for many people. In order to examine the impact of fertility challenges and treatment, Fertility Network UK commissioned Middlesex University to conduct a survey to examine the impacts of fertility challenges and treatment in order to update the survey conducted in 2016. The online survey was conducted between April and July 2022 to examine the psychological, emotional and relationship impacts of treatment, funding and support issues, and experiences of combining treatment and work. Specifically, Fertility Network UK gathered information on: - Emotional and psychological impacts of experiencing fertility challenges and of treatment - How it affects relationships with partner, friends, family and colleagues - How it impacts work and career, and how supportive employers are - Access to counselling and other supports - Access to NHS-funded fertility treatment. Fertility Network UK wished the survey to be applicable to a broad range of individuals including those who have fertility challenges but are not having treatment, those who have received or are currently having treatment or who are planning or awaiting treatment, and those who have completed their fertility journey, whether it has been successful or they have had to accept involuntary childlessness.

Key Findings: Mental health

• 4 out of 10 respondents experienced suicidal feelings.

• Approaching half (47%) of respondents experienced feelings of depression often or all the time, while the vast majority (83%) felt sad, frustrated and worried often or all the time.

Finances

• Two-thirds of patients (63%) had to pay for their own medical treatment.

• The average cost of investigations and treatment was £13,750. • Around 1 in 10 couples (12%) spent more than £30,000 and a few (0.5%) spent over £100,000.

Career

• 15% either reduced their hours or left their job.

• Over a third (36%) of respondents felt their career was damaged as a result of fertility treatment, and the majority (58%) felt concerned that fertility treatment would affect their career prospects.

• Only a quarter (25%) reported the existence of a supportive workplace policy

• Less than half (45%) of respondents felt they received really good support from their employer.

Relationships

• The majority of respondents (59%) reported some detrimental impact of fertility problems and/or treatment on their relationship with their partner.

Information & Support

• Approaching half (44%) of respondents sought help from Fertility Network UK, the nation's leading fertility charity.

• Three-quarters of respondents (75%) felt their GP did not provide sufficient information about fertility problems and treatment and 7% were not sure. Less than one-fifth (18%) were satisfied with the information GPs provided.

• The majority of respondents (78%) would have liked to have counselling if it was free. Half of respondents (51%) did have counselling, but most of these (59%) had to fund some of it themselves.

Trial registration number: XXXX

Abstract citation ID: dead093.208
O-171 Psychological support: the light in the darkness of my infertility journey

S. Dumont[1]

[1]*Fertility Europe, Patient association, Saint-Maur des Fosses, France*

Sandrine Dumont shares her own personal patient experience : « my infertility journey started at the age of 32, when I was finally diagnosed with endometriosis after years of pain. I went through one year of natural but monitored baby trials, 4 artificial inseminations, a break-up with my then partner, and 3 IVF with my new partner : these 3 IVF took place during a short period of 5 months. First IVF was in a public hospital, who kicked us out after first IVF failed. Other two IVF took place in a private hospital in Paris, who incidentally diagnosed PCOS : both IVF were also unsuccessful. I was physically and psychologically exhausted, and then decided to take a break from fertility treatments. We expected information and guidance from both fertility centers to help us through this. It never came.

During all these years, we've been appalled with how very few information we were given from all the medical staff. We were never prepared for failure at all. We felt treated badly, even in a rude and sometimes brutal way, by some professionals. Patient care was almost inexistent. We felt like a mere number on a list, not worth being explained anything or just answered our legitimate and simple questions about our own case. We were left alone with our doubts and questions: how could we make an enlightened decision as whether to continue treatments or not, with no information to nurture this decision ?

It seemed an endless dark tunnel with no exit. Then came guilt, loneliness and depression. I searched for external psychological support to help us get through this: this was not easy, since psychological weakness was something to be very ashamed of at the time, in French society. Psychological support turned out to be the light in our darkness. I found a psychiatrist to whom I went weekly for almost two years. This is what it took to heal : accepting failure, accepting my own limits to what I was ready to endure to try to be a mother, accepting to be different from what is expected of a woman in our traditional French society. This therapy was a lifesaver. There also came the association Collectif Bamp, thanks to whom I met a two women in the same situation as me. Knowing I was not alone just changed everything : it was the first day of the rest of my life.

Today, 10 years later, I am now an active member of Bamp association : I try to be there for patients as Bamp was there for me. I share my experience, so people don't feel alone. I advocate pyschological support and real patient care.

SELECTED ORAL COMMUNICATIONS

SESSION 56: OOCYTE PRESERVATION: MISSION ACCOMPLISHED?

Tuesday 27 June 2023 Auditorium 10–12 14:00 - 15:00

Abstract citation ID: dead093.209
O-172 Room and directions for improvement in oocyte cryopreservation

M.J. De Los Santos[1]

[1]*IVI Valencia, IVF Laboratory, Valencia, Spain*

Cryopreservation and more specifically oocyte vitrification has become a before and after in the field of Assisted Reproduction Techniques. Despite great efforts to improve slow oocyte freezing protocols, the levels of reproducibility, survival rates and clinical outcomes achieved by oocyte vitrification have never been reached by any of these old approaches.

However, once this barrier was overcome, new challenges emerged. We must reassert ourselves on issues such as the efficacy, efficiency and safety of a technique that is now fully implemented in IVF laboratories around the world.

Continuos monitoring and improvement as part of the quality management system is mandatory, to keep the the standards of qualilty, identify factors that may affect survival rates and look for room for improvemnt and finetune the protocols.

But also it is important to provide answers to women who wish to preserve their fertility for medical or non-medical reasons. They have key questions such as how many oocytes to store, what the survival rate will be after warming, will the cryopreserved oocytes be able to fertilize and develop as good as the fresh counterparts, how does the number of oocytes used affect the cumulative live birth rate?

Also for women undergoing ART, oocyte vitrification may represent a successful strategy. For example, patients with low response or women at risk of ovarian hyperstimulation, the two sides of the coin, may benefit from oocyte cryopreservation. The former, by storing and accumulating oocytes that allow them to obtain success rates comparable to those of normal responders while avoiding the adverse effects of a low response; the latter, by postponing insemination over time, investing in safety.

Yet embryo vitrification can offer good alternatives to oocyte vitrification so both options can be explored and compared

Trial registration number: XXXX

Abstract citation ID: dead093.210
O-173 Cellular and developmental damage from oocyte cryopreservation

P. Comizzoli[1]

[1]*Smithsonian's National Zoo and Conservation Biology Institute, Veterinary Hospital, Washington, U.S.A.*

Compared to somatic or sperm cells, mammalian oocytes are much more sensitive to cryopreservation mainly because of the large volume, cytoskeleton, and presence of the zona pellucida. Connections with the surrounding cumulus cells also are challenging to preserve in immature oocytes. Besides the genetic and epigenetic information enclosed in the nucleus, oocytes contain organelles and cytoplasmic factors that are necessary for the early embryo development. Therefore, exposure to cryoprotectant and freezing temperatures can easily damage the oocyte's complex cellular structure and developmental competence, which leads to poor success in fertilization, lower embryo quality, and reduced pregnancy rates. Using the domestic cat as a model, our laboratory has studied damages in cumulus-oocyte complexes (immature oocytes) occurring at each step of the cryopreservation process. Specifically, this included the impact of cryoprotectants on microtubules, the effect of osmotic changes on the overall structures, as well as sensitivity of the nuclear chromatin and epigenetic patterns to freezing temperatures. Based on those findings, it has been possible to design mitigating solutions like customized ultra-rapid freezing or vitrification protocols, Laser-warming methods, or post-warming reanimations. New horizons have also been explored to move away from issues and limitations related to cryopreservation. Approaches like storage of oocytes for the long-term at non-freezing temperatures are currently being developed based on encouraging data generated in preliminary studies. For instance, incorporation of trehalose into the oocyte followed by microwave-assisted dehydration can allow to remove enough water while creating a protective trehalose glass compatible with survival and storage at non-freezing temperatures. Interestingly, transcriptomic and proteomic studies have already shown that stresses and damages induced by dehydration versus cryopreservation are different and may be easier to overcome.

SELECTED ORAL COMMUNICATIONS

SESSION 57: NURSE-MIDWIFE LED FERTILITY CARE AROUND THE GLOBE

Tuesday 27 June 2023 Hall D5 14:00 - 15:00

Abstract citation ID: dead093.211

O-174 The transition from fertility to maternity care services - a Royal College of Nursing publication

F. Steyn[1]

[1]*Peppy Health, Nursing, London, United Kingdom*

Study question: Is there a need for guidance for nurses and midwives involved in the delivery of care for patients transitioning from fertility service to maternity services.

Summary answer: There is a potential knowledge gap that exists in the transition of care from fertility to maternity services.

What is known already: Once a patient becomes pregnant through fertility treatment in the UK, they are usually discharged out into NHS maternity care services via their general practitioner. This means that the maternity service staff are often unaware of the time it has taken to conceive, fertility treatments and procedures, fertility medications and emotional trauma that the patient has been through already and may not be aware of some of the complexities surrounding the fertility treatment journey.

Study design, size, duration: A project group of 14 key contributors was invited to be part of the publication that included fertility nurses, midwives, early pregnancy specialist nurses and patient representative members. An initial literature review took place and each person contributed from their specialist area. The project to produce the guidance took 9 months of planning, reviewing and meeting to discuss content with a final publication in September 2022.

Participants/materials, setting, methods: An initial literature review of the project subject did not highlight any existing research or publications in this area. A forum governance bid was submitted to produce a 45 page online resource aimed at fertility nursing staff, early pregnancy care staff and maternity services staff. The key project team contributed from their specialist areas and was reviewed by stakeholder groups.

Main results and the role of chance: Pregnancy following fertility treatment can be an exciting, challenging and anxious time for expectant parents. This projects aim was to primarily raise awareness of possible pathways of care for women and others (their partners/support networks) as they travel through fertility treatment and pregnancy, and how they can best be supported by the health care professionals they encounter along their journey. A 45 page online resource was produced and disseminated to fertility, early pregnancy and maternity staff via stakeholder groups and a webinar to launch the publication.

Limitations, reasons for caution: No limitations identified. The publication is available for all staff working in all three areas and provided guidance for all patient pathways including those from the LGBTQIA community.

Wider implications of the findings: No wider implications.

Trial registration number: Not applicable

Abstract citation ID: dead093.212

O-175 The Positive Reappraisal Coping Intervention: how it works in recurrent pregnancy loss

S. Bailey[1], **A. Parkinson**[2], **J. Steer**[2], **J. Boivin**[3]

[1]*University Hospitals Southampton NHS Foundation Trust / University of Southampton, Gynaecology, Southampton, United Kingdom*
[2]*Cardiff University at time of analysis, School of Psychology, Cardiff, United Kingdom*
[3]*Cardiff University, Cardiff Fertility Studies Research Group- School of Psychology, Cardiff, United Kingdom*

Study question: How does the Positive Reappraisal Coping Intervention (PRCI) sustain coping during the early stages of a new pregnancy in women with recurrent pregnancy loss (RPL)?

Summary answer: The PRCI improved psychological well being by sustaining coping and providing psychological respite from the stress of uncertainty.

What is known already: Increased levels of maternal anxiety and uncertainty are common during the early stages of a new pregnancy following RPL when women wait for confirmatory scan of ongoing pregnancy, however psychological support is often limited. The PRCI is a self-administered coping tool developed for uncontrollable and unpredictable situations like waiting, theorised to achieve its benefits by promoting positive feelings alongside negative feelings in unrelenting stressor situations, sustaining coping. It has been shown to have benefits for those awaiting the outcome of fertility treatment and those experiencing RPL by increasing positive emotions, positive appraisals of the situation and emotional quality of life.

Study design, size, duration: We performed secondary analysis of qualitative data from a two-centred feasibility randomised controlled study on the effectiveness of the PRCI for psychological well being of women with RPL. Participants (n = 75) were recruited over a two-year period. Participants for the qualitative component of the study (n = 14), participated in semi-structured interviews exploring their experience of pregnancy following RPL, perception of study methods and the intervention.

Participants/materials, setting, methods: Participants were recruited from the Recurrent Miscarriage Clinic in two tertiary referral hospitals in the UK. Women who had experienced three or more pregnancy losses were eligible to participate. Semi-structured interviews were conducted face to face. Data for the secondary analysis were interview data from the intervention arm of the study, that received the PRCI. Interview transcripts were analysed using NVivo 12 software and the approach of Braun and Clarke (2006).

Main results and the role of chance: This secondary analysis focused on improving understanding of the mechanisms by which the PRCI was perceived to be effective at promoting maternal psychological well being during the challenging early stages of a new pregnancy following RPL. Inductive thematic analysis of the qualitative data identified three themes on which the main theme of 'PRCI sustains coping' was based, namely that PRCI:

- prevented further falling or spiralling down by stimulating questioning of negative thoughts and encouraging time out for oneself
- encouraged an 'I can do this' attitude, acting as a reminder of the positives and stimulating positive thinking in this experience
- was perceived a helpful coping resource to lean on

The study identified that the PRCI was a 'go to' resource in taxing moments of need, conveying benefits to maternal psychological well being by restoring depleted coping resources, stimulating positive coping, and providing psychological respite from the stressor of uncertainty experienced during the early stages of a new pregnancy. There were no apparent downsides of use. These insights are consistent with theory of how positive reappraisal helps people carry on coping in challenging situations.

Limitations, reasons for caution: Since this is a secondary analysis, data was utilised from a study that was not originally designed to investigate the perceived mechanism of the PRCI, and as such the data should be interpreted cautiously.

Wider implications of the findings: PRCI has the potential to be made more widely available as an effective, safe, convenient, and low-cost intervention to provide emotional support in RPL clinics. It might also be a useful coping technique in other clinical contexts where patients/carers experience increased psychological strain because of prolonged periods of uncertainty.

Trial registration number: ISCTN43571276

Abstract citation ID: dead093.213

O-176 Knowledge and belief about fertility preservation for medical and social reasons among Iranian female students

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Study question: What is the knowledge and beliefs of the female students in Tehran universities regarding the oocyte cryopreservation for medical and social reasons?

Summary answer: This study highlighted the positive attitude but insufficient knowledge about medical and social egg freezing and the ideal timing of childbearing in female students.

What is known already: Emerging research indicates that physiologic or pathologic ovarian aging limits the female reproductive capacity and oocyte cryopreservation (OC) is a rapidly evolving branch of reproductive medicine. Given the growing numbers of young women surviving cancer, along with increasing numbers of high educated women postponing childbearing for educational or professional pursuits, there will be a growing demand for egg freezing services to guarantee the pregnancy in near future. Egg freezing can prevent age-related infertility, but it also can cause new potentially ethical and financial problems and the neonatal and maternal risks of childbearing at an advanced maternal age.

Study design, size, duration: Totally, 1279 students from Tehran universities were included in this cross-sectional survey between March and August 2022.

Participants/materials, setting, methods: The survey was distributed through an online forum and advertised on social media groups targeting students in Tehran universities. Childless students in master's and doctoral degrees with the age of ≤ 38 years were included. Bachelor's students were not included. Knowledge and beliefs about medical and non-medical oocyte cryopreservation were assessed through Fertility Preservation Survey (FPS) instrument. To analyze the data, descriptive statistical methods (mean and standard deviation) and analytical statistics (One-way ANOVA) were used.

Main results and the role of chance: The majority of participants were 30-34 years (41.1%, M: 26.38 ± 4.9), not married (77.2%), master's student (77.7%), and hoped to have 2 children in the future (40.5%). Most of participants expected to be "30-34 years" when they become pregnant with their first child (34.4%) and "35-39 years" when they give birth to their last child (39.1%). The students agreed with preserving fertility with medical (93.3%) and social (86.9%) indications and believed medical (95.1%) and social (87.4%) costs of cryopreservation should be covered by health system. Participants believed in the routine providing of information about egg freezing to women of childbearing age as part of regular healthcare visits by health care professionals (95.5%). Women stated if they decide not to use eggs to become pregnant, they would consider donating them for fertility research (67.1%), to a friend or family member with fertility problems (58.1 %) and to infertile couples (42.7%). The overall correct response to knowledge questions was 57.7% which is relatively moderate. The marital status ($P = 0.028$), university faculty ($P = 0.025$) and occupation ($P = 0.048$) were related to knowledge. Married (M: 7.40 ± 1.98) and unemployed students (M: 7.24 ± 2.24) had more knowledge and students of Art faculty had lower knowledge than other faculties (M: 6.36 ± 2.22).

Limitations, reasons for caution: The results would not be generalizable to all female students because the research project's advertisement may have been ignored by students who are not interested in childbearing or it might have been noticed by students who are more interested in childbearing and preserving their fertility.

Wider implications of the findings: The findings are valuable in planning fertility preservation services. The supportive policies for childbearing could be expanded to cover the costs of fertility preservation. Midwives could play an important role in increasing the knowledge about age-related infertility and oocyte cryopreservation and help students in their reproductive life planning.

Trial registration number: not applicable

Abstract citation ID: dead093.214

O-177 The importance of an onco-fertility program for pediatric oncology patients

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Study question: How to start an onco-fertility program in a pediatric oncology unit as standard of care

Summary answer: An active onco-fertility program helps to offer the best option for future fertility for pediatric oncology patients. Awareness of fertility risk is necessary.

What is known already: Infertility after treatment for pediatric oncology is an important late effect Of these patients, 25-35 % classify as high risk (HR) for infertility. With the increasing survival chance (>80%) this late effect plays an important role.

Study design, size, duration: An onco-fertility program was started in 2018 with the start of a centralized hospital for pediatric oncology patients (yearly 600 patients) navigated by a nurse-practitioner. The program runs with intense collaboration between the different specialties. All new patients are identified. Fertility-risk is based on the international fertility guidelines.

Participants/materials, setting, methods: All patients are informed on fertility risk by their oncologist. All HR children are additionally counseled by the onco-fertility nurse-practitioner and referred for further counseling to gynecology for ovarian tissue cryopreservation(OTC) or urology for sperm cryopreservation or testicular biopsy (in research setting). Monthly the onco-fertility working-group members discuss difficult cases and research in the field. Survivors have the possibility for fertility consultations.

Main results and the role of chance: Awareness of treatment (chemotherapy, radiotherapy and surgery) on fertility in children has increased from 2018 till now 2023 among oncologists, nursing staff, carers of the child, and the patients themselves. We offer fertility preservation mainly in the high risk for infertility group but also for low risk groups we offer information and counseling . In the outpatient clinic for survivors, there is an increasing number of consults yearly on fertility questions From 2018 till 2022 we saw a fourfold of consults.

The number of high risk for infertility over the years is stable around 30%. Over the years we see an increasing number of patients who have fertility preservation done in time In the girls this increased from 32% in 2018 to nearly 40% in 2022. For the boys it increased from 37% in 2018 to 57% in 2022

Limitations, reasons for caution: This is an ongoing programme in a large pediatric oncology unit in a first world country where this programme is possible. insurance is well organized We realize that in other countries it is much more difficult to run this program

Wider implications of the findings: In 75% of survivors risk of infertility is the most important late effect. Therefore running an onco fertility program at the start of the patient journey offering the patient the best options for fertility preservation will help the patient in future to improve their quality of life concerning fertility issues.

Trial registration number: not applicable

POSTER DISCUSSION SESSION
SESSION 58: STEM CELLS
Tuesday 27 June 2023 Hall D2 14:00 - 15:00

Abstract citation ID: dead093.215
P-793 Human trophoblastic spheroid derived from human expanded potential stem cells (hEPSC) for the identification of adhesion molecules for embryo implantation.

Y.L. Lee[1], H. Ruan[2], A.C.H. Chen[2], S.W. Fong[2], R.H.W. Li[2], E.H.Y. Ng[2], W.S.B. Yeung[2]

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Study question: Are cell surface proteins on trophoblastic spheroid BAP-EB derived from hEPSC involved in the endometrial epithelial cell attachment?

Summary answer: E-cadherin (CDH1) and Neuropilin-1 (NRP1) are two potential embryonic surface molecules involved in the human implantation process.

What is known already: Study of the mechanisms of human implantation in vivo is challenging. The availability of high-quality human embryos for in vitro research is limited. We have recently derived morula-like hEPSC lines (hEPSC-em) from donated human embryos. Trophoblastic spheroids (em-BAP-EB) derived from hEPSC-em resemble human trophectoderm during the early implantation process. The em-BAP-EBs attach specifically onto receptive endometrial epithelial cells, suggesting its usefulness as human embryo surrogate for studying embryonic surface molecules involving in early implantation process.

Study design, size, duration: hEPSC-em was subjected to embryoid body formation and induced to differentiate into trophoblastic spheroids (em-BAP-EB) for 48h and 72h. Endometrial aspirates were collected from infertile women in their natural cycles 2 or 7 days after LH surge (LH+2/+7). Endometrial epithelial cells (EEC) were isolated and cultured for the attachment assays. Biotin-labelled apical surface proteins on em-BAP-EB were identified by mass spectrometry. Antibody blocking assays were performed to confirm the biological functions.

Participants/materials, setting, methods: The attachment rates of em-BAP-EB onto EEC were compared between pre-receptive (LH+2 day) and receptive (LH+7 day) phases. The differentially expressed apical surface protein on em-BAP-EB-48h and -72h were subjected to plasma membrane proteins (PMP) identification and pathway analysis. The gene and protein expression patterns of the target proteins were analyzed by single cell RNA sequencing (scRNA-seq) and western blotting respectively. The functional roles of the target proteins were studied by antibody blocking during attachment

Main results and the role of chance: The attachment rates of em-BAP-EB-72h onto receptive stage (LH+7, n=17) EEC were significantly higher than those onto pre-receptive stage (LH+2, n=5) EEC. Gene Ontology analysis indicated that the PMP with induced expression levels on em-BAP-EB-72h when compared to -48h (72h>48h) were enriched for "protein binding" and "cadherin binding" in molecular functions, and "cell-cell adhesion" in biological processes. Among them, 28 PMP were trophectoderm-specific. CDH1 and NRP1 were selected for further analysis. Analysis based on a published dataset showed that significantly higher expressions of *CDH1* and *NRP1* were detected in polar trophectoderm as compared to mural trophectoderm of E7 human blastocyst. *CDH1* and *NRP1* were significantly up-regulated in trophectoderm-like em-BAP-EB by scRNA-seq analysis. Live-cell immunocytochemistry demonstrated more intense apical surface expression of CDH1 and NRP1 on em-BAP-EB-72h as compared to -48h. Antibody blocking experiments indicated that pre-treatment of the em-BAP-EB-72h with specific antibody individually against the ectodomains of CDH1 and NRP1 significantly reduced the attachment rates onto receptive endometrial epithelial cells. The data suggested that CDH1 and NRP1 are two potential apical surface molecules essential for blastocyst attachment.

Limitations, reasons for caution: The lack of embryonic compartment in the em-BAP-EB and the absence of other key players like decidualized stromal cells and immune cells in the coculture model might not fully represent the early attachment process. The limited sample size in this study may also limit the interpretation of the data.

Wider implications of the findings: Despite advancement in in vitro fertilization, the success rate remains unsatisfactory. It is not known if embryological factor(s) other than embryo aneuploidy are involved. The current data may suggest the potential roles of CDH1 and NRP1 as non-chromosomal embryological markers of its implantation potential.

Trial registration number: Not applicable

Abstract citation ID: dead093.216
P-794 Autologous stem cell-derived mitochondria transfer show therapeutic advantages in human embryo quality rescue

H. Shen[1], Z. Jiang[1]

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Study question: To fill gaps in the evidence base on the autologous superior mitochondria donor cells, and its therapeutic effect on human embryo development among infertile population.

Summary answer: Autologous urine-derived mesenchymal stromal cells (USC) mitochondria transfer may be an effective strategy to improve embryonic development, especially in infertile females with advanced age.

What is known already: Mitochondria are vital for the initial development of oocytes and embryos. Transferring autologous mitochondria into oocytes of infertile females is a novel and feasible strategy for infertile problems without genomic safety and ethical concerns. Stem cells have biological advantages, but research and evidence in this area are quite scarce.

Study design, size, duration: In vitro fertilization/Intracytoplasmic sperm injection (IVF/ICSI) patients at the Assisted Reproductive Technology (ART) Centre of Peking University People's Hospital (Beijing, China) were divided into young group (<35) and advanced age group (≥35) according to age. Oocytes obtained from each age group were randomly assigned to the conventional ICSI group and the mitochondria ICSI group.

Participants/materials, setting, methods: Mitochondrial morphology, mitochondrial DNA (mtDNA) copy number, mitochondrial activity, metabolic capacity are analyzed among autologous adipose, marrow, urine-derived mesenchymal stromal cells (ADSC, BMSC and USC) and ovarian germline granulosa cells (GC). Whole mitochondrial genome sequencing was performed to validate mtDNA biosecurity. The effect of mitochondrial transfer was evaluated by human early embryonic morphology, euploidy, mitochondrial content, mitochondrial membrane potential (MMP), cytosolic reactive oxygen species (ROS) and Ca^{2+} levels.

Main results and the role of chance: Among many types of human primary cells, urine-derived mesenchymal stromal cells (USC) demonstrate a non-fused spherical mitochondrial morphology and low oxidative stress status, which resembles the oocyte stage. Moreover, USC mitochondrial content, activity and function are all higher than other cell types and less affected by age, and it also exhibits a biphasic metabolic pattern similar to the pre-implantation stage of embryonic development. After the biosafety identification of USC mitochondrial genome, human early embryos after USC mitochondria transfer showed improvements in mitochondrial content, activity, and cytoplasmic Ca^{2+} levels. Further, aging embryos also showed improved

embryonic morphological indicators, euploidy rates, and oxidative stress status.

Limitations, reasons for caution: Our study is an in-vitro basic research on human early embryos, further basic mechanisms and clinical trials are still needed to confirm its clinical therapeutic results.

Wider implications of the findings: Autologous USC mitochondria transfer provides evidence and possibility for the autologous treatment in the infertile females without invasive and ethical concerns.

Trial registration number: Beijing Natural Science Foundation (grant no. 7222197); State Key Program of the National Natural Science Foundation of China (grant no. 21737001)

Abstract citation ID: dead093.217
P-805 Gene expression of human endometrial organoids hormonally treated in vitro for 28 days

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[4]Karolinska University Hospital, Department of Reproductive Medicine- Division of Gynecology and Reproduction, Stockholm, Sweden

Study question: Does gene expression of human endometrial organoids (EO) mimic the *in vivo* response to a 28- days substitutive hormonal treatment?

Summary answer: Gene expression profile of human EO exposed to 28-day hormone treatment showed correspondence with the phases of the menstrual cycle *in vivo*.

What is known already: The recent development of human EO demonstrated their translational potential for reproductive medicine. Current studies testing functional response to hormones of EO are based on short-term responsiveness to estrogen (E2), progesterone (P4) and differentiation factors up to 10 days. It is unknown how organoids can be used to faithfully reproduce and/or model a complete menstrual cycle of 28 days. High-throughput analyses on endometrium have identified specific markers available for characterizing differentiation and functional aspects related to secretory activity and receptivity to implantation.

Study design, size, duration: Experimental research was carried out including 4 endometrial biopsies donated by women with normal endometrium function, recruited at an academic center for assisted reproduction between 2022-06-15 to 2022-07-28. These endometrial biopsies were processed for organoid derivation and culture. The EO were hormonally treated *in vitro* and analyzed for gene expression.

Participants/materials, setting, methods: Human endometrial biopsies were enzymatically digested to isolate endometrial glands, embedded in matrigel and cultivated in a spinning bioreactor to achieve organoid formation. Thereafter EO were treated as following: 1) E2: (day 0–28); 2) E2 + P4: E2 (day 0–28), P4 (day 14–28); 3) E2 + P4 + dibutyryl cyclic Adenosine monophosphate (dbcAMP): E2 (day 0–28) + P4 (day 14–28) + dbcAMP (day 14–28) or 4) control group.

Main results and the role of chance: A total of 10 markers were selected for functional analysis and 3 showed a pattern of interest. The target gene expression included Forkhead box O1 (FOXO1), involved in regulating endometrium receptivity in human; progesterone associated endometrium protein (PAEP), a major protein in glandular endometrium secretions; and secreted phosphoprotein 1 (SPP1), an extracellular cytokine that increases in endometrium during the receptive phase.

Gene expression of FOXO1, PAEP and SPP1 showed a significant increase in the organoids exposed to complete hormonal cycle, compared to controls. PAEP and SPP1 gene expression was lower up to day 21 but upregulated to 15-fold relative to non-treated group by day 28. This pattern recapitulates a similar response to the physiological conditions *in vivo* in the human endometrium. Although gene expression patterns followed similar trends, individual biopsies had slight variations in gene expression along 28-day hormone treatment.

The 28 day-hormone treatment can stimulate EO for recapitulating histological and functional aspects. The dynamics resembled a timely correlation with gene expression changes accounting for some specific markers mostly involved in receptivity, and thus reinforcing the potential application of EO for further research purposes.

Limitations, reasons for caution: In vitro organoid culture conditions may have some influence over responsiveness and gene expression that requires further optimization to reproduce uterine environment in vitro and for other conditions related to infertility in a completely defined culture system.

Wider implications of the findings: Our findings enable the opportunity to expand experimental research over endometrial disorders affecting different stages of the menstrual cycle and also to improve research related to embryo-endometrium interactions.

Trial registration number: This research has been funded by grants from The Swedish Cancer Society, The Swedish Childhood Cancer Foundation, The Cancer Research Funds of Radiumhemmet, The Swedish Cancer Association BRO, the Stockholm County Council (CIMED) and Karolinska Institutet.

Abstract citation ID: dead093.218
P-803 3D human endometrium from noninvasively retrieved primary endometrial cells is a reliable model to mimic the implantation window

F.P. Luongo[1], I. Ortega Baño[1], G. Morgante[1], A. Luddi[1], P. Piomboni[1]

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Study question: Can human endometrial organoids (hEOs) from a noninvasive source (menstrual blood-MB) mimic the physiology of a receptive endometrium

Summary answer: Endometrial organoids from MB functionally respond to stimuli and express markers of receptivity and can be modulated.

What is known already: 3D models are considered a new step in promoting precision medicine and an advanced tool for studying endometrial biology, endometrial-associated diseases, and understanding the complex mechanisms that comprise endometrial-embryo crosstalk. So far, both 2D and 3D models are usually obtained from endometrial biopsy. The recent discovery of molecules crucial for successful embryo implantation has offered researchers valuable insights in this field. However, important questions about the molecular mechanisms that drive this process remain to be deciphered.

Study design, size, duration: hEOs are usually obtained from endometrial biopsy, in this case a noninvasive source(MB) was used to isolate endometrial epithelial (Ep) and stromal (hESC) cells. The hEOs were generated to study the morphological changes that occur in vivo during the menstrual cycle, particularly in the window of implantation (WOI). The hEOs obtained from MB were exposed to hormones treatments to mimic WOI and were also treated with the progesterone blocker mifepristone to reverse the decidualization process.

Participants/materials, setting, methods: 3D hEOs were prepared from 6 healthy volunteers and cultured for 4 days in expansion medium supplemented with 10^{-8} M E_2 + 10^{-6} M P_4 and 500 uM cAMP. As a control, hEOs were treated with the progesterone blocker mifepristone to reverse the decidualization process. Finally, hEOs were washed with PBS and fixed for immunofluorescent staining or for scanning-electron-microscopy (SEM) or stored at -80 °C for gene expression analysis.

Main results and the role of chance: In this study, we were able to establish a reliable 3D model using both epithelial and stromal cells with a less invasive approach to mimic the mid-secretory phase. Immunofluorescence staining of vimentin (marker of stromal cells) and cytokeratin 19 (marker of epithelial cells) confirmed that organoids are formed by both Ep and hESCs. Using SEM, we closely observed the luminal surface of hEOs in proliferative phase, where the inner side of Ep cells was characterized by the presence of pinopods, a specific marker of the implantation window. In addition, hEOs

express PP14, an implantation marker that has been shown to be significantly up-regulated during WOI. Finally, the expression of FKBP5, regulated by cAMP, and ZBP16, regulated by progesterone, increased significantly during decidualization, while their expression was up-regulated at dawn by mifepristone, a progesterone blocker, thus confirming that hEOs are an effective model of WOI.

Limitations, reasons for caution: All results need to be validated in a larger cohort and further implemented with other cell types (e.g., endothelial and immune cells) to implement this model for drug testing and therapy

Wider implications of the findings: Effective and reliable modeling of embryonic implantation is needed to mimic the cascade of molecular events that occurs in vivo. This model can be used for personalized medicine without the invasiveness of biopsy.

Trial registration number: Not applicable

SELECTED ORAL COMMUNICATIONS
SESSION 59: OPTIMISATION OF FERTILISATION
Tuesday 27 June 2023 Hall A 15:15 - 16:30

Abstract citation ID: dead093.219
O-178 Conventional IVF results in better embryo development when compared with ICSI in patients without severe male factor

M.D. Cuquerella[1], S. Rafael[1], S. Veganzones[1], M. Vidaurreta[1], M.I. Calventus[1], I. Cristobal[1]

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Study question: Which fertilisation technique offers better embryo development results in patients without severe male factor?

Summary answer: Conventional IVF offers better results regarding embryo development than ICSI in patients without male factor when applying both techniques in the same cohort of oocytes.

What is known already: ICSI was originally indicated for severe male factor. Nevertheless, it is currently used for many other indications so that it has become the most used technique. One of the reasons for the use of ICSI is the fear of a fertilisation failure when using conventional IVF (cIVF).

We have to consider that ICSI is more expensive and invasive. For this reason, it is important to know whether there is a justification to use it in these cases.

Study design, size, duration: It is a randomized prospective study where 68 IVF/ICSI cycles were analysed. The study comprised 812 oocytes and 469 embryos and was conducted from January to December 2022 in the Assisted Reproduction Unit of a tertiary hospital.

In each cycle, we performed a mixed technique using cIVF and ICSI in the same cohort of oocytes.

MII rate, fertilisation rate and embryo development were analysed.

Participants/materials, setting, methods: All consecutive cycles with at least 6 fresh oocytes and semen parameters suitable for cIVF were offered to participate.

In each cycle, oocytes were divided into two groups in order of collection and the fertilisation technique (cIVF or ICSI) was randomly assigned for each group.

All the embryos were cultured to blastocyst stage.

Data of maturity, fertilisation and embryo development were individually registered for every oocyte.

Data were statistically analysed by applying multilevel regression models.

Main results and the role of chance: The patients average age was 36.3 ± 3.0 (28 to 40) and the number of oocytes per cycle was 11.9 ± 5.1 (6 to 26).

396 oocytes (48.8%) were allocated to cIVF group and 416 (51.2%) to ICSI group.

The percentage of mature oocytes was significantly higher in cIVF group than in ICSI group (88.9% vs 81.5%, $p = 0.017$), which is consistent with the later assessment of maturity in cIVF.

There were no statistically significant differences between cIVF and ICSI groups in terms of fertilisation rate (59.6% vs 56.0%) and high-quality blastocyst rate (A+B) (9.3% vs 8.7%) when analysed per oocyte.

We observed clinical but not statistical differences in blastocyst rate per oocyte (30.6% cIVF vs 23.6% ICSI, $p = 0.09$). However, blastocyst rate per fertilised oocyte showed statistically significant differences (51.3% cIVF vs 41.6% ICSI, $p = 0.047$).

Additionally, we found statistically significant differences between cIVF and ICSI in the rate of usable blastocysts (transferred or cryopreserved) per oocyte (26.3% vs 18.3%, $p < 0.01$) and per fertilised oocyte (44.1% vs 32.2%, $p < 0.01$).

In 57.8% of the cycles, the best blastocyst was obtained by cIVF and in 42.2% by ICSI.

Fertilisation failure was observed in 3 patients with cIVF and 1 patient with ICSI. All of them had ≤4 mature eggs.

Limitations, reasons for caution: This study includes the 68 patients who met the recruitment requirements until this moment. It is a part of a wider work, which will analyse a higher number of oocytes and pregnancy and perinatal outcomes. For this reason, the sample size is a limitation to the interpretation of the results.

Wider implications of the findings: Our findings encourage the use of cIVF when there is no severe male factor. Considering that cIVF is cheaper and less invasive than ICSI, the exclusive use of ICSI in these patients does not seem to be justified according to our results.

Trial registration number: not applicable

Abstract citation ID: dead093.220
O-179 Fertilization topologies that occur seldom or not at all in nature give rise to distinct functional classes of mouse embryos

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Study question: Would new embryo properties emerge if fertilization was imposed on oocytes in regions, such as the animal pole, where it does not take place naturally?

Summary answer: Oocyte fertilization at the animal pole, vegetal pole or equator results in 2-cell embryos with distinguishable transcriptomes and functional peculiarities.

What is known already: There is fertilization bias in embryos used in basic and clinical research, because: 1) sperm–oocyte fusion hardly occurs at the surface above the meiotic spindle – the animal pole; 2) the region opposite the spindle - vegetal pole - is poorly accessible to sperm due to tiny perivitelline space. These regions are also avoided during intracytoplasmic sperm injection (ICSI) for fear of damaging the spindle or losing the sperm nucleus into the 2nd polar body. Pole materials are held non-essential for mouse development, but this conclusion relies on the 2nd polar body, which is an unreliable topological marker.

Study design, size, duration: Oocytes were rotated using a micromanipulator fitted with Nomarski optics, using the meiotic spindle as a landmark. Between 9:30 and 10:30 am single sperm heads were microinjected (ICSI) at the animal or vegetal pole (treatments) vs. the naturally prevalent equatorial region (control). This way two fertilization topologies and possibly also two classes of zygotes were created that are otherwise seldom or not at all represented in natural fertilization, in vitro insemination or conventional ICSI.

Participants/materials, setting, methods: Metaphase II oocytes were collected from 8-week-old B6C3F1 mice stimulated with 10 I.U. eCG+hCG. Sperm heads from a single batch of cryopreserved CD1 semen were deposited via ICSI in the cortex at the animal pole, vegetal pole or half-way between poles i.e. equatorially. Zygotes were cultured in KSOM(aa) and analyzed (triplicate or more) for: cleavage rates, transcriptomes at the 2-cell stage (RNAseq), blastocyst germ layers (immunostaining for trophectoderm, primitive endoderm, epiblast), and postimplantation development.

Main results and the role of chance: Although full development was supported irrespective of ICSI site, embryos clustered by site, as revealed by single-cell RNAseq of 21, 21 and 13 two-cell embryos whose oocytes were fertilized at the animal pole, vegetal pole or equator, respectively. When examining the sister blastomeres together, 462 genes of the shared transcriptome were differently expressed between ICSI sites, with the equatorial class contributing most to the difference (adj.p<0.05, Wilcoxon test). This was true also when examining the sister blastomeres separately: inter-blastomere differences of the equatorial class exceeded those of the pole classes (72% vs. 14% of differently expressed genes). Ontology analysis of the differently expressed genes using *Enrichr* pointed at the endomembrane system – an acquaintance of oocyte polarity studies (PMID 10545249; PMID 29746690). Follow-up of 2-cell embryos to blastocysts revealed that sister blastomere contribution to each germ layer was less balanced in the equatorial class, as measured by linear correlation of cell numbers (e.g. equatorial R^2=0.00 vs. polar R^2>0.23 for the epiblast). Summing up, it is difficult to reconcile these data with a mainstream view that fertilization at the animal pole is harmful. Rather they support that the topology of fertilization defines functional classes of 2-cell embryos with distinguishable transcriptomes.

Limitations, reasons for caution: This is an animal study. Mouse ICSI uses mercury-loaded piezo-driven needles, human ICSI does not. The higher consistency of using the one and same batch of cryopreserved spermatozoa was traded off against lower developmental rates. Single-cell resolution posed a limit on RNAseq depth. Results need confirmation in other mouse strains.

Wider implications of the findings: The higher blastomere similarity observed after ICSI at the vegetal pole compared to ICSI at the equator is not consistent with the prevalent model of first zygotic cleavage that is driven by the topology of the two apposing pronuclei.

Trial registration number: not applicable

Abstract citation ID: dead093.221
O-180 What is the true potential of 1PN embryos? A report on utilisation and live birth rates following PGT-A and biparental testing

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Study question: What is the developmental potential of monopronuclear (1PN) embryos, and does allowing their use only after confirming biparental inheritance offer a clinical solution for them?

Summary answer: 1PN embryos demonstrate clear utilisation, euploidy, and live birth rates, that warrant their routine culture, and use following biparental inheritance testing, in the clinical setting.

What is known already: Embryos only displaying a single pronuclei (1PN) are widely regarded to be abnormal and not suitable for clinical use. It is therefore common practice to discard these embryos following fertilisation check. However, 1PN embryos have shown the ability for normal blastocyst development, albeit at a decreased rate, and the ability to produce live births.

To bridge the gap between discarding an "abnormal" 1PN embryo, and the knowledge that it can result in a live birth, our clinic utilises PGT-A with biparental inheritance testing to assess the fitness of a 1PN embryo for transfer.

Study design, size, duration: A retrospective analysis was performed of 5010 1PN embryos derived from IVF (2612) or ICSI (2398) insemination at Genea clinics across Australia between January 2017 and November 2022.

Blastocyst development, euploidy, parental inheritance, pregnancy, and live birth rates were analysed.

Participants/materials, setting, methods: Embryos were defined as being 1PN by the appearance of one pronuclei at fertilisation check 16-18 hours post insemination. Time lapse footage was reviewed on day 3 of culture to eliminate the late appearance of a second pronucleus. Suitable embryos underwent trophectoderm biopsy for PGT-A and biparental inheritance testing. Only euploid embryos with confirmed biparental inheritance were available to patients for frozen embryo transfer.

Main results and the role of chance: 1PN embryos derived from ICSI insemination were found to have a significantly lower blastocyst development potential of 17.4% compared to 33.7% for IVF embryos ($p < 0.0001$). The utilisation rate was also significantly lower at 8.1% for ICSI vs 21.2% for IVF ($p < 0.0001$).

The IVF embryos showed a higher rate of euploidy following PGA-T testing, 50.9% for IVF vs 37.9% for ICSI ($p < 0.0001$).

Uniparental inheritance, an indication the 1PN appearance was caused by parthenogenesis, was only observed in 6.7% of the total embryos tested, with IVF embryos having a considerably lower rate of 1.8% vs 20.5% for ICSI ($p < 0.0001$).

There was no significant difference between the 2 groups for pregnancy rate or live births, following single embryo transfer, with 58.6% for IVF vs 62.9% for ICSI ($p = 0.6$) and 39.6% for IVF vs 45.7% for ICSI ($p = 0.5$) respectively. These levels were comparable to normally fertilised (2PN) euploid embryos across Genea clinics in the same time period. During the study period there have been 175 1PN embryos transferred that has resulted in 69 live births from embryos that otherwise would have been discarded.

Limitations, reasons for caution: The use of timelapse imaging from the time of insemination for ICSI embryos, as opposed to 16-18 hours post insemination for IVF embryos may play a role in the type of 1PN embryos that are being identified in each group.

Wider implications of the findings: This study demonstrates that not all 1PN embryos are abnormal and their routine use in the clinical IVF lab in conjunction with appropriate testing should be re-evaluated.

Trial registration number: not-applicable

Abstract citation ID: dead093.222
O-181 Utilisation of monopronucleated (1PN) embryos and potential clinical benefits for patients

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Study question: Is it beneficial to culture 1PN embryos for clinical use?

Summary answer: Culturing 1PN embryos to blastocyst for potential clinical use is beneficial to all patients, especially those with low prognosis.

What is known already: 1PN embryos are those with only a single pronucleus visible at the time of fertilisation check. It is generally assumed that such embryos are haploid, having only 23 chromosomes in each of their cells. Given that haploid embryos are not viable, current guidelines recommend only transfering embryos where two pronuclei are visualised. Embryo biopsy at the blastocyst stage, followed by testing with next-generation sequencing (NGS), combined with analysis of DNA polymorphisms, can reveal whether 1PN embryos are truly haploid, or whether normal fertilsation has occurred. Previous studies have shown that in some cases a diploid chromosome number may be present.

Study design, size, duration: A new clinical policy was put into practice to culture all 1PN embryos to day 5/6. This is an ongoing study initiated in April 2022. The data reported are from an eight month period, up until December 2022. During this time, we cultured 288 1PN embryos from 203 patients. Trophectoderm biopsy specimens were shipped to a specialist genetics laboratory where they underwent PGT-A using a method that assesses chromosome copy number and polymorphisms in tandem.

Participants/materials, setting, methods: If embryos were assessed at fertilisation check and found to be 1PN they were not discarded, but rather they were maintained in culture. If any such embryos successfully produced a blastocyst the patients received counselling from their fertility consultant and an embryologist, giving them the options to either go ahead with transfer (only IVF derived), have the embryos genetic status clarified using PGT-A, freeze without testing, or to discard the embryo.

Main results and the role of chance: Of the 288 1PN embryos cultured (IVF and ICSI derived) 85 displayed signs of blastulation (29.5%). 134 of these were derived from ICSI with 20 forming blastocysts (14.9%). 154 were derived from IVF with 65 forming blastocysts (42.2%). Three IVF derived 1PN embryos have been transferred. Currently one is an ongoing singleton pregnancy, one is an ongoing twin pregnancy and the third has resulted in a live birth. 35 embryos were biopsied and underwent genetic testing to confirm their ploidy status. Six of these were ICSI derived, four of which were found to be haploid and two diploid. The other 29 tested embryos were derived from IVF. Of these, 27 were found to be diploid (93.1%) and two were shown to be triploid (6.9%). None were confirmed as haploid. Traditionally, PGT-A methods using NGS examine relative chromosome copy number, but in cases of haploidy/triploidy all of the chromosomes are decreased/increased in number, meaning there is no change in the relative number of individual chromosomes. We established embryo ploidy status using an advanced PGT method, genotyping thousands of polymorphisms scattered across the genome, which are essential for accurate diagnosis of haploidy/triploidy.

Limitations, reasons for caution: The sample size for genetically tested ICSI derived 1PN's is currently too small to clearly determine whether culture and testing of these embryos is beneficial to the patient. Patients who decided to transfer an embryo categorised as 1PN were made fully aware of the potential risks in doing so.

Wider implications of the findings: These findings show that the culture of 1PN embryos is clinically beneficial to a large number of patients, especially those with poor prognosis who would otherwise have had a failed cycle. Genetic testing demonstrates that IVF derived 1PN embryos that reach the blastocyst stage are likely to have fertilised normally.

Trial registration number: *

Abstract citation ID: dead093.223

O-182 The clinical value of rescued MI-oocytes. Retrospective analysis of 625 MI-oocytes versus 2124 sibling MII-oocytes obtained in 285 fresh donor ICSI cycles

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Study question: What are the developmental competence and clinical value in ICSI cycles of retrieved metaphase-I oocytes matured in vitro (rescued-MI) to the metaphase-II (MII) stage?

Summary answer: Rescued MI-oocytes showed lower developmental but similar post-implantation competence than their sibling MII-oocytes, thereby contributing to a + 9.5% relative-increase in the cumulative-live-birth rate per completed-cycle.

What is known already: MI-oocytes are typically excluded from ICSI. Nevertheless, some authors reported their use in cancer or poor responder patients. On average, 15-20% of the oocytes retrieved in IVF are immature, but 45% of them may mature within a few hours of culture. These rescued-oocytes showed lower developmental, chromosomal and reproductive competence. Regardless, they might be valuable for some patients. To date, most of the studies were conducted including patients using own oocytes, thus implicitly involving the bias of infertility and poor prognosis. Here we aimed at outlining the clinical value of rescued-MI oocytes in the context of egg donation cycles.

Study design, size, duration: Observational study conducted at a private IVF center including 284 fresh oocyte donation cycles (Jan-2020 to Dec-2021). Two hours after oocyte-retrieval, all MI-oocytes identified after denudation were cultured for 1-2 additional hour(s). If reaching the MII-stage, they were inseminated via ICSI with ejaculated sperm, as for sibling MII-oocytes. Single culture in continuous media was conducted. MI- and MII-derived embryonic cohorts were monitored and compared. One-hundred-fifty-three cycles in the same period had no MI-oocyte available.

Participants/materials, setting, methods: Two-hundred-eighty-four cycles were conducted from 272 recipients (42.1 ± 4.0 years) with fresh oocytes retrieved from 215 donors (25.5 ± 5.0 years). All sperm samples were ejaculated (42.3% fresh; 15% oligoasthenoteratozoospermic). The number of MI- and MII-oocytes after denudation was 2.2 ± 1.3 (range:1-7) and 7.5 ± 1.6 per cycle, respectively. 1.7 ± 1.2 (0-6) MI-oocytes matured in vitro (rescue-rate: 78.1 ± 33.4%) and were inseminated. Embryological data were compared between sibling MI- and MII-derived cohorts. Clinical data and relative contributions of MI-oocytes were also reported.

Main results and the role of chance: The mean fertilization rate per cohort of inseminated MI- and MII-oocytes were 54.3 ± 43.0% and 74.4 ± 18.1%, respectively (paired t-test<0.01). The mean blastulation rate per cohort of MI- and MII-derived zygotes were 53.1 ± 44.4% and 59.1 ± 25.9%, respectively (paired t-test=0.1). Overall, 0.5 ± 0.7 (0-4) MI-derived blastocysts were obtained adding up to the 3.2 ± 1.7 MII-derived ones, thereby resulting in a + 20.8 ± 40.1% (0 to + 300%) relative increase per cycle. Overall, 268 (94%), 247 (89.7%), and 188 (66.2%) cycles had ≥1, ≥2 and ≥3 MII-derived blastocyst(s), respectively. These rates showed a + 1.1% (N = +3), +4.9% (N = +12) and +7.4% (N = +24) relative increase due to MI-derived blastocysts use. To date, 19 out of 63 MI-derived blastocysts transferred resulted in a live-birth (30.2%, 95%CI 19.6-43.2) versus 124 out of 399 MII-derived ones (31.1%, 95%CI 26.6-35.9; p = 0.99). We reported 4 miscarriages out of 27 clinical pregnancies (14.8%, 95%CI 4.8-34.6) versus 38 out 162 (23.5%, 95%CI 17.3-30.9, p = 0.45), respectively. To date, 70% (N = 199/284) of the cycles was concluded, with a cumulative-live-birth-rate of 61.3% (N = 122/199). This rate showed a + 9.5% (N = +18) relative increase due to MI-derived blastocysts use. Among 85 non-completed cycles, 19 (22.4%) have MI-derived blastocysts still available.

Limitations, reasons for caution: Retrospective study. A cost-effectiveness analysis is warranted, especially because MI-oocytes clinical use involves additional workload and costs. 31% (N = 44/140) of patients with ≥1 live-birth have supernumerary cryopreserved MI-derived blastocysts. Many cycles are not concluded. Gestational/perinatal outcomes were not reported. More data with own eggs and/or including chromosomal testing are needed.

Wider implications of the findings: Rescuing MI-oocytes might increase the number of blastocysts available per cycle, and possibly the cumulative-live-birth-rate. Nevertheless, this practice involves a higher workload and the production of more supernumerary blastocysts. Mostly poor prognosis couples might benefit from their use; therefore a couple-specific decision-making process is desirable.

Trial registration number: n/a

SELECTED ORAL COMMUNICATIONS

SESSION 60: NEW CONCEPTS: AI IN OVARIAN STIMULATION

Tuesday 27 June 2023 Hall D3 15:15 - 16:30

Abstract citation ID: dead093.224

O-183 Forecast of Total and Mature Oocyte Number for Three Different Trigger Days using Machine Learning Prediction Models

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Study question: Can an Artificial Intelligence (AI) algorithm accurately predict the number of total\mature (MII) oocytes retrieved during an antagonist protocol cycle for different trigger days.

Summary answer: AI algorithm can accurately predict the number of total and MII oocytes for three different trigger days in an antagonist protocol.

What is known already: Two recent studies used machine learning prediction models for determining the number of MII oocytes retrieved during ovarian stimulation. These studies predicted MII with mean absolute error (MAE) 2.87-3.11 and R2 0.62-0.64. However, currently no studies show results for predicting total and MII oocytes two days in advance.

Study design, size, duration: The data used for developing this algorithm consists of 9,622 antagonist protocol cycles performed in a large center serving over 50 physicians, between August 2017 and November 2022. This dataset contains a subset of 5,517 cycles with information about the maturity of each retrieved oocyte representing ICSI cycles and egg freezing (MII dataset).

Participants/materials, setting, methods: Two sets of prediction models were developed using XGBoost, to estimate the number of total and MII oocytes, each with three individual predictors for different possible trigger days: today, tomorrow, in two days. Parameters used include estradiol and LH levels, follicles' size, stimulation days, gonadotropin dosage, and patients' characteristics. Accuracy of the model was evaluated by comparing the predictions to the actual values on 20% of the data that was reserved as a test set.

Main results and the role of chance: The accuracy of the models for the total number of oocytes evaluated using the entire test set, while the models for the number of MII oocytes evaluated using the MII dataset test.

The performance of the prediction models for the number of oocytes: (1) trigger today, R2=0.72, MAE=2.59 (2) trigger tomorrow, R2=0.66, MAE=2.95 (3) trigger in two days, R2=0.64, MAE=3.12.

Of the prediction models for the number of MII oocytes: (1) trigger today, R2=0.68, MAE=2.24 (2) trigger tomorrow, R2=0.62, MAE=2.53 (3) trigger in two days, R2=0.59, MAE=2.54.

Further evaluation was done on a refined subset, excluding cycles with a very large difference between the number of follicles in the last ultrasound and the number of oocytes retrieved (~10%).

The performance of the prediction models for the number of oocytes: (1) trigger today, R2=0.81, MAE=2.14 (2) trigger tomorrow, R2=0.73, MAE=2.42 (3) trigger in two days, R2=0.69, MAE=2.77.

Of the prediction models for the number of MII oocytes: (1) trigger today, R2=0.74, MAE=2.03 (2) trigger tomorrow, R2=0.66, MAE=2.25 (3) trigger in two days, R2=0.62, MAE=2.38.

Limitations, reasons for caution: To ensure accurate predictions, it is important to only provide predictions on days when a trigger can be performed. Thus, an outlier detection mechanism is required to identify predictable days. This requirement is a limitation when using the prediction models for the number of total or MII oocytes in real-time.

Wider implications of the findings: Estimating the number of oocytes retrieved for different trigger days may assist in selecting the optimal trigger day and potentially improving outcomes. Additionally, if the estimated number of oocytes retrieved for different trigger days are similar, it may provide more flexibility in choosing the trigger day.

Trial registration number: HMC-0011-22

Abstract citation ID: dead093.225

O-184 Validation of a model capable of estimating the minimum number of mature oocytes needed to obtain at least one euploid blastocyst regarding female age

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Study question: Which is the minimum number of mature oocytes needed to obtain at least one euploid blastocyst regarding female age in In Vitro Fertilization (IVF) treatments?

Summary answer: The validated model estimates with a 74% accuracy the probability of having one euploid blastocyst regarding the number of mature oocytes and female age.

What is known already: Female age is significantly and directly related to embryo aneuploidy rates, thus lowering the chances of success in IVF treatments. The current delay in motherhood has led to a large proportion of women of advanced maternal age seeking infertility treatment. The aim of the present study is to determine the number of metaphase II (MII) oocytes needed to obtain at least one euploid blastocyst regarding female age. This information will help to decide the best strategy for each patient, taking also into account other variables such as ovarian reserve, semen quality and oocyte quality.

Study design, size, duration: Retrospective analysis of IVF cycles with preimplantational genetic testing for aneuploidies (PGT-A) performed over the last 5 years in an infertility clinic in Spain, from January 2017 to March 2022.

Participants/materials, setting, methods: Patients undergoing an IVF cycle in an infertility clinic with own or donated oocytes, regardless semen origin. Only trophoectoderm biopsies performed on day 5 or 6 of development and analyzed using Next Generation Sequencing (NGS) were included. PGT-A was used for reasons such as advanced maternal age, implantation failure and recurrent miscarriage. PGT-A cycles due to a known abnormal karyotype were excluded. Endpoints were analized using binary logistic regression models.

Main results and the role of chance: A total of 3840 IVF-PGT-A cycles meeting the inclusion criteria were performed in the study period. Of them, 939 cycles were discarded due to the absence of any biopsied/analyzed embryo (final sample size=2901). A model for the probability of having at least one euploid blastocyst (pEB) regarding female age and the number of MII oocytes retrieved was created with 80% of the sample (n = 2320) and validated in the remaining 20% (n = 581). The validation of this model showed that it was capable of estimate with an accuracy of 73.88%. The pEB was directly related to the number of MII oocytes retrieved (odds ratio (OR) 1.130, confidence interval (IC) 95% (1.110-1.150); $p < 0.001$), but indirectly related to female age (OR 0.751, IC95% (0.725-0.778); $p < 0.001$). The ROC curve showed a significant predictive value of the number of MII oocytes (area under the curve (AUC):0.8041 (0.7882-0.82)) for this model. A mathematical formula was created for the calculation of pEB using this model. The number of MII oocytes needed for a pEB of 75% regarding female age (female age:number MII) was 35:6, 36:9, 37:12, 38:15, 39:18, 40:21, 41:24, 42:27, 43:30, 44:32, 45:35, 46:38, 47:41, 48:44, 49:47, 50:50.

Limitations, reasons for caution: The main limitation of this study is its retrospective design. In addition, it's important to keep in mind that many other variables should be taken into consideration along with maternal age in the assessment of the number of oocytes needed to obtain at least one euploid blastocyst in IVF treatments.

Wider implications of the findings: Results from this study may constitute a useful tool for both clinical and patient. The clinician may be able to better decide the best strategy for each patient, while the patient will understand more easily this information, helping her morally with the treatment.

Trial registration number: 2204-VLC-040-CR

Abstract citation ID: dead093.226

O-185 A clinically robust machine learning model for selecting the first FSH dose during controlled ovarian hyperstimulation: incorporating clinical knowledge to the learning process

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Study question: Can we improve the selection of the first dose of follicle stimulating hormone (FSH) for IVF patients by incorporating clinical evidence with machine learning?

Summary answer: A significant improvement in correct dose prescription is achieved when using a FSH dosing model that integrates a novel machine learning methodology with clinical knowledge.

What is known already: The appropriate selection of the initial FSH dose during controlled ovarian hyperstimulation (COH) is key for ART success. An optimal number of oocytes should be obtained for achieving pregnancy, while also avoiding complications for the patient. While clinical protocols achieve good results for the majority of patients, further refinements in individualized FSH dosing may improve safety while reducing the risk of poor response. Machine learning techniques have been applied to dose/response problems with promising results. However, the observational datasets used to train the models are often incomplete and biased, leading to less clinically robust predictions.

Study design, size, duration: We used a database of 2713 first cycles performed between January 2011 and December 2019, across five clinics to train the model. Predictors included age, body mass index (BMI), anti-Müllerian hormone (AMH) levels and antral follicle count (AFC). First FSH dose and number of mature oocytes retrieved were also recorded. Another 273 cycles performed between January 2020 and September 2021 were used for validation. Dosing was performed by 41 clinicians (mean 12 years of experience).

Participants/materials, setting, methods: The model was developed in Python and trained while incorporating available clinical evidence. The relationship between FSH dose and response (number of mature oocytes) was specified by assuming a positive linear function. Rules were incorporated to penalize dose changes that resulted in poorer outcomes compared to the clinician, and to discourage large dose modifications by the model. These same rules were applied during the validation phase. Comparisons were assessed by Chi-squared test with Bonferroni correction.

Main results and the role of chance: Mean maternal age was 37.1 ± 4.9 years. Patients had a mean BMI of 23.8 ± 4.2, AFC of 11.9 ± 7.7 and AMH of 2.4 ± 2.3. They were prescribed a first dose of 247.0 ± 59.0 IU of FSH and obtained 7.3 ± 5.3 mature oocytes after pick-up. An optimal outcome was defined as 10-15 mature oocytes retrieved, and 300 IU was the maximum FSH dose allowed. In the validation dataset, we observed that 22.9% patients could have received a higher FSH dose (<10 mature oocytes collected), 69.3% were correctly prescribed (10-15 mature oocytes or no change in dose was required), while 7.9% needed a decrease in FSH dose (>15 mature oocytes). In the machine learning model 9.4% of patients required a dose increase, 80.5% were correctly prescribed and 7.9% needed a decrease in dose. Accordingly, the model rescued 11.6% of patients that would have otherwise achieved suboptimal outcomes. Ultimately, when compared to the clinicians, the model significantly reduced the number of patients that required a dose increase, and significantly improved the number of patients that were prescribed a correct dose ($p < 0.05$).

Limitations, reasons for caution: The model assigned a non-conservative dose increase to 2.3% of patients, potentially leading to a risk of ovarian hyperstimulation syndrome. Moreover, hyper-responders were underrepresented in the database, resulting in no significant improvements in the dose/outcome relationship for this subgroup.

Wider implications of the findings: Coding clinical knowledge into the training of clinically relevant machine learning models ensured adherence to scientific evidence, and improved model performance while making conservative changes. This approach is critical for the safe and robust clinical implementation of interventional models.

Trial registration number: not applicable

Abstract citation ID: dead093.227

O-186 The implementation of a novel algorithm for monitoring ovulation in an IVF mixed protocol may simplify stimulation monitoring

J. Ben-Guigui[1], R. Hemmings[2], F. Bissonnette[3], I.J. Kadoch[2]

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Study question: Which patients are eligible to skip ovulation monitoring before day 10 in In Vitro Fertilization (IVF) and what are the financial implications?

Summary answer: Thanks to our innovative gonadotropin prescription algorithm, patients with low ovarian reserve and maximum ovarian stimulation may not require monitoring until day 10 of IVF.

What is known already: In most fertility clinics, the standard procedure for IVF is early monitoring on day 6 of ovarian stimulation. Our focus is on personalizing the stimulation protocol by identifying the optimal dose of gonadotropin. With the aid of our unique algorithm, we aim to streamline monitoring. Frequent ultrasound examinations required for IVF can impose restrictions and create a burden for patients, resulting in increased costs for both patients and physicians.

Study design, size, duration: In a retrospective study conducted from April 2021 to August 2022 at the OVO clinic in Montreal (Canada), participants included all patients over 18 years old who underwent an IVF stimulation cycle utilizing an antagonist protocol and a customized mixed protocol prescription consisting of follitrophin delta and human menopausal gonadotropin, based on the patient's weight and anti-Mullerian hormone level. The sudy was approved by Veritas IRB, an independent ethic committee. Tracking number of study :2023-3189-13659-2

Participants/materials, setting, methods: In the study, gonadotropin dosages were assigned either based on our algorithm (group 1) or at the physician's discretion (group 2). Ultrasound and hormonal analysis were performed on day 6. Following the physician's evaluation, adjustments to the gonadotropin dosage were made if necessary. The common trigger criteria was the presence of at least three follicles measuring between 16 and 22mm.

Main results and the role of chance: The study included 714 patients meeting the inclusion criteria. In group 1, there was a high percentage of patients stimulated with the maximum doses (80.3% compared to 22.5% in group 2). No dosage modifications or incidents of ovarian hyperstimulation syndrome were observed in group 1. Only 4.5% of patients ($n = 16$) triggered ovulation before day 10 (on day 9). In the non-maximal dose population, patients treated according to the algorithm (group A) experienced significantly fewer changes to the gonadotropin dosage at day 6 compared to patients treated based on the physician's discretion only (group B) (24.6% vs 46.9%, $p < 0.001$). The dosage of gonadotropins was notably more frequent adjusted in group B (46.9% vs 24.6%, $p < 0.001$). A higher risk of ovarian hyperstimulation syndrome was significantly more frequent in the non-maximal dose population compared to the maximal dose population (64.6% vs 27.5%, $p < 0.01$). These findings suggest that ovulation monitoring may not be necessary in the population stimulated to the maximum dose. This could result in 478 ultrasound and blood tests being avoided in the context of this study.

Limitations, reasons for caution: The data was obtained through a retrospective analysis. However, not monitoring before day 10 could pose a risk of delaying the cancellation of the IVF cycle, resulting in unnecessary treatment continuation.

Wider implications of the findings: This significant study indicates that, thanks to our algorithm, monitoring only on day 10 would be adequate for patients with low ovarian reserve who are stimulated to the maximum dose. Further validation of this strategy can be conducted through a randomized prospective study or with the use of different gonadotropins.

Trial registration number: non applicable

Abstract citation ID: dead093.228

O-187 The combination of machine learning and pharmacogenetics for the prediction of sub-optimal ovarian response

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Study question: Can an artificial intelligence (AI)-based model predict the risk of suboptimal ovarian response using patient genetic data?

Summary answer: AI-based predictive models can identify relevant predicting factors for suboptimal ovarian response, including several genetic variants.

What is known already: A substantial proportion of women classified as "normo-responders" may not reach the optimal range of oocytes retrieved after COS. Polyzos and Sunkara (2015) defined a new category of women (15-36%) with a worse prognosis and a "suboptimal" level of ovarian response in which the number of oocytes retrieved ranges between 4 and 9 oocytes.

The explanation for this behavior has been suggested to be genetic and indeed variants in FSH receptor and LH subunit-B have been identified that predispose to lower ovarian sensitivity to stimulation. Therefore, these women might require higher doses of gonadotropins or longer stimulations.

Study design, size, duration: This observational study included a retrospective analysis of 1370 ovarian stimulations from egg donors performed between March 2018 and April 2022. The oocyte donor candidates were selected according to our clinic donation program requirements and ASRM and ESHRE guidelines for oocyte donation. They were stimulated following a progesterone-primed ovarian stimulation protocol. All donors started stimulation with 150-300 IU/day of FSH according to AFC and BMI. A GnRH agonist was used for final oocyte maturation.

Participants/materials, setting, methods: Oocyte donors were healthy women between 18 and 35 years old (n = 504). In order to establish the predictive machine learning models, patient characteristics and controlled ovarian stimulation data were recorded in a data frame. In addition, donors were genotyped for 31 variants corresponding to 16 genes associated with ovarian response.

The association between the different variables and risk of suboptimal ovarian response was analysed using SPSS (v23.0) and R (v. 4.2.0) statistical software.

Main results and the role of chance: The oocyte donors had a mean age of 25.4 ± 4.0 and a high ovarian reserve (AFC: 18.4 ± 7). The mean number of oocytes retrieved after ovarian stimulation was 16.3 ± 7.5, of which 13.1 ± 6.4 were mature. Despite being young women with good ovarian reserve, 16.4% of the stimulations were suboptimal (4-9 oocytes retrieved).

Classical statistical method (binary logistic regression) and 5 different supervised classification machine learning algorithms (multi-layer perceptron, support vector-machines, k-nearst neighbors, random forest and eXtreme Gradient Boosting (XGBoost)) were used to establish a prediction model for suboptimal ovarian response.

The model with the highest AUC value was the XGBoost (0.876). The accuracy, sensitivity and specificity were 0.798, 0.809 and 0.787 respectively. The variables that had the greatest predictive power in the best machine learning algorithm (XGBoost) were: age, BMI, AFC and total gonadotrophin dose. In addition, four genetic variants were identified that modified the risk of a suboptimal ovarian response:

-*ESR2* (oestrogen receptor; c.*39G>A).

-*LHB* (LH beta subunit; c.82T>C/p.Trp28Arg).

-SOD2 (superoxide dismutase; c.47T>C/p.Val16Ala). A mitochondrial enzyme that catalyses the detoxification and protection against redox damage that can occur in COS.

-and TP53 (tumour suppressor protein; c.215C>T/p.Pro72Leu) that exerts a protective effect on DNA damage in folliculogenesis.

Limitations, reasons for caution: Our investigation was performed with retrospectively collected data, and hence it will be of importance to collect data prospectively to confirm that the new identified variants are associated with suboptimal ovarian response also in the IVF population.

Wider implications of the findings: The combination of AI and pharmacogenetics has led to the identification of genetic variants that might predispose to suboptimal ovarian response. Women who carry these variants (erroneously considered as normo-responders) could be candidates for personalization of their ovarian stimulation treatment with higher doses of gonadotrophins and/or longer stimulations.

Trial registration number: Not applicable

SELECTED ORAL COMMUNICATIONS

SESSION 61: NOVEL DIAGNOSTIC AND THERAPEUTIC PATHS FOR UTERINE FIBROIDS

Tuesday 27 June 2023 Hall D1 15:15 - 16:30

Abstract citation ID: dead093.229

O-188 Decoding uterine leiomyoma tumorigenesis using single-cell transcriptomics and single-cell proteomics

A. Machado-Lopez[1], R. Pérez-Moraga[1], P. Punzón-Jiménez[1], J. Llera-Oyola[1], M. Gálvez-Viedma[1], D. Grases[2], P. Aragon-Fernandez[3], E. Satorres[4], B. Roson[1], E.M. Schoof[3], E. Porta-Pardo[2], C. Simón[1], A. Mas[1]

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Study question: Can single-cell transcriptomics and proteomics contribute to a better understanding uterine of leiomyoma tumorigenesis?

Summary answer: We demonstrate the significant involvement of the MAPK, PI3K-Akt, and proteoglycan pathways in smooth muscle, endothelial and perivascular cells in leiomyoma tumorigenesis.

What is known already: Uterine leiomyomas (LM), also known as fibroids, are benign tumors of the uterus that arise from the myometrium. Previous studies have shown cellular heterogeneity in both myometrium and uterine leiomyomas, although cell spatial location within the tissue has not been shown. Further, no data describing single-cell-level proteomic differences and key pathways involved in uterine LM tumorigenesis have yet been reported.

Study design, size, duration: A prospective, observational, and biomedical study of cohorts was conducted at Hospital La Fe (Valencia, Spain) for one year. Single-cell RNAseq (scRNA-seq; n = 16) and single-cell proteomic (scP; n = 16) analyses were performed on eight sample pairs of LM and matched myometrium (MM), to generate a high-resolution transcriptomic and proteomic map decoupled from cell type, state, and spatial location.

Participants/materials, setting, methods: After obtaining informed consent, LM and MM samples were collected from eight patients between 35-50 years undergoing hysterectomies. Part of the samples were preserved in paraffin for spatial transcriptomics using VISIUM (10x Genomics). While the remaining tissues were dissociated into single-cell suspensions and subjected to Chromium Controller and Orbitrap Eclipse Tribid mass spectrometry for scRNA-seq and scP, respectively. All data were analyzed using publicly available R/Python tools.

Main results and the role of chance: After restrictive quality control filtering, we analyzed a total of 52,599 and 5,909 cells by scRNA-seq and scP, respectively. While LM and MM possessed similarities in terms of cellular composition, they displayed differential expression of genes and proteins across all the cell populations studied, particularly in smooth muscle, endothelial and perivascular cells. In LM samples, these cell populations displayed impaired MAPK signaling, which acts as a signal integrator for growth factors, estrogen, and vitamin D. We also observed alterations in the PI3K-Akt and

proteoglycan pathways in LM smooth muscle and perivascular clusters, which relate to cell proliferation and tumor growth. Additionally, we encountered a subset of consistently dysregulated genes in all LM populations, which may suggest the existence of a shared tumorigenic pathway independent of cell type. Spatial transcriptomics further demonstrated relationships between cells and their relative locations within the tissue, which we validated by immunofluorescence. Together, our results highlight the relevance of specific cell populations in LM tumorigenesis.

Limitations, reasons for caution: This study involved a sample cohort limited to Caucasian women; therefore, further studies including more patients, and addressing racial disparities will help to generalize these findings to a broader population.

Wider implications of the findings: Our work describes an unprecedented transcriptomic and proteomic analysis of LM and MM at single-cell resolution, which supports a novel understanding of myometrial tumorigenesis.

Trial registration number: NCT04214457

Abstract citation ID: dead093.230
O-189 Extracellular vesicles derived from human myometrial cells support endometrial mesenchymal stromal/stem cells

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Study question: Whether other forms of intercellular communication such as extracellular vesicles (EVs) participate in stem cell maintenance in the human endometrium.

Summary answer: The present study revealed the involvement of EVs as a novel intercellular communication method between endometrial mesenchymal stem cells (eMSC) and their surrounding niche.

What is known already: In human endometrium, many studies have demonstrated the importance of EVs in mediating various physiological as well as pathological processes. Our group has demonstrated that the myometrial cells are candidate niche cells of eMSC modulating their biological function. We also demonstrate the Notch signaling pathway is involved in endometrial stem cell regulation. Although classical Notch signaling relies on direct cell contact dependent interactions, this pathway can also be activated at a distance by EVs containing Notch ligands. Therefore, we hypothesized that certain Notch ligand(s) are packaged into the myometrial EVs to mediate stem cell functions.

Study design, size, duration: Sequential beading with magnetic beads coated with anti-CD140b and anti-CD146 antibodies was used to isolate eMSC ($CD140b^+CD146^+$ cells) from endometrial tissues from proliferative (n = 15) and secretory (n = 10) phase. Myometrial derived EVs (n = 17) were isolated by ultracentrifugation. The EVs were characterized by transmission electron microscopy and western blotting.

Participants/materials, setting, methods: Endometrial samples obtained from women aged 41 – 52 years undergoing total abdominal hysterectomy. EMSC were cocultured with myometrial EVs and the percentage of eMSC was analysed by flow cytometry. Blockage the secretion of EVs was performed by transfection of Rab27a siRNA. Western blot analysis and gene silencing approach validated the role of Notch signaling in eMSC. The therapeutic features of transplanted eMSC/myometrial EVs was determined using the mouse injured endometrial model.

Main results and the role of chance: EVs released from myometrial cells can be internalized by eMSC, leading to a significant effect on stemness of eMSC. Pharmacological inhibition of Notch signaling with DAPT or silencing of Notch 1 nullified these stimulatory effects. Myometrial EVs contains a high amount of the notch ligand – JAG1, thus inducing a strong Notch activity in eMSC. When JAG1 was silenced in the myometrial EVs, the self-renewal and clonogenic activity was reduced. Combined transplantation of eMSC with myometrial EVs improves the therapeutic effect of eMSC in endometrial regeneration in vivo. The observed therapeutic feature was potentially achieved by elevating the cell proliferation and suppressing apoptosis in the injured mouse endometrium.

Limitations, reasons for caution: Whether other signaling pathway are involved in the stimulatory effect of myometrial EVs in eMSC activities remains unknown. Also, EVs derived from other endometrial cell types i.e. epithelial cells may also regulate eMSC function.

Wider implications of the findings: Our findings revealed that myometrial EVs stimulated the biological function of eMSC via the Notch signaling. In the near future, it would be important to explore whether Notch signaling pathway plays a role in the injured mouse endometrium transplanted with eMSC/myometrial EVs.

Trial registration number: Not applicable

Abstract citation ID: dead093.231
O-190 Establishment of non-invasive prediction models for diagnosis of the subtypes and tissue composition of uterine leiomyomas by machine learning using MRI data

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Study question: We investigated whether machine learning models using MRI data can predict the subtypes and tissue composition of uterine leiomyomas.

Summary answer: Our machine learning models using MRI data were able to predict the subtypes and tissue composition of uterine leiomyomas with high accuracy.

What is known already: Recently, somatic mutations in the Mediator complex subunit 12 (MED12) gene were found to be a biomarker of uterine leiomyomas, which is detected in about 70% of uterine leiomyomas. Uterine leiomyomas are classified into two subtypes with or without the MED12 mutation. These subtypes differ in the ratio of smooth muscle cells and fibroblasts and in the amount of collagen fibers. In addition, sensitivities to female hormones differ between smooth muscle cells and fibroblasts. Thus, the effect of therapeutic drugs (GnRH analogs and selective progesterone receptor modulators) may differ depending on the subtypes and tissue composition of uterine leiomyomas.

Study design, size, duration: We analyzed 90 uterine leiomyoma nodules (MED12 mutation-positive and negative = 62 and 28) obtained from 51 women who underwent surgery at our hospital between 2020 and 2022. Seventy-one uterine leiomyomas (MED12 mutation-positive and negative = 49 and 22) were assigned to the primary dataset to establish the prediction models. Nineteen uterine leiomyomas (MED12 mutation-positive and negative = 13 and 6) were assigned to the test dataset to validate the prediction model utility.

Participants/materials, setting, methods: For each leiomyoma, the tumor signal intensity was quantified by five MRI sequences (T2WI, ADC, T1map, T2*BOLD, MTC) for evaluating the collagen amount. After surgery, genotyping of MED12 was examined and Trichrome staining was performed to quantify the collagen amount. Using these results, we established the prediction models based on machine learning by applying support vector classification and logistic regression for subtype prediction, and support vector regression and Ridge regression for tissue composition prediction.

Main results and the role of chance: The signal intensity of all five MRI sequences differed significantly between the subtypes. The cross-validation within the primary dataset showed that support vector classification and logistic regression models using five MRI sequences and MED12 genotyping data were highly predictive of the subtypes (AUC: 0.984 and 0.995, respectively). The validation using the test dataset showed that both models were able to predict the subtypes for all uterine leiomyomas (AUC: 1.000, both). This result showed higher accuracy than each MRI sequence's cut-off value alone. On the other hand, four MRI sequence values (other than ADC) showed a significant correlation with the collagen amount. However, to improve

accuracy, we added the ADC data and the result of the subtype predicted by the preceding models to the predictors for the collagen amount. Support vector regression and Ridge regression models using five MRI sequences and Trichrome staining data were shown to be highly predictive of the collagen amount in cross-validation within the primary dataset (R2: 0.570 and 0.525, respectively) and in validation using the test dataset (R2: 0.648 and 0.675, respectively). Moreover, the prediction models using only T2WI and ADC, which are taken in general clinical practice, were as accurate as using five MRI sequences.

Limitations, reasons for caution: In this study, we used limited data by using one specific MRI machine in a single center to establish the prediction models. We have not verified that similar results would be obtained under different conditions. Therefore, further training and evaluation in larger cohorts are required for clinical use.

Wider implications of the findings: The prediction models established in this study may be useful in clinical support such as predicting the effect of drug therapy and selecting therapeutic drugs, such as GnRH analogs and selective progesterone receptor modulators. Their utilities will be confirmed by validation using our prediction models in studies involving drug therapy.

Trial registration number: not applicable

Abstract citation ID: dead093.232

O-191 Effects of relugolix combination therapy (Relugolix-CT) on uterine fibroid (UF) and uterine volume through 52 weeks: post hoc analysis of the LIBERTY Long-Term Extension study

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Study question: How does baseline UF and uterine volume impact the proportion of women achieving meaningful UF and uterine volume reductions with Relugolix-CT through 52 weeks?

Summary answer: Women with larger UF and uterine volumes at baseline may be more likely to experience clinically meaningful reductions of UF and uterine volume with Relugolix-CT.

What is known already: In the randomised, Phase 3 LIBERTY 1 and 2 pivotal studies, the efficacy and safety of once-daily Relugolix-CT (relugolix 40 mg, estradiol 1 mg, norethisterone acetate 0.5 mg) were demonstrated in premenopausal women (aged 18–50 years), with a significant reduction in UF-associated heavy menstrual bleeding (HMB) vs placebo, and preserved bone mineral density (BMD) through 24 weeks. The open-label, 28-week LIBERTY Long-Term Extension (LTE) study continued to demonstrate sustained menstrual blood loss reductions and preservation of BMD, alongside reductions in UF and uterine volume.

Study design, size, duration: The LIBERTY 1 and 2 studies of Relugolix-CT were conducted in 770 premenopausal women with UF-associated HMB. Women were randomised 1:1:1 to receive Relugolix-CT or placebo for 24 weeks, or delayed Relugolix-CT (relugolix 40 mg alone for 12 weeks, then Relugolix-CT for 12 weeks). Women who completed the pivotal trials were eligible to participate in the LIBERTY LTE study, receiving open-label Relugolix-CT for an additional 28 weeks.

Participants/materials, setting, methods: Endpoints of the LTE included change from baseline to Week 52 in UF and uterine volume. This *post hoc* analysis assessed the proportion of women who experienced a clinically meaningful reduction in UF or uterine volume of $>25\%$ or $>50\%$ through 52 weeks of Relugolix-CT. Subgroup analyses were performed to descriptively summarise the impact of UF and uterine volume at baseline ($<25cm^3$ or $\geq 25cm^3$; $<300cm^3$ or $\geq 300cm^3$, respectively) on change in UF and uterine volume.

Main results and the role of chance: In total, 477 women enrolled in the LTE, 363 completed 52 weeks of treatment, and 163 received Relugolix-CT continuously up to 52 weeks. At baseline, the mean (standard deviation) volume of the largest UF was 80.0 (145.1) cm^3; mean (SD) uterine volume was 386.7 (320.5) cm^3 in the Relugolix-CT group.

At Week 24, 52.6% and 30.3% of women in the Relugolix-CT group experienced $>25\%$ and $>50\%$ reduction in UF volume, respectively. These proportions increased to 61.1% and 36.6%, respectively, at Week 52. For uterine volume, 36.1% and 4.5% experienced a $>25\%$ and $>50\%$ reduction at Week 24, increasing to 41.2% and 9.6% at Week 52.

At Week 52, of 81 women with a baseline UF volume of $<25cm^3$, 52.4% and 27.0% experienced a $>25\%$ and $>50\%$ reduction in UF volume, respectively; 69.1% and 45.6% of 81 women with a baseline UF volume of $\geq 25cm^3$ experienced $>25\%$ and $>50\%$ reductions in UF volume, respectively.

At Week 52, of 96 women with a baseline uterine volume of $<300cm^3$, 38.5% and 6.4% experienced a $>25\%$ and $>50\%$ reduction in uterine volume; 44.8% and 13.8% of 67 women with a baseline uterine volume of $\geq 300cm^3$ experienced $>25\%$ and $>50\%$ reductions in uterine volume.

Limitations, reasons for caution: The study was conducted as an open-label study without a control group over the 28 weeks of the extension period. The present data are from a *post hoc* analysis of LIBERTY data without predefined criteria.

Wider implications of the findings: Treatment with Relugolix-CT led to a substantial reduction of UF and uterine volume in a large proportion of women with UF-associated HMB. Women with larger UF and uterine volume at baseline may be more likely to experience substantial reductions of UF and uterine volume.

Trial registration number: NCT03049735, NCT03103087, NCT03412890

Abstract citation ID: dead093.233

O-192 Does tubal flushing by hysterosalpingo-foam sonography and hysterosalpingography affect tubal patency? Results from a randomized clinical trial

D. Kamphuis[1], N. Van Welie[1], M. Van Hooff[2], H. Verhoeve[3], J.P. De Bruin[4], F. Mol[5], M. Van Baal[6], B. Van de Laar[3], C. Lambalk[1], J. Stoker[7], M. Van Wely[5], P. Bossuyt[8], B. Mol[9], K. Dreyer[1], V. Mijatovic[1]

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Study question: Does tubal flushing by hysterosalpingo-foam-sonography (HyFoSy) or hysterosalpingography (HSG) affect tubal patency in infertile women?

Summary answer: Tubal flushing by HyFoSy and HSG does not increase the number of women with visible bilateral tubal patency.

What is known already: HyFoSy and HSG are two frequently used methods to visualize tubal patency by flushing a contrast fluid through the Fallopian tubes, while the uterus and Fallopian tubes are imaged using respectively fluoroscopy and ultrasound. Tubal flushing by HSG, specifically with oil-based contrast, improves live birth rates in infertile women. The mechanism of this fertility-enhancing effect is still not completely understood. The potential fertility-enhancing effect of other tubal flushing methods, for example HyFoSy, are studied less frequently. Here, we investigated whether tubal flushing affect tubal patency, and therefore could explain the fertility-enhancing effect of tubal flushing.

Study design, size, duration: This is a secondary analysis of the FOAM-trial, a multi-center RCT in which women were assigned to undergo tubal flushing by HSG and HyFoSy in randomized order. They either had HyFoSy first and then HSG or the other way around. Here, we assess whether tubal flushing by either HSG or HyFoSy led to differences in tubal patency. We also investigate whether the type of contrast used during HSG (oil -and water based) influenced tubal patency.

Participants/materials, setting, methods: We studied infertile women with indication for tubal patency testing. Women with anovulatory cycles, endometriosis or with a partner with male infertility were excluded. The main outcome was the number of women with bilateral tubal patency. This outcome, was compared in two analyses: 1) Tubal flushing by HyFoSy versus no flushing, with HSG as reference test, and 2) Tubal flushing by HSG (with oil – or water contrast) versus no flushing, with HyFoSy as reference test.

Main results and the role of chance: Between May 2015 and January 2019, 1,160 women were included. There were 957 women who underwent HyFoSy with interpretable results and 1,081 women who underwent HSG with interpretable results.

Tubal flushing by HyFoSy versus no tubal flushing, with HSG done in all women as reference test, evaluating the effect of tubal flushing by HyFoSy, resulted in a comparable number of women with visible bilateral tubal patency: 467/537 (87%) vs. 472/544 (87%) ; (RR 1.00; 95%CI: 0.96-1.05).

Tubal flushing by HSG (with both water –and oil-based contrast) versus no tubal flushing, with HyFoSy done in all women as reference test, evaluating the effect of tubal flushing by HSG, did not show an increase of women with visible bilateral tubal patency: 394/471 (84%) vs. 428/486 (88%); RR 0.95; (95%CI:0.90-1.00).

Tubal flushing by HSG with oil-based contrast versus no flushing, with HyFoSy as reference test, resulted in a comparable number of women with visible bilateral tubal patency: 283/330 (86%) vs. 271/308 (88%); RR 0.97; (95%CI:0.92-1.04). Tubal flushing by HSG with water-based contrast versus no flushing, with HyFoSy as reference test, did show a decrease of women with visible bilateral tubal patency: 108/137 (79%) vs. 156/171 (91%); RR 0.90 (95%CI:0.83-0.98).

Limitations, reasons for caution: It needs to be noted that subtle improvements of tubal patency, caused by initial tubal flushing with either HyFoSy or HSG, cannot be detected on respectively ultrasound and fluoroscopy images and were therefore not assessed in this study.

Wider implications of the findings: The therapeutic effect of tubal flushing cannot be explained by dissolving visible obstruction in the Fallopian tubes. This suggests that the therapeutic effect of tubal flushing mainly applies to women with anatomically normal Fallopian tubes.

Trial registration number: NTR4746

SELECTED ORAL COMMUNICATIONS

SESSION 62: IS PLOIDY DETECTION OUR STRONGEST TOOL IN REPRODUCTIVE MEDICINE?

Tuesday 27 June 2023 Hall D4 15:15 - 16:30

Abstract citation ID: dead093.234

O-193 Opening the black box: why do euploid blastocysts fail to implant? A systematic review and meta-analysis

D. Cimadomo[1], L. Rienzi[12], A. Conforti[3], E. Forman[4], S. Canosa[5], F. Innocenti[1], M. Poli[67], J. Hynes[4], L. Gemmell[4], A. Vaiarelli[1], C. Alviggi[3], F.M. Ubaldi[1], A. Capalbo[7]

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Study question: What are the embryonic, maternal, paternal, clinical and laboratory features associated with the live-birth-rate (LBR) after euploid blastocyst transfer?

Summary answer: Delayed blastulation, poor blastocyst quality, maternal age ≥38 years, obesity, previous repeated-implantation-failure (RIF), poor or multiple manipulations may reduce the LBR per euploid blastocyst transfer.

What is known already: Euploidy assessed at the blastocyst stage through comprehensive-chromosome-testing (CCT) technologies during IVF cycles with preimplantation genetic testing for aneuploidies (PGT-A) represents the strongest predictor of embryo implantation potential. Nevertheless, its positive predictive value hardly exceeds 50-60%. The current gap of knowledge on the causes of euploid blastocysts' reproductive failure is known as "the black box of implantation".

Study design, size, duration: Systematic search conducted up to August-2021 with the keywords "(blastocyst OR day 5 embryo OR day 6 embryo OR day 7 embryo) AND (euploid OR chromosomally normal OR preimplantation genetic testing) AND (implantation OR implantation failure OR miscarriage OR abortion OR live birth OR biochemical pregnancy OR recurrent implantation failure)". Among 1608 items, prospective/retrospective studies and randomized controlled trials (RCTs) investigating associations with LBR and/or miscarriage rates (MR) after non-mosaic euploid blastocyst transfers were scrutinized.

Participants/materials, setting, methods: 335 retrospective, 30 prospective papers and 7 RCTs were included. PRISMA guideline, PICO model, and ROBINS-I and ROB 2.0 scoring for bias, were adopted. Funnel plots and trim and fill methods were used to visually inspect bias regarding the LBR. Categorical data were combined with a pooled-OR. The random-effect model was used for meta-analyses. Between-study heterogeneity was addressed using I^2. Whenever not suitable for the meta-analysis, the included studies were simply described for their results.

Main results and the role of chance: Inner-cell-mass C-grade (7 studies, OR:0.37, 95%CI:0.27-0.52, $p < 0.01$), trophectoderm C-grade (9 studies, OR:0.53, 95%CI:0.43-0.67, $p < 0.01$), overall blastocyst quality <BB (8 studies, OR:0.4, 95%CI:0.24-0.67, $p < 0.01$) developmental delay (18 studies, OR:0.56, 95%CI 0.49-0.63, $p < 0.01$) and, by qualitative analysis, some morphodynamic abnormalities pinpointed through time-lapse microscopy (abnormal cleavages, spontaneous collapse, longer tM, tB, and blastulation) were all associated with poorer reproductive outcomes. Slightly lower LBR was reported for women ≥38 years (7 studies, OR:0.87, 95%CI:0.85-1.00, $p = 0.05$), while maternal BMI>30 involved both lower LBR (2 studies, OR:0.66, 95%CI:0.55-0.79, $p < 0.01$) and higher MR (2 studies, OR:1.8, 95%CI:1.08-2.99, $p = 0.02$). The experience of previous RIF was also associated with lower LBR (3 studies, OR:0.72, 95%CI:0.55-0.93, $p = 0.01$). By qualitative analysis, abnormal progesterone level prior to transfer impaired LBR and MR. Among clinical policies, vitrified-warmed transfer was more effective than fresh (2 studies, OR:1.56, 95%CI:1.05-2.33, $p = 0.03$). Lastly, multiple vitrification-warming cycles (2 studies, OR:0.41, 95%CI 0.22-0.77, $p < 0.01$) and, by qualitative analysis, high biopsy cellularity may slightly impact the LBR, while simultaneous zona opening and biopsy protocol showed higher LBR than the day3 hatching-based protocol (3 studies, OR:1.41, 95%CI:1.18-1.69).

Limitations, reasons for caution: The associations outlined mostly issue from retrospective and/or few studies or studies with a limited sample size, therefore the level of evidence is low or very low. Moreover, some findings represent "prognosis without promise" because the poorer outcome of euploid blastocysts is not clinically actionable (e.g., women ≥38 years).

Wider implications of the findings: Future research should target: (i) the mechanisms involved in reproductive aging beyond *de novo* chromosomal abnormalities, and how environment and nutrition may accelerate or exacerbate their consequences; (ii) uterine evaluation and blastocyst-endometrial dialogue; (iii) standardization of embryo assessment and IVF protocols; (iv) additional (non-)invasive tools for embryo selection.

Trial registration number: http://www.crd.york.ac.uk/PROSPERO/ (registration number CRD42021275329)

Abstract citation ID: dead093.235

O-194 Blastulation timing in embryos is influenced by ploidy and is a predictor of transfer outcome

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Study question: Is there an association of the timing of blastulation (TE biopsy day) on embryo ploidy and pregnancy outcome after frozen embryo transfer?

Summary answer: Blastocysts expanded on day 5 are more likely to be euploid and result in an ongoing pregnancy after single euploid frozen embryo transfer (FET).

What is known already: Embryo selection for ET is commonly based on static morphological parameters for patients undergoing in vitro fertilization (IVF). Embryo quality on the day of biopsy in combination with preimplantation genetic testing (PGT) are considered important predictors for successful implantation. At present, coherent data regarding any association between the timing of blastulation with embryo ploidy and pregnancy outcomes following single FET are lacking. The aim of our study was to investigate the correlation of the culturing method and blastulation day (day 5/6/7) with embryo ploidy and outcomes of matched d5 vs d6 vs d7 FET cycles.

Study design, size, duration: This retrospective cohort study was performed at the CReATe Fertility Centre and its Reproductive Genetics Laboratory, between January 2019-December 2022. 29660 blastocysts from 5302 cycles (3645 patients) were analyzed using high resolution NGS PGT-A (10Mb resolution for copy number variations and 30%-70% for mosaicism). Pregnancy outcome data from 3942 single-FETs were analyzed. Average age of ovum provider was 32.9 ± 5.8 and sperm provider 39.1 ± 6.4. Clinical, laboratory and demographic data were obtained for statistical analysis.

Participants/materials, setting, methods: Embryos were allocated into four groups based on static morphological grading before biopsy on either d5 (n = 8312), d6 (n = 19183) or d7 (n = 2165): Good (>1/2BB), n = 5648, Average (1BB, 2BB), n = 10492, Poor (<1/2 BB) n = 12219 and Early blast, n = 1301. Embryo ploidy in each group and pregnancy outcomes from single euploid FET were compared between matched cycles of day 5 biopsy to days 6 and 7.

Main results and the role of chance: PGT-A analysis of 29660 embryos showed 53.1% were euploid, 33.9% were aneuploid and 13.0% were mosaic. There were significantly higher proportion of euploid embryos among those biopsied on d5 compared to d6 and d7 with average morphology grade (p = 0 O.R.=1.35979; p = 0.008098 O.R.=1.34442), poor (p = 0 O.R.=1.76481; p = 0 O.R.=2.73903) and early grades (p = 0 O.R.=2.71891; p = 0 O.R.=2.74271), but not among embryos with good grade (p = 0.244399 O.R.=1.07276; p = 0.182084 O.R.=1.38189).

Ongoing pregnancy rates were significantly higher for d5 vs d6 biopsied embryos, for all morphology grade groups: good (p = 0.030036 O.R.=1.26598), average (p = 0 O.R.=1.80782), poor (p = 0.000303 O.R.=1.89235) and early blasts (p = 0.049501 O.R.=3.30556). D5 blasts had higher ongoing pregnancy rates compared to d7 among average and poor morphology grade as well as early blast (p = 0.000419 O.R.=8.93182, p = 0.000036 O.R.=7.29464, p = 0.010034, respectively), except among good grade embryos (p = 0.885598 O.R.=0.9028). Continuous culture of embryos in Embryoscope™ versus conventional incubator did not influence the ploidy (n = 24321; n = 4756, respectively) or the pregnancy outcome after FET (n = 3134; n = 752, respectively) while comparing between each day of biopsy.

Limitations, reasons for caution: Although this is the largest sample size study to investigate the association of blastulation with ploidy and pregnancy outcomes, the sample size for d7 embryos is relatively low and results should be confirmed in a bigger study.

Wider implications of the findings: Prioritization of euploid embryos for transfer should consider the timing to blastulation and embryos biopsied on d5 should be considered before those biopsied on d6 or d7. This data has potential to shed light on the impact of normal chromosomal content on pre-implantation embryo development.

Trial registration number: not applicable

Abstract citation ID: dead093.236

O-195 Fetal ploidy status in pregnancy loss evaluated by cell-free fetal DNA in maternal blood versus direct sequencing of the pregnancy tissue

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Study question: Is cffDNA in maternal blood applicable for fetal ploidy evaluation in early pregnancy loss, and what are the strengths and limitations of the method.

Summary answer: With a sensitivity of ~85% for aneuploidies and ~93% specificity, cffDNA-based testing is a robust method for evaluation of fetal ploidy status in PL.

What is known already: Pregnancy loss (PL) is an under investigated condition without precise prognostic models or evident treatments. From previous studies using karyotype and chromosomal microarray analysis it is described that approximately half of PLs are caused by fetal aneuploidy, but international guidelines still refrain from recommending routine genetic evaluation of PL. Traditional fetal cytogenetics are expensive and dependent on collection of pregnancy tissue containing fetal or chorionic tissue to avoid inconclusive results and maternal contamination. Consequently, chromosomal investigation of the lost fetus is lacking in most literature about PL and couples are currently not getting an explanation of their loss.

Study design, size, duration: As a part of the prospective Copenhagen Pregnancy Loss (COPL) cohort, 1000 consecutive women with PL (spontaneous miscarriage, missed miscarriage or anembryonic sac) before GA 22 weeks were recruited between Nov 12, 2020, and May 1, 2022. Results from the first 333 women were used to validate cffDNA-based testing compared with direct sequencing of the collected pregnancy tissue. Additional 667 women were included to evaluate cffDNA performance and result distribution in 1000 PLs

Participants/materials, setting, methods: Blood for cffDNA-based testing were drawn in STRECK © tubes before treatment was initiated or within 24 h after complete passage of pregnancy tissue. Pregnancy tissue was collected from the vacuum system if surgical treated or by home collection if medically treated and classified as fetal, chorionic villi or unknown tissue and sequenced at University of Copenhagen. Genome wide cffDNA-based testing and fetal fraction of DNA by SeqFF was performed at Hvidovre Hospitals NIPT Center.

Main results and the role of chance: Among invited candidates, the participation rate was 73%. Mean maternal age was 33.9 years (SD 5.2). Gestational age at inclusion ranged from 35 days to 149 days measured from last menstrual period with a mean of 70.5 days (SD 16.5), or 10 weeks+1 day. Mean maternal BMI was 24.6 kg/m^2 (SD 4.6) and 233 (23%) conceived after fertility treatment.

In 19 (6%) of the initial 333 women, collection of the pregnancy tissue failed for practical or psychological reasons. DNA was isolated from fetal tissue or chorionic villi in 228 (73%) cases, or from unknown tissue in 86 (27%) cases and thereby at a high risk of being maternal contaminated.

The sensitivity of cffDNA-based testing compared with the pregnancy tissue sequencing result was 85% (95% CI 79–90), specificity of 93% (88–96), accuracy of 89% (85–92), and Cohen's coefficient 0.78 showing substantial agreement.112 (11%) of 1000 total cffDNA analyses were inconclusive due to low fetal fraction (SeqFF<0.015 or < 0.025) or low sequencing quality. In instances of a conclusive result, 446 (50%) were euploid, 405 (46%) were aneuploid, and 37 (4%) contained multiple aneuploidies. The most common abnormal karyotypes identified were trisomy 16 (n = 91), monosomy X (n = 86), and trisomy 22 (n = 41).

Limitations, reasons for caution: The used platform for cffDNA-based testing does not report fetal polyploidy, uniparental disomy, and copy number variants found in approximately 10% of early PLs. Moreover, blood for cffDNA-based testing must be drawn with the pregnancy tissue in situ or within 24 hours limiting the window for testing.

Wider implications of the findings: Considering the difficulties of pregnancy tissue collection, it is relevant to introduce a pregnancy tissue-independent alternative. The validity of cffDNA-based testing for fetal ploidy status found in this study shows the potential of the method to improve clinical management and research in the field of PL.

Trial registration number: H-18024745

Abstract citation ID: dead093.237

O-196 Evidence-based management of mosaic embryos - a single Centre experience from prospective transfer and birth outcomes of 565 mosaic embryos

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Study question: What are the pregnancy and neonatal outcomes of prospective transfer of 565 mosaic embryos and what are the key determinants influencing the outcome?

Summary answer: Mosaic (diploid/aneuploid) embryos have a significant developmental potential with good neonatal outcomes allowing personalized management and evidence based prioritization of embryos for transfer.

What is known already: Since the first report of healthy babies born after mosaic transfer, the gradual accumulation of knowledge on mosaic embryo transfers in the International Registry of Mosaic Embryo Transfers provided the necessary reassurance for clinical management of mosaic embryos. Large and comprehensive data from single centres with consistent clinical genetic counselling and laboratory practices for preimplantation and prenatal testing offer valuable contribution to the current practices. The aim of this study was to evaluate the pregnancy and neonatal outcomes after mosaic embryo transfers and to evaluate for associations between embryo genetic aberrations, static morphology and specific outcomes.

Study design, size, duration: This is a retrospective cohort study that took place in a single academic IVF centre (The CReATe Fertility Centre, Toronto, Canada), where we analysed the results from high resolution PGT-A, embryo morphologic features and pregnancy outcomes of 3529 frozen embryo transfers from 2016-2022. Associated patient demographic data, transfer outcome and neonatal outcome of 2964 euploid and 565 mosaic embryo transfers were analyzed.

Participants/materials, setting, methods: All patients received genetic counselling prior to frozen embryo transfer. High resolution NGS PGT-A was performed using the Illumina platform and BluGnome and NxClinical for data analysis. Clinically relevant findings were aberrations >10Mb and mosaicism from 25%-75%. Comparison of clinical and morphological characteristics of euploid, low-level mosaic (25%-<50% aneuploid cells in trophectoderm biopsy), and high-level mosaics (50% -75%) was performed with R-statistical package, Mathlab and SSPS software. $p < 0.05$ with CI 95% was considered significant.

Main results and the role of chance: Of the total 565 mosaic embryos transferred 77.1% had mosaicism levels ≥25%-<50% (Group1-n=436): 54.8% had segmental chromosome mosaic (SCM)gain/loss, 45.2% had whole chromosome mosaicism(WCM)(trisomy/monosomy); and 22.9% had mosaic levels of ≥ 50-<70% (Group2-n=129)(50.4% with SCM and 49.6% with WCM). The overall ongoing pregnancy rate(OPR) for mosaic embryos was 33% and the miscarriage rate was 6%.

Compared with euploid embryos, mosaic embryos have significantly lower implantation (p = 0,OD0.62-CI[0.5-0.7]) and OPR(p = 0,OD0.5-CI[0.4-0.6]). Pregnancy outcomes are determined by level of mosaicism: Group1 have better implantation(p = 0.005,OD1.8-CI[1.18-2.78]) and OPR compared to Group2. High level of mosaicism independently affected the OPR of SCM(p = 0.003) and WCM(p = 0.02) embryos. WCM had lower implantation rates compared to SCM (p = 0.005,OD1.6-CI[1.15-2.27]). Mosaic monosomies had the lowest impanation and OPR of all mosaic embryos (14% and 8%,respectively). Within Group1 and Group2 good static morphology was associated with higher OPR for SCM and WCM embryos. Birth outcomes were available for 118 babies from mosaic-FET and from 802 euploid-FET. There is no difference in duration of pregnancy and birth weight between mosaic and euploid-FET. No gross-fetal anomalies were reported in the mosaic group at birth. There was one termination<20GW due to fetal anomalies and one stillbirth. For euploid transfers, 11 had fetal anomalies, there were 7-terminations and 2-stillbirths.

Limitations, reasons for caution: Although this is the largest single centre study to date that evaluated genomic, morphology and outcome data of mosaic transfers, the numbers are still low to analyze the impact of specific chromosomal aberrations on pregnancy outcome.

Wider implications of the findings: Mosaic embryos should be considered for transfer and prioritized based on the level of mosaicism, type of chromosomal aberration and static morphology. The implantation potential is lower than euploid embryos however established pregnancies result in healthy babies born at term and normal birth weight.

Trial registration number: CReATe Fertility Centre, Toronto, Canada

Abstract citation ID: dead093.238

O-197 Vertical transmission of maternal mitochondrial DNA via extracellular vesicles (EVs) modulates embryo bioenergetics during the periconceptional period

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Study question: Does maternal endometrial mitochondrial (mt) DNA cargo of EVs modulate embryo bioenergetics during embryo implantation?

Summary answer: We demonstrate the vertical transmission of maternal mtDNA within endometrial-derived EVs and their uptake by the trophoblast which reduces mitochondrial respiration and ATP production.

What is known already: The release and uptake of membrane-enclosed compartments with specific cargos, commonly known as EVs, represents a novel cell-to-cell communication mechanism in physiological and pathogenic conditions. EVs are generally classified into three populations based on their biogenetic pathways, composition, and physical characteristics: apoptotic bodies (ABs), microvesicles (MVs), and exosomes (EXOs). Among other contents, EVs contain single- and double-stranded DNA, with their relative abundance varying depending on the cell and vesicle type. The vertical

transmission of EV-associated DNA has been proposed as a novel genetic material transfer mechanism that may impact genome evolution and tumorigenesis.

Study design, size, duration: Prospective observational multicenter analysis in which EVs were obtained from endometrial fluid from healthy donors aged 18–35 years (n = 10) during the receptive phase of their natural cycle and under hormonal replacement therapy in pre-receptive (P + 2), receptive (P + 5), post-receptive (P + 8) stages (n = 13).

Participants/materials, setting, methods: Endometrial EVs isolated using ultracentrifugation and classified according to `parameters obtained from electron microscopy, Western blotting, and size distribution analysis. DNA copy number identified using high throughput sequencing. EV-associated DNA tagged with 5-ethynyl-2'-deoxyuridine was followed by confocal imaging after co-incubation of EVs with murine embryos (n = 200). ATP levels assessed using the FLASC luciferase reporter system, and the Seahorse XFE96 extracellular flux analyzer used to measure embryo oxygen consumption rate (OCR) (n = 400).

Main results and the role of chance: The human endometrium secretes all three EV types - ABs, MVs, and EXOs - into the human endometrial fluid. Deep sequencing revealed that EVs encapsulated nuclear and mtDNA. When analyzing endometrial biopsies, we observed the reduced mtDNA content of endometrial cells and the activation of mitochondrial clearance mechanisms, which coincided with the time of embryo implantation together with specific enrichment in endometrial MVs secreted during the periconceptional period. EVs were internalized and DNA was transferred to the cytoplasm and nuclei of trophectoderm of murine embryos. We analyzed ATP concentrations in murine embryos and found a significant reduction in ATP levels following the coculture of embryos with a combination of all EVs types compared to control embryos cultured without endometrial EVs ($p < 0.001$). Finally, we demonstrated a reduction in the OCR in embryos treated with endometrial EVs obtained during the receptive phase compared with the pre-receptive phase. In conclusion, maternal EVs modulate the bioenergetics of the preimplantation embryo by increasing the embryo's metabolic rate and oxygen consumption during the periconceptional period.

Limitations, reasons for caution: These results were obtained using a combination of a human endometrial model and a murine embryo model.

Wider implications of the findings: Our results suggest that the vertical transmission of maternal mtDNA encapsulated within EVs to the trophectoderm might energetically assist the preimplantation embryo through the implantation process.

Trial registration number: N/A

SELECTED ORAL COMMUNICATIONS

SESSION 63: OUTCOMES OF ART CHILDREN

Tuesday 27 June 2023 Auditorium 10-12 15:15 - 16:30

Abstract citation ID: dead093.239

O-198 Obstetric and neonatal outcomes after natural frozen embryo transfer; results from a follow-up study of the Antarctica-2 RCT

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Study question: Do obstetric and neonatal outcomes of women who conceived through home-based monitoring of the ovulation in natural cycle frozen embryo transfer (NC-FET) differ compared to hospital-controlled monitoring of the ovulation in NC-FET using a hCG trigger?

Summary answer: We found no differences in obstetric and neonatal outcomes when home-based monitoring versus hospital-controlled monitoring of the ovulation in women undergoing NC-FET.

What is known already: NC-FET is associated with lower risk of adverse obstetric and neonatal outcomes compared with artificial cycle FET. The question of how to prepare the endometrium for FET, has now gained even more importance and taken on the dimension of safety into account as it should not simply be reduced to the basic question of effectiveness.

Study design, size, duration: Between April 10, 2018 and April 13, 2022, 1464 women were included in the Antarctica-2 RCT (n = 732 home-based monitoring and n = 732 hospital-controlled monitoring). For this study we performed a follow-up study in order to investigate obstetric and neonatal outcomes of the participating women with live births after the Antarctica-2 trial.

Participants/materials, setting, methods: We included only singleton livebirths. Main outcomes were gestational age, preterm birth (PTB), very preterm birth (very PTB), birth weight, large for gestational age (LGA) and small for gestational age (SGA). Additional outcomes included: gestational diabetes (GDM), hypertensive-disorders-of-pregnancy (HDP), pre-eclampsia (PE), abnormal placentation (including placenta previa and accrete), hospital admission during pregnancy, congenital anomalies and neonatal death. We calculated risk ratios and absolute risk differences (95% CI) using per protocol analysis.

Main results and the role of chance: Both studyarms resulted in similar live birth rates. Singleton live birth occured in 146 of 732 women (19.9%) following home-based and in 148 of 732 women (20.2%) following hospital-controlled monitoring. The mean gestational age in weeks was 39.0 (SD 0.3) for the home-based monitoring group versus 39.3 (SD 0.2) for the hospital-controlled monitoring group. We found no significant differences in preterm birth (5/146 versus 11/148, RR 1.00, 95% CI 0.90 – 1.10) or very preterm birth (1/146 versus 2/148, RR 1.00, 95% CI 0.90 – 1.10) for home-based versus hospital-based monitoring. Very preterm birth only occurred once in the home-based monitoring group. The mean birth weight was 3504.0 (SD 47.7) for the home-based monitoring group versus 3532.1 (SD 48.1) for the hospital-controlled monitoring group. We found no significant differences in LGA (20/146 versus 18/148, RR 1.00, 95% CI 0.90 – 1.10) and SGA (12/146 versus 12/148, RR 1.00, 95% CI 0.90 – 1.10) for home-based versus hospital-based monitoring. Also, no differences were found in HDP, PE, abnormal placentation, GDM, hospital admission during pregnancy and congenital anomaly. One neonatal death due to pulmonary hypoplasia occurred in the home-based monitoring group after very PTB.

Limitations, reasons for caution: The outcomes of HDP, PE, abnormal placentation, GDM and hospital admission during pregnancy were patient-reported and confirmed by hospital-files after contacting the participating centers.

Wider implications of the findings: NC-FET is the preferred treatment in women with ovulatory cycles undergoing FET. When home-based monitoring is compared to hospital-controlled monitoring in NC-FET we found no difference in obstetric or neonatal outcomes.

Trial registration number: not applicable

Abstract citation ID: dead093.240

O-199 Imprinting disorders in singletons conceived by assisted reproductive technology in Sweden

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Study question: Is singleton birth from assisted reproductive technology (ART) associated with imprinting disorders (IDs) independent of parental infertility and other background factors?

Summary answer: Singletons conceived using intracytoplasmic sperm injection (ICSI) and frozen embryos have higher risks of Beckwith-Wiedemann syndrome (BWS), Prader-Willi syndrome (PWS) and Silver-Russell syndrome (SRS).

What is known already: Previous studies have suggested elevated risk of IDs in children conceived with ART compared with children conceived with spontaneous conception (SC). It is not clear to what extent the observed associations could be explained by parental infertility and related risk factors. Knowledge on specific ART procedures and their association with IDs is also limited.

Study design, size, duration: A nationwide register-based cohort study was carried out in which all liveborn singletons in 1997-2017 in Sweden were included with follow-up to 2018.

Participants/materials, setting, methods: Among 1 998 825 singletons, 60 210 were conceived using ART. The International Classification of Diseases (ICD) version 10 was used to identify three distinct ID groups: PWS/SRS, BWS, and central precocious puberty (CPP). Cox regression, combined with inverse probability treatment weights to account for birth year, parity, pregnancy loss history, parental age and country of origin, was used to compare ART singletons with all SC singletons and those born to couples with known infertility.

Main results and the role of chance: A total of 1012 children were diagnosed with the IDs of interest (654 PWS/SRS, 255 BWS, and 109 CPP), and 49 of them were conceived through ART. Compared with all SC singletons, higher risk of PWS/SRS and BWS was observed in ART singletons and the weighted hazard ratios (wHRs) were 1.57 [95% CI, 1.09-2.26] and 2.57 [95% CI, 1.60-4.12], respectively. The elevated risks remained when comparison was restricted to SC singletons of couples with known infertility, though the wHR for BWS was somewhat attenuated (1.87, 95% CI: 1.04-3.36). No difference in risk of CPP was observed between singletons conceived with and without ART irrespective of parental infertility. Further subgroup analysis revealed that ICSI in combination with frozen embryo transfer was responsible for the higher risks of PWS/SRS (wHR 6.32, 95% CI: 3.34-11.95) and BWS (wHR 9.04, 95% CI: 4.34-18.82).

Limitations, reasons for caution: The use of the Swedish ICD-10 did not allow distinction of PWS and SRS. The number of CPP cases in ART singletons was too small (N = 3) to make inference.

Wider implications of the findings: This study found that ART-conceived singletons, particularly those conceived using ICSI and frozen embryos, had a higher risk of IDs independent of parental infertility. However, the underlying mechanism is not clear, and the role of type and severity of infertility warrants further investigation.

Trial registration number: not applicable

Abstract citation ID: dead093.241

O-200 Cardiometabolic health in Danish children aged 7-10 years born after assisted reproductive technology with frozen and fresh embryo transfer

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Study question: Is cardiometabolic health in children conceived after frozen embryo transfer (FET) different from children conceived after fresh embryo transfer (fresh-ET) and natural conception (NC)?

Summary answer: FET-children had similar anthropometrics, glucose- and lipid profile in childhood compared to fresh-ET and NC. Blood pressure was higher in FET-girls compared to fresh-ET-girls.

What is known already: Children conceived after assisted reproductive technology (ART) with FET are more often born large-for-gestational age (LGA) while children born after fresh-ET are at risk of being small-for-gestational age (SGA).In general, children born LGA or SGA are at increased risk of obesity, diabetes and cardiovascular disease later in life. Smaller studies on the whole ART-population have raised concerns about premature vascular aging, increased risk of insulin resistance and higher blood pressure. The long-term cardiometabolic health of children born after ART and especially FET is scarcely explored.

Study design, size, duration: This study was part of the large cohort study "Health in Childhood following Assisted Reproductive Technology" (HiCART) which included 606 singletons (292 boys) born between December 2009 and December 2013: 200 children were conceived after FET; 203 children were conceived after fresh-ET; and 203 children were conceived naturally and matched for birth year and sex. The study period lastet from January 2019 to September 2021.

Participants/materials, setting, methods: The children were 7-10 years of age at examination and underwent a clinical examination with anthropometric measurements, pubertal staging and blood pressure measurement. Furthermore a whole-body dual-energy x-ray absorptiometry-scan (DXA) was performed and a fasting blood sample was drawn. Anthropometric measurements and blood pressure were converted to standard deviation scores (SDS) using a Danish reference. The three study groups were compared pairwise using univariate linear regression model.

Main results and the role of chance: Data is presented as mean (SD). Children conceived after FET had significantly higher birth weight (SDS) (0.20 SDS (1.09)) compared to children conceived after fresh-ET (-0.22 SDS (1.00), mean difference: 0.42 SDS (95% CI: 0.21; 0.62)) and NC-children (-0.16 SDS (1.09), mean difference: 0.35 SDS (95% CI: 0.14; 0.57)).

At 7-10 years height (SDS), weight (SDS) and BMI (SDS) were similar between the groups. Fat percentage (DXA) and waist-to-height ratio were also comparable between the groups.

Markers of glucose metabolism were similar in the groups including fasting glucose, C-peptide, HbA1c and insulin resistance (HOMA-IR). Lipid profiles including, cholesterol, LDL and HDL were also similar.

Blood pressure was comparable between the three groups, but stratified on sex girls conceived after FET had significantly higher systolic blood pressure (SDS) (0.75 SDS (0.84)) compared to girls concieved after fresh-ET (0.49 SDS (0.61), mean difference: 0.25 SDS (95% CI: 0.05; 0.45)) and significantly higher diastolic blood pressure (SDS) (FET: 0.54 SDS (0.57), fresh-ET: 0.38 (0.58), mean difference: 0.16 SDS (0.00; 0.31)). Puberty had started in 17% of the girls, equally distributed in the three groups.

Limitations, reasons for caution: As the participation rate was between 18-42% in the three groups, selection bias cannot be excluded. We currently do not have information regarding endometrial preparation protocol in the FET-cycles which may bias the results. Due to multiple testing type I errors cannot be excluded.

Wider implications of the findings: Higher birth weight in children conceived after FET did not translate into differences in anthropometrics, glucose- or lipid profile in children ages 7-10 years. However, the higher systolic- and diastolic blood pressure (SDS) found in girls concieved after FET compared to fresh-ET is of concern and should be further explored.

Trial registration number: NNF18OC0034092, NFF19OC0054340

Abstract citation ID: dead093.242

O-201 Growth of singletons born after frozen embryo transfer until early adulthood: a Finnish register study

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Study question: Does the growth of children born after frozen embryo transfer (FET) differ from those born after fresh embryo transfer (ET) or natural conception (NC)?

Summary answer: Boys born after FET have a higher risk of overweight compared to fresh ET.

What is known already: FET is associated with a higher mean birth weight and an increased risk of large-for-gestational-age (LGA) compared to fresh ET and NC, thus raising questions of possible growth disturbances later in life. Previous studies on early childhood growth after FET have reported partly contradictory results. Moreover, data on the adolescent growth after FET are scarce, reporting no significant differences.

Study design, size, duration: This observational cohort study, based on national population-based registers, includes 1825 and 2933 singletons born after FET and fresh ET in Oulu and Helsinki city areas in Finland between 1995 and 2006. A 10% sample of NC controls ($n = 31\,136$) from the same birth years, matched for area of residence, was obtained from the Finnish Medical Birth Register. Growth data were obtained from the Register of Primary Health Care visits.

Participants/materials, setting, methods: Mean heights, weights, and body mass indices (BMI) were compared between the groups, stratified by sex, between ages 7 and 18, using general linear model. The proportions of overweight (age- and sex-adjusted ISO-BMI for children ≥ 25) were analyzed using multiple linear regression, adjusting for birth year, preterm birth, maternal age, parity, and socioeconomic status. Additionally, the odds for overweight were calculated for all ages combined, adjusting for multiple measurements using generalized estimating equations.

Main results and the role of chance: Growth measurements for each age were available for 5.9-30.6% of boys and 4.7-29.5% of girls. The mean and median number of measurements was three per child. No significant differences in height were found between FET and fresh ET. FET boys were significantly taller compared to NC between ages 11 and 14. For boys, the mean proportions of overweight for FET, fresh ET and NC were 28%, 22% and 26%, respectively ($p < 0.001$ for FET vs fresh ET, $p = 0.014$ for FET vs NC and $p < 0.001$ for fresh ET vs NC). For all ages combined, the adjusted odds ratio (aOR) of overweight was 1.14 (95% CI 1.02-1.27) for FET compared to fresh ET, and 1.08 (95% CI 0.99-1.18) for FET compared to NC. For girls, the mean proportions of overweight for FET, fresh ET and NC were 18%, 19% and 22% ($p = 0.169$ for FET vs fresh ET, $p < 0.001$ for FET vs NC and $p < 0.001$ for fresh ET vs NC). For all ages combined, the aOR of overweight was 0.92 (95% CI 0.81-1.06) for FET compared to fresh ET and 0.89 (95% CI 0.80-0.99) for FET compared to NC.

Limitations, reasons for caution: Unfortunately, we were not able to adjust for parental anthropometric characteristics. The growth data were not available for the entire cohort, and the proportion of children measured at the start and end of the follow-up was limited. Mainly cleavage stage embryos were transferred, and slow freezing was used.

Wider implications of the findings: The lack of significant height differences between FET and fresh ET offers reassurance of the safety and feasibility of FET. However, the risk of overweight among FET boys warrant further research as to verify the results in larger cohorts, and further investigate the mechanisms that explain this gender-specific finding.

Trial registration number: not applicable

Abstract citation ID: dead093.243

O-202 Early reproductive outcomes of the first birth cohorts conceived with in-vitro fertilisation in Sweden

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Study question: Are early reproductive outcomes of individuals conceived with IVF different from the general population or from spontaneously conceived individuals born to couples with infertility?

Summary answer: The first birth cohorts from IVF were more likely to experience infertility and use assisted reproduction also when accounting for parental factors and infertility

What is known already: In-vitro fertilisation has helped countless couples overcome involuntary childlessness, but little is yet known of the reproductive outcomes of their offspring. Deviations could occur as a result of the transmission of, or reaction to, parental traits and behaviors, or adverse effects of the procedures. A few small studies of young adults conceived with intra-cytoplasmic sperm injection due to male factor infertility have indicated lower sperm quantity (but not quality) compared to spontaneously conceived males, and no differences in reproductive hormones or antral follicle count in females.

Study design, size, duration: This nation-wide cohort study considered all individuals in the Swedish Medical Birth Register, born between 1985 and 1996 (N = 1,291,795). Linkage of population-registers allowed prospective follow-up of potential reproductive outcomes, migration from Sweden or death, until the end of 2017.

Participants/materials, setting, methods: Parental infertility and IVF use was identified from maternal self-report and IVF-clinic reporting to the Medical Birth Register, which was also used for outcome ascertainment, along with registers over family relations, outpatient specialist care, and assisted reproduction. Individuals conceived with IVF were compared to spontaneously conceived individuals of the same age. Comparisons were also adjusted for parents' age, education and country of origin and further restricted to only concern individuals whose parents experienced infertility.

Main results and the role of chance: Compared to spontaneously conceived of the same age, individuals conceived with IVF were less likely to have had any biological children across all studied birth cohorts (Hazard ratio (HR) 0.67 95% Confidence interval (CI) 0.62–0.74 overall), but adjustment for parental factors and comparison to individuals with parental infertility attenuated the differences to the null. Among individuals followed until at least age 30, those that had been conceived with IVF were more likely to have received a diagnosis of infertility (HR 2.03, 95% CI 1.15–3.58), also after adjustment for parental factors (HR 2.08, 95% CI 1.18–3.66) and restriction with respect to parental infertility (HR 1.82, 95% CI 1.03–3.24). While there was no substantial difference in mean age, the first recorded birth was more likely to have been preceded by infertility and IVF assistance when individuals had been conceived with IVF themselves (Odds ratio (OR) 2.89, 95% CI 1.47–5.66 and OR 3.83, 95% CI 1.48-9.93 respectively in adjusted comparison to individuals with parental infertility). Corresponding elevated risks were not observed in any of the birth cohorts for which follow-up did not extend beyond their 20s (born 1988-1996).

Limitations, reasons for caution: Since not even the first IVF birth cohorts have completed their expected reproductive period, these findings merely represent an indication of the early reproductive outcome of the first generation conceived with IVF in Sweden. Information on type and severity of parental infertility was not available.

Wider implications of the findings: Finding the earliest birth cohorts conceived with IVF at elevated risk of infertility and of using IVF for their first birth, also when accounting for parental infertility, stresses the need for further monitoring as more cohorts enter and pass through the expected reproductive period.

Trial registration number: Not applicable

Abstract citation ID: dead093.244
O-203 Unexplained infertility: diagnosis by exclusion

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Study question: What is the optimal work-up to establish the diagnosis of unexplained infertility, based on the best available evidence in the literature?
Summary answer: The ESHRE Guideline on Unexplained Infertility provides 35 recommendations on the diagnosis of unexplained infertility.
What is known already? The diagnosis of unexplained infertility is made when no abnormalities of the female and/or male reproductive systems are clearly identified. UI is inevitably a diagnosis by exclusion, after "standard" investigations. However, a real standardization of the diagnostic work-up is still lacking.
Study design, size, duration: The guideline was developed according to the structured methodology for the development of ESHRE guidelines. First, a group of experts formulated the key questions. Literature searches and assessments were then performed. Papers written in English and published up to 24 October 2022 were included in the review. Prior to publication, the guideline was reviewed by independent international reviewers.
Participants/materials, setting, methods: Based on the collected evidence, recommendations were formulated and discussed until consensus was reached within the guideline group. A stakeholders' review was organized after the finalization of the draft. The revised version was approved by the guideline group and the ESHRE Executive Committee.
Main results and the role of chance: This guideline aims to help clinicians in delivering the optimal care for couples with unexplained infertility. Since unexplained infertility is a diagnosis of exclusion, this lecture will focus on the diagnostic procedures that couples should/could go through during the infertility work-up, and will analyse the need for additional diagnostic tests to establish the diagnosis of unexplained infertility. The guideline has been in stakeholder review and is prepared for submission in 2023.
Limitations, reasons for caution: Most diagnostic tests and interventions in couples with unexplained infertility are not well studied. For a large proportion of these tests, evidence was very limited and of very low quality. Further studies are definitely needed in this field, possibly providing reliable data for revisiting the current recommendations.
Wider implications of the findings: Based on the best available evidence, the guideline provides clinicians with clear advice on how to optimize the diagnostic approach to couples with unexplained infertility. In addition, a list of research recommendations is included to prompt further studies in the field.
Study funding/competing interest(s) The guideline was developed and funded by ESHRE, that covered expenses associated with the guideline meetings, with the literature searches and with the dissemination of the guideline. The guideline group members did not

Abstract citation ID: dead093.245
O-204 Treatment of unexplained infertility: IUI for all?

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Study question: What is the recommended management for couples presenting with unexplained infertility, based on the best available evidence in the literature?
Summary answer: The ESHRE Guideline on Unexplained infertility makes 19 recommendations on treatment of couples with unexplained infertility.
What is known already?: Unexplained infertility (UI) is diagnosed in the absence of any abnormalities of the female and/or male reproductive systems after "standard" investigations. The management of UI is traditionally empirical. The efficacy, safety, costs, and risks of treatment options have not been subjected to robust evaluation.
Study design, size, duration: The guideline was developed according to the structured methodology for the development of ESHRE guidelines. Following formulation of key questions by a group of experts, literature searches and assessments were undertaken. Papers written in English and published up until 24 October 2022 were included. Prior to publication, the guidelines were reviewed by independent international reviewers.
Participants/materials, setting, methods: Based on the available evidence, recommendations were formulated and discussed until consensus was reached within the guideline group. A stakeholder review was organized after finalization of the draft. The final version was approved by the guideline group and the ESHRE Executive Committee.
Main results and the role of chance: This guideline aims to help clinicians provide the best care for couples with unexplained infertility. The first-line treatment for couples with unexplained infertility was deemed to be intrauterine insemination (IUI) in combination with ovarian stimulation. This lecture will focus on the rationale behind this recommendation and the place of additional and alternative options for the treatment of unexplained infertility.

The guideline has been in stakeholder review and is prepared for submission in 2023.

Limitations, reasons for caution: Most interventions in couples with unexplained infertility have not been subjected to robust evaluation. For a large proportion of these treatments, evidence was very limited and of very low quality. More evidence is required, and the results of future studies may require the current recommendations to be revised.
Wider implications of the findings: The guideline provides clinicians with clear advice on best practices in the care of couples with unexplained infertility, based on the best evidence currently available. In addition, a list of research recommendations is provided to stimulate further studies in the field.
Study funding/competing interest(s): The guideline was developed by ESHRE which funded the guideline meetings, the literature searches and dissemination. The guideline group members did not receive any financial incentives; all work was provided voluntarily.

POSTER DISCUSSION SESSION
SESSION 65: ANDROLOGY
Tuesday 27 June 2023 Hall D2 15:15 - 16:30

Abstract citation ID: dead093.246
P-016 WHO 2021-based comprehensive appraisal of sperm factor parameters' association with embryological and clinical outcomes. A single center study of 4013 PGT-A cycles

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Study question: What is the impact of sperm parameters and paternal age on all embryological and clinical outcomes during ICSI-cycles with aneuploidy testing?

Summary answer: Reduced basal/post swim-up motility and patients with concentration+morphology+motility <5th-percentile (based on WHO-2021) are associated with poorer embryological outcomes and cumulative-live-birth-rate per concluded PGT-A cycle.

What is known already: Previous studies reported an association between oligoasthenoteratozoospermia (OAT), obstructive azoospermia (OA) and non-obstructive azoospermia (NOA) and poorer fertilization/blastulation rates, but no impact on euploidy rates at the blastocyst stage. Similarly, the clinical outcomes were mostly independent from sperm characteristics when euploid blastocysts are transferred. The data about paternal age effect are controversial, varying between absent and severe embryological/clinical impact. Moreover, little is known about the association between each semen analysis parameters and embryological/clinical outcomes. Lastly, most of the evidence to date was based on WHO 2010 criteria, recently updated by WHO 2021, and did not mention cumulative-live-birth-rates (CLBR) per cycle.

Study design, size, duration: Retrospective study at a single private center involving 4013 ICSI+PGT-A cycles with own-oocytes conducted by 3101 couples (2013-2021). The primary embryological outcome was euploid-blastocyst-rate (EBR) per cohort of inseminated oocytes. The primary clinical outcome was CLBR per concluded cycle (i.e., LB achieved or no transferable/all transferred blastocysts). Intermediate embryological/clinical outcomes were also tested. All parameters were analyzed as continuous variables. For some analyses we categorized paternal age (<38/38-44/>44 years) and sperm factor (WHO-2021 parameters<5th percentile).

Participants/materials, setting, methods: GnRH-antagonist ovarian-stimulation, ICSI, trophectoderm biopsy without zona-pellucida drilling in day3, qPCR/NGS-based analysis to report non-mosaic aneuploidies (segmental aneuploidies reported from 2017 onwards; N = 1991 cycles) and vitrified-warmed euploid single-blastocyst-transfers were conducted. Semen analysis (volume, concentration, total sperm per ejaculate, basal/post swim-up motility, morphology), sperm characteristics (ejaculated/FNA/TESE; fresh/frozen), DNA-fragmentation-index (DFI, outlined via Tunel-test; N = 188 cycles), paternal BMI and age were investigated for their association with embryological/clinical outcomes adjusted for confounders via linear/logistic regressions.

Main results and the role of chance: Maternal and paternal age were 38.9 ± 3.2(range:23-45) and 41.9 ± 5.7(25-72) years, 10 ± 6.3(1-44) cumulus-oocyte-complexes were retrieved and 7.3 ± 4.6(1-32) metaphase-II oocytes inseminated. Cycles showing all parameters >5th-percentile were 47.1%(N = 1890), concentration<5th-percentile were 3.7%(N = 149), motility<5th-percentile were 3.9%(N = 155), morphology<5th-percentile were 10.1%(N = 405), concentration+motility<5th-percentile were 1.7%(N = 70), concentration+morphology<5th-percentile were 6.2%(N = 248), motility+morphology<5th-percentile were 5.0%(N = 70), concentration+motility+morphology<5th-percentile were 19.9%(N = 797), OA and NOA were 1.4%(N = 56) and 1.0%(N = 41), respectively. The only confounder upon EBR per cohort of metaphase-II oocytes was maternal age. Only basal/post swim-up motility was associated with this outcome. When categorized, only concentration+morphology<5th-percentile (-2.7%,95%CI -5.1 to -0.3%, adjusted-p=0.03), concentration+morphology+motility<5th-percentile (-4.0%,95%CI -5.5 to -2.6%, adjusted-p<0.01), OA (-5.5%,95%CI -10.3 to -0.7%, adjusted-p=0.03) and NOA (-5.8%,95%CI -11.4 to -0.1%, adjusted-p=0.05) showed significantly poorer results. Maternal age and number of metaphase-II oocytes were the confounders upon the chance to obtain ≥1 euploid blastocyst and ≥1 LB among concluded cycles. Only basal/post swim-up motility was associated with this outcome. When categorized, only concentration+morphology+motility<5th-percentile showed significantly lower chances (multivariate-OR:0.74,95%CI 0.61-0.90, adjusted-p<0.01 and multivariate-OR:0.74,95%CI 0.59-0.92, adjusted-p<0.01). No association was reported between semen parameters/sperm factor categories and 1PN/3PN rates, day5-7 blastocyst-rate, morphological quality, outcomes per euploid transfer. Paternal age categories were associated with day5-blastocyst (N = 1131/3308,34.2%, N = 1179/3644,32.4%, and N = 919/3127,29.4%) and AA-blastocyst rates (N = 1732/3308,52.4%, N = 1815/3644,49.8%, and N = 1387/3127,44.4%) in men <38/38-44/>44 years, respectively (p < 0.01).

Limitations, reasons for caution: Some confounders were unbalanced among the study groups but accounted for in linear/logistic regressions. 9% of the cycles were not concluded by the time of abstract drafting. The sample size in the groups of surgically-retrieved sperm is limited, in part because NOA patients due to genetic causes were not included.

Wider implications of the findings: By comprehensively accounting for sperm factor characteristics, this report provides reproductive professionals with useful figures to counsel infertile couples about their chance of success during IVF. These estimates are useful for the decision-making process regarding the most effective clinical strategies to apply. Further studies including more NOA/OA patients are warranted.

Trial registration number: not applicable

Abstract citation ID: dead093.247
P-047 A novel mutation of DRC1 gene causes multiple morphological abnormalities of the sperm flagella and male infertility in humans

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Study question: Can novel mutations of *DRC1* gene affect the sperm morphology in humans and how about the outcomes of assisted reproductive therapy of the affected patients?

Summary answer: An infertile man with multiple morphological abnormalities of the sperm flagella caused by *DRC1* biallelic mutations has good outcomes of fertility after intracytoplasmic sperm injection.

What is known already: The nexin-dynein regulatory complex (N-DRC) functions by linking neighboring doublet microtubules within motile cilia and flagella, stabilizing the axonemal structure and thereby regulating ciliary motility. As a core of N-DRC protein, the disruptions of *DRC1* gene have been identified to cause multiple morphological abnormalities of the sperm flagella (MMAF) and male infertility in humans and mice. *DRC1* mutations usually

resulted in decreased flagellum axoneme stability, thereby causing flagellar structural disorder.

Study design, size, duration: The 35 patients with MMAF were recruited from the center for reproductive medicine from August 2019 to June 2022. They were diagnosed with primary male infertility caused by abnormal morphologies of sperm flagella and decreased sperm motility. Genetic testing, pedigree analysis, sperm morphological analysis, functional assays, and assisted reproductive therapy were performed in 2022.

Participants/materials, setting, methods: We identified and confirmed the *DRC1* mutation through a 22-gene next-generation sequencing panel and sanger sequencing. Papanicolaou-staining, scanning electronic microscope, and transmission electronic microscope were performed to reveal the changes of sperm morphology and ultrastructure. Immunostaining was conducted to show the effect of *DRC1* mutation on the expression and localization on sperm structural proteins. After the ovarian stimulation, a time-lapse monitoring system was applied for the assisted reproductive therapy of *DRC1* mutant patient.

Main results and the role of chance: Biallelic mutations in *DRC1* were identified in a 39-years-old proband who suffered from primary infertility for 9 years due to severe asthenoteratozoospermia. Pedigree analysis of the non-consanguineous family manifested an autosomal recessive inheritance pattern. Papanicolaou-staining, scanning and transmission electronic microscopy showed the abnormalities of sperm flagella and severe disorganization of the axoneme in *DRC1*-deficient male compared to control subject. Immunostaining with sperm-specific markers, TOM20, DNAI1, and DNALI1, showed the composite changes of flagella morphology and molecular components. After intracytoplasmic sperm injection (ICSI), the rate of fertilization was 71.4% (5/7), the rate of embryo cleavage was 100% (5/5), the rate of transferable embryo was 60% (3/5), respectively. Following frozen-thawed embryo transfer, the wife of the proband became pregnant.

Limitations, reasons for caution: Additional cases are needed to investigate the gene-disease relationship between *DRC1* mutations and male infertility owing to abnormal sperm morphology and motility.

Wider implications of the findings: This study expands the mutant spectrum of *DRC1* and describes a good fertility outcome of assisted reproduction therapy with ICSI for the *DRC1* mutant patient. Together with the available information regarding male infertility, it provides new information for the genetic diagnosis and counseling of MMAF in the future.

Trial registration number: Not applicable

Abstract citation ID: dead093.248
P-045 Effects of seminal BPA levels on human sperm parameters and gene expression

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Study question: Are high BPA levels in human seminal fluid associated with low sperm metrics and changes in gene expression?

Summary answer: A trend towards an inverse relationship between seminal BPA and sperm count was observed. Expression of androgen receptor mRNA was down-regulated with high BPA exposure.

What is known already: Bisphenol A (BPA) is a widespread industrial chemical, used as the key monomer of polycarbonate plastics and epoxy resins. While some countries have introduced bans/stringent regulation for BPA, it continues to be widely used especially in North America, where its use is only limited in baby products. BPA has been detected in human seminal fluid and has been correlated with decreased sperm counts and motility - crucial factors in determining male fertility, which continue to decrease. We have demonstrated that *in vitro* BPA exposure can reduce motility, mitochondrial membrane potential, and capacitation in mature bovine spermatozoa.

Study design, size, duration: In collaboration with CCRM, Toronto, seminal fluid samples were collected from 90 patients undergoing fertility assessment between 2021 and 2023. Typical evaluations such as sperm counts, motility, and morphology were recorded by the clinic (following WHO guidelines). Samples were then shipped to the University of Guelph for further investigations. Information on outcomes for patients who underwent subsequent IVF cycles like fertilization rates and pregnancy rates were also recorded at the clinic.

Participants/materials, setting, methods: At the University of Guelph, seminal BPA levels were measured through an enzyme-linked immunosorbent assay (ELISA; My BioSource), then compared to sperm metrics obtained by the clinic. Next, a GeNorm analysis was performed to determine the most stable reference genes for subsequent qPCR analysis. The relative mRNA expression of three key genes related to male reproduction (androgen receptor (AR), cyp17a1 and cyp11a1) were quantified and compared between groups with high and low BPA content.

Main results and the role of chance: BPA concentrations in the seminal fluid samples ranged between 0-3000 pg/mL, with most samples containing 35-1000 pg/mL. When samples were graphed according to increasing BPA levels, no relationships were observed between BPA and motility or morphology rates. However, a negative trend between BPA and sperm count was detected, although this was not significant. Samples were then divided into high- (total 15 samples with >700 pg/mL) and low- (total 15 samples with <100 pg/mL) BPA content groups. The GeNorm M value indicated that HPRT1 and HMBS were the most stable reference genes across the groups. qPCR analysis on three biological replicates in technical triplicates showed that androgen receptor mRNA expression was significantly higher in sperm exposed to low seminal BPA compared to the high BPA exposure group ($p = 0.03913$). This finding is particularly interesting as AR expression has previously been positively correlated with sperm count, motility and morphology. cyp17a and cyp11a mRNA expression was undetectable in human spermatozoa under our experimental conditions. Future steps include increasing the sample size to increase the power of the correlation studies between sperm quality parameters and ART success rates. Furthermore, other fertility-related genes will be investigated at both the mRNA (qPCR) and protein levels (flow cytometry).

Limitations, reasons for caution: Relatively small sample size; duration of study must be extended to gain information on outcomes of ART cycles; many samples in the mid-range of BPA are excluded from gene expression studies.

Wider implications of the findings: These results can ultimately display a relationship between BPA exposure and male fertility and provide further insights into the molecular mechanisms through which BPA exerts its effects. Ultimately, this research can drive changes to guidelines/exposure limits for BPA, especially in North America.

Trial registration number: not applicable

Abstract citation ID: dead093.249
P-088 The Paternal Clock: Shedding Light on the Relationship Between Paternal Age and Sperm DNA Fragmentation

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Study question: What is the impact of advanced paternal age (APA) on sperm DNA fragmentation index (DFI)?

Summary answer: Sperm DFI levels remain relatively stable until the age of 35 and increase progressively beyond that age.

What is known already: APA can have a negative impact on male fertility and the health of offspring. Previous studies have shown that APA is linked to poor conventional sperm parameters, including decreased semen volume, sperm count, motility, morphology, as well as poor sperm DNA integrity. Additionally, APA has been associated with reduced natural or assisted reproduction and perinatal outcomes and a higher risk of genetic and chromosomal abnormalities in the offspring.

Study design, size, duration: A retrospective cohort study of 4250 consecutive semen samples from men undergoing infertility evaluation at the OVO clinic, in Montreal, Canada, between April 2016 and December 2022. Participants were stratified into seven age groups: <26 (n = 36; 0,8%), 26-30 (n = 500; 11,8%), 31-35 (n = 1269; 29,9%), 36-40 (n = 1268; 29,8%), 41-45 (n = 732; 17,2%), 46-50 (n = 304; 7,2%), >50 years (n = 141; 3,3%). The mean age was 37.4 ± 6.4 years (range 18-71 years).

Participants/materials, setting, methods: The study was population-based and included male patients from all ages, ethnicities, and medical histories. Semen samples were collected after 2-3 days of abstinence. For patients who underwent more than one DFI testing, only the first sample was included, and any duplicates were excluded. DFI was evaluated by flow-cytometry based TUNEL assay using the APO-Direct Kit. Data were analyzed using one-way ANOVA and T3 Dunnett post-hoc multiple comparison test, as well as Pearson's correlation coefficient.

Main results and the role of chance: The results show a significant positive correlation between %DFI and age ($r = 0.19$, $p < 0.001$). Mean %DFI levels were relatively stable in men aged <26 to 35 years (17.9%, 18.1% and 18.1% in men aged <26, 26-30 and 31-35, respectively), with %DFI increasing progressively beyond age 35 (20.7%, 22.5%, 25.7% and 27.9% in men aged 36-40, 41-45, 46-50 and >50, respectively). The mean %DFI in the 36-40 age group was significantly higher than in the 31-35 age group ($p < 0,001$). Our study has uncovered that %DFI follows an exponential curve starting at age 35, indicating that the %DFI accelerates significantly as men age beyond their mid-30s.

Limitations, reasons for caution: This retrospective analysis has inherent limitations that may introduce confounding variables. The patient clinical background, such as medical history and lifestyle factors was not assessed. Also, the studied cohort consisted of a population under investigation for infertility and may not be representative of the general male population.

Wider implications of the findings: The study demonstrates the age-related increase in sperm %DFI and suggests that there may be an age cut-off below which sperm %DFI is stable and beyond which sperm %DFI increases. This information may be useful to medical specialists that offer sperm DNA testing to infertile couples.

Trial registration number: not applicable

Abstract citation ID: dead093.250

P-014 Inhibition of mitochondrial uncoupling proteins irreversibly arrests human spermatozoa motility

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Study question: Are mitochondrial uncoupling proteins (UCPs) expressed in human spermatozoa and if yes, what is their role?

Summary answer: UCP1, UCP2, and UCP3 are expressed in human spermatozoa. The inhibition of UCPs irreversibly arrests human spermatozoa motility without compromising viability.

What is known already: UCPs are expressed in the mitochondrial inner membrane, where they act as channels between the intermembrane space and the matrix. Six UCP homologs had already been identified in mammals (UCP1-6). UCPs act as regulators of reactive oxygen species (ROS) production, general cellular redox state, and mitochondrial function. The altered expression or function of UCPs is positively linked with the onset of metabolic diseases, such as obesity and diabetes mellitus. Male infertility is closely related to metabolic diseases since the testis is susceptible to metabolic alterations and oxidative stress, however, the expression and function of UCPs in human spermatozoa are unknown.

Study design, size, duration: We performed a control-versus-treatment study. High motility spermatozoa were isolated through density gradient centrifugation from human normozoospermic seminal samples (n = 16) and incubated with genipin, a selective UCP inhibitor (0, 0.5, 5, and 50 μM), for 3 h at 37 °C. Cells and culture media were collected for analysis.

Participants/materials, setting, methods: UCP1-3 protein expression was detected by western blot and immunofluorescence. After UCPs inhibition, spermatozoa viability and motility were assessed. Mitochondrial membrane potential and ROS production were evaluated. Media were collected and the metabolic profile and antioxidant potential were analysed by ^{1}H-NMR and FRAP, respectively.

Main results and the role of chance: We were able to identify the expression of UCP1, UCP2, and UCP3 in human spermatozoa. UCP1-3 are mainly located at the equatorial segment of the head, whereas UCP1 and UCP2 are also expressed at the spermatozoa midpiece, where mitochondria are located. The inhibition of UCPs by 50 μM genipin, resulting in the UCP3 inhibition, led to the complete and irreversible loss of motility that persisted despite washing or incubation with theophylline, a cAMP activator. These effects were associated with decreased mitochondrial membrane potential and lactate production. Interestingly, the loss of motility did not compromise spermatozoa viability. In addition, the inhibition of UCPs led to no alterations concerning ROS production, possibly due to the decreased mitochondrial activity and genipin antioxidant properties.

Limitations, reasons for caution: This is an in vitro study with a relatively small sample size. Genipin is considered a specific yet a general inhibitor of UCPs. The development and use of specific inhibitors for each homolog will further disclose their role in human spermatozoa motility and bioenergetics.

Wider implications of the findings: UCPs are expressed in human spermatozoa. UCPs are important regulators of human spermatozoa motility and metabolism. The discovery and characterization of UCPs' role in human spermatozoa open the path for studies on ROS-related pathways and bioenergetics physiology of human spermatozoa.

Trial registration number: 'not applicable'

SELECTED ORAL COMMUNICATIONS
SESSION 66: ALL ATTENTION TO THE OOCYTE

Tuesday 27 June 2023 Hall D3 17:00 - 18:00

Abstract citation ID: dead093.251

O-205 Poor mitochondrial metabolism impairs meiosis and contributes to reduced oocyte maturation rates in patients with advanced maternal age

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Study question: Is there a relationship between poor mitochondrial metabolism and impaired meiotic progression of germinal vesicle (GVs) oocytes in women of advanced maternal age (AMA)?

Summary answer: Poor mitochondrial metabolism impairs meiotic progression in human oocytes, contributing to a lower oocyte maturation capacity in women of AMA.

What is known already: High aneuploidy rates and impaired metabolism are characteristic features of AMA oocytes. Studies in mouse suggest that

successful meiotic maturation and metabolic competency may be functionally linked, however this relationship has not been clearly established in human. Metabolic dynamics can be visualized by indirect measurements through mitochondrial staining and quantified more directly using Fluorescence Lifetime Imaging Microscopy (FLIM). This live-imaging approach can generate metabolic timelapse profiles of oocytes throughout meiosis. We performed an extensive characterization of oocyte metabolism in maturing GVs, obtained from both young and AMA patients, to establish the importance of mitochondrial metabolism during meiosis.

Study design, size, duration: A total of 401 GVs from young (≤30 years, n = 264) and AMA (>37 years, n = 137) women were included in the study. GVs were matured in vitro in G2-plus medium for 30 hours. Maturation was determined by the presence of an extruded polar body. Oocytes were used for FLIM (n = 175) and immunofluorescence (IF) analysis (n = 226). Further, mitochondrial metabolism loss of function studies (n = 64) were performed by treating young GVs with 1μM Trifluoromethoxy-carbonylcyanide-phenylhydrazone (FCCP) for 30 minutes.

Participants/materials, setting, methods: The proteins Dihydrolipoamide-S-Acetyltransferase (D-LAT) and Translocase-of-outer mitochondrial-membrane (TOMM20) were analysed in young and AMA oocytes by IF (Arbitrary-Unit, AU) to assess mitochondrial activity and localization, respectively. Fluorescence mean intensities were quantified with ImageJ and compared by t-test; maturation rates were compared by Chi-squared test. FLIM comprehensive metabolism (NADH; FAD^{+}) were taken at GV stage. Different FLIM parameters (fluorescence intensity, fraction bound, short/long lifetime) were evaluated individually and combined into the Redox ratio (NADH intensity/FAD^{+} intensity).

Main results and the role of chance: Mitochondrial staining showed a common pattern in young and AMA GV oocytes, with a uniform localization of mitochondria in the ooplasm (TOMM20) and a subcortical localization of active organelles (D-LAT). These patterns were confirmed by in-live FLIM analysis. The total mitochondria abundance was comparable between AMA and young GV oocytes (intensity of 61674 ± 24322 AU in young, 32186 ± 33414 AU in AMA, p = 0.195), however, active mitochondria were diminished in AMA compared to young GVs both by IF and FLIM (intensity of 78614 ± 58534 AU in young, 12517 ± 10187 AU in AMA, p = 0.003; Redox ratio in young 2e+00 ± 0.15, in AMA 1e+00 ± 0.16, p = 2.969e-05). Notably, young oocytes matured at a significantly higher rate (86.3%; 63/73) than AMA oocytes (62.3%; 38/61; p = 0.002). Moreover, FLIM imaging revealed that GVs with a higher metabolism were more likely to complete meiosis to MII (Redox ratio 2e+00 ± 0.17 in GVs matured to MII, 1e+00 ± 0.18 in non-matured, p = 1.054e-06). Following treatment with FCCP, young GVs showed a similar phenotype to AMA oocytes, with a significant decrease in mitochondrial activity (intensity of 78614 ± 58534 IU in untreated and 11554 ± 16131 IU in treated GVs, p = 0.019), and an associated drop-in maturation rate. Ultimately, only 39.5% (17/43) of the young treated GVs were able to accomplish meiotic maturation.

Limitations, reasons for caution: Maturation rates were assessed by the presence of an extruded PB and variations in spindle assembly timings may have been overlooked. The quantification of mitochondrial activity in loss of function studies was assessed only by IF staining.

Wider implications of the findings: Our findings demonstrate the presence of a functional link between oocyte metabolism and impaired meiosis, which may contribute to the decline of oocyte quality with age. Given that live-metabolic imaging is able to discern GVs that progress through meiosis, this approach may represent a valuable non-invasive tool in oocyte selection.

Trial registration number: not applicable

Abstract citation ID: dead093.252

O-206 Growth hormone supplementation reduces aneuploidy and improves the quality of aged oocytes by activating the JAK2-ERK1/2 pathway

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Study question: Does growth hormone (GH) supplementation improves the quality of oocytes in aged mice via reducing aneuploidy?

Summary answer: Daily injections of GH for 8 weeks reduces aneuploidy by activating JAK2-ERK1/2 pathway and improves the quality of oocyte in aged mice.

What is known already: Aging-induced low-quality oocytes, which are mainly caused by aneuploidy, decrease female fertility. However, few treatments are available to improve the poor quality of oocytes in aged women. Age-related decline in GH levels may be related to the insufficient reproductive potential in aged women. Several studies have reported that GH improves assisted reproductive technology outcomes in poor ovarian response patients, although its role is still controversial. In animal models, GH supplementation improved the oocyte quality by enhancing mitochondrial function. However, whether GH reduces the aneuploidy rate in aged oocytes, and if so the underlying mechanisms, are unclear.

Study design, size, duration: The aged (8-month-old) C57BL/6J female mice were used in the study. For the *in vivo* experiments, the aged mice were intraperitoneally injected with GH (GenSci, Changchun, China; 1.6 mg/kg body weight) or an equivalent volume of normal saline for 8 weeks; For the *in vitro* experiments, germinal vesicle (GV) oocytes from aged mice were treated with GH (200 ng/mL) or PBS in M16 medium.

Participants/materials, setting, methods: We counted oocytes by immunohistochemistry to assess ovarian reserve. The expression of growth hormone receptor and mitochondrial genes were measured by quantitative real-time PCR. Time–lapse incubator was utilized to record the developmental potential of early embryos. Immunofluorescence was performed to assess parameters of oocyte quality (mitochondrial functions, spindle/chromosome defects, and DNA damage). Chromosome spread and DNA sequencing were used to analyze aneuploidy rate. We performed quantitative proteomics analysis to identify the potential targets of GH.

Main results and the role of chance: Firstly, we found that GH promoted GHR expression in aged oocytes (30.64 ± 1.70 vs 21.68 ± 1.08, $P < 0.001$). GH ameliorated aging-induced decline in ovarian reserve of aged mice, with increased ovarian index (0.040% ± 0.0031 vs 0.023% ± 0.0019, $P < 0.01$), and number of antral follicles (8.67 ± 1.2 vs 3.33 ± 0.88, $P < 0.05$) compared to the controls. In addition, GH improved the quality of aged oocytes, representing restored mitochondrial functions ($P < 0.05$), decreased DNA damage (23.37 ± 1.76 vs 38.35 ± 2.52, $P < 0.001$) . As expected, GH promoted the fertilization rate (39.9% ± 1.10 vs 14.4% ± 0.62, $P < 0.001$) and early embryo development ($P < 0.01$) of aged oocytes. Of note, GH recovered the spindle/chromosome defects (42.6% ± 2.12 vs 63.9% ± 1.83, $P < 0.01$, and reduced aneuploidy rate (20.9% ± 1.61 vs 39.3% ± 1.84, $P < 0.01$) in aged oocytes. GH activated ERK1/2 expression to decrease aneuploidy rate in aged oocytes. In addition, JAK2 might be involved in the regulation of GH on ERK1/2 expression in aged oocytes. Altogether, GH restored age-related decline in oocyte number, and decreased the occurrence rate of aneuploidy by activating JAK2-ERK1/2 pathway, thereby improving the quality of aged oocytes.

Limitations, reasons for caution: The molecular action mechanism of GH to regulate aneuploidy remains to be determined. Besides, future work should be extended to human oocyte to determine whether this mechanism is conserved between mice and humans

Wider implications of the findings: Our work expounds a theoretical basis for application of GH to improve the fertility of aged women. Besides, the results also feed new ideas for the prevention and treatment of oocyte quality decline in assisted reproductive technology.

Trial registration number: not applicable

Abstract citation ID: dead093.253

O-207 zp1 mutation affects oocyte energy metabolism and leads to oocyte apoptosis

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Study question: How does the zp1 variation leads to apoptosis oocytes in antral follicular and infertility in females?

Summary answer: Mutant zp1 induces abnormal pyruvate transport leading to the accumulation of reactive oxygen species (ROS), which activates the mitochondria-mediated apoptosis pathway leading to oocytes apoptosis.

What is known already: Previous research reported a zp1 mutation in human infertility, and we develop a homologous rat strain to investigate the pathogenic effect. The ovaries of homozygous rat posses growing and fully grown oocytes, the oocytes completely lack a zona pellucida. Only 1-2 eggs were recovered from oviducts of per superovulated homozygous rat, and were not surrounded by a zona pellucida thus lost their fertilization capacity in vitro.Further studies showed that the homozygous female rats not only lacked ZP but also TZPs (an important communication bridge between granulosa cells and oocytes), and the caval follicles showed obvious apoptotic signals.

Study design, size, duration: In this study, 10 Sprague-Dawley rats were used at 8 weeks of age (5 wild-type and 5 mutant respectively).

Participants/materials, setting, methods: We used CRISPR/Cas 9-mediated genomic editing technology to develop a rat model carrying the homologous 8bp deleted mutation in Zp1 gene(Zp1mt/mt). The rats were sacrificed by cervical vertebra dislocation and their ovaries were isolated immediately. Then using bulk RNA sequencing of ovaries from Zp1mt/mt and wild-type female rats to identify the differential expression gene between the two groups.The differentially expressed genes enriched from KEGG were verified by qRT-PCR and in vitro functional experiments.

Main results and the role of chance: Total of 293 DEGs were obtained, of which 144 were up-regulated and 149 were down-regulated. The results of biological process showed that up-regulated genes were mainly involved in redox reaction and negative regulation of cell migration.Down-regulated genes are mainly involved in the follicular growth and development,and related to oocyte energy metabolism.

In order to verify the DEG results obtained from RNA sequencing, the differentially expressed gene *Gcgr*, *Plcβ2*, *Aox3* and *Hpgd* enriched from KEGG in cell metabolism were verified by qRT-PCR. The results showed that the differentially expressed gene results were consistent with the expression trend of sequencing data.The results showed that there was no significant difference in the expression of *Pkm* in granulosa cells, while there was a significant difference in the expression of *Pdk4* in Zp1mt/mt oocytes.

For ROS detection, 52 eggs of 4 *Zp1*mt/mt rats and 48 eggs of 6 wide-type rats were analyzed for reactive oxygen species. The results showed that the IOD of mutant eggs was significantly higher than that of wild-type,indicating that ROS content in mutant eggs was significantly increased, which was statistically significant.The detection of apoptotic protein expression in eggs shows that the apoptosis probability of Zp1mt/mt eggs is significantly higher than that ofwild-type.

Limitations, reasons for caution: This study provides more experimental basis and data support for the involvement of ZP in oogenesis, but the effect of ZP deficiency on the dynamic transcriptional regulation of developing oocytes-granulosa cells and the specific molecular mechanism remain to be further explored.

Wider implications of the findings: The work provides key insights into the crucial features of the transcriptional regulation in Zp1 mutant animal ovaries, and offers important clues for exploring the potential mechanism of abnormal oocyte development due to ZP deficiency.

Trial registration number: none

Abstract citation ID: dead093.254

O-208 Maternal spindle transfer restores the developmental competence of in vitro aged oocytes with diminished metabolic activity identified by hyperspectral imaging

N. Costa-Borges[1], A. Parra[2], E. Mestres[1], M. Acacio[1], C. Castello[1], A. Seriola[2], S. Ojosnegros[2], G. Calderón[1]

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Study question: Can the developmental competence of poor-quality oocytes identified by hyperspectral imaging be restored by maternal spindle transfer?

Summary answer: Maternal spindle transfer can be efficiently assisted by hyperspectral imaging to identify and subsequently repair the developmental competence of poor-quality oocytes.

What is known already: Oocyte cytoplasmic dysfunctions (including, but not limited to the mitochondria) are a major cause for poor embryonic development. Given that maternal spindle transfer (MST) allows the replacement of the entire cytoplasm of an oocyte, it is a promising method for the enhancement of oocyte developmental competence. However, accurate diagnostic tools for oocyte quality assessment are lacking. Recently, a novel hyperspectral imaging approach has been shown to classify oocytes non-invasively based on their metabolic profile, which could assist in the evaluation of oocyte developmental potential prior to MST.

Study design, size, duration: Oocytes were obtained from young female mice and analyzed blindly by hyperspectral imaging either immediately after collection (fresh group, n = 122), after overnight culture (*in-vitro* aged group, n = 58) or on a group of MST oocytes (n = 36), where the meiotic spindle from *in-vitro* aged oocytes was transferred into enucleated fresh oocytes. Oocytes were inseminated by ICSI and cultured *in-vitro* individually. Correlations between the oocyte's metabolic profiles and rate of development to the blastocyst stage were performed.

Participants/materials, setting, methods: Oocytes were collected from 6-10 weeks-old superovulated B6CBAF1 females. MST was performed in manipulation medium supplemented with cytochalasin B. After enucleation, karyoplasts were exposed to an inactivated Sendai virus solution to promote membrane fusion. ICSI was performed using a Piezo-Drill actuator. Hyperspectral imaging was done in the near-infra-red regime to avoid photoxicity. Image processing was done using dimensionality reduction, which was then feed to an Akaike Information Criterion algorithm to classify the different metabolic subpopulations.

Main results and the role of chance: Fresh oocytes showed metabolic profiles that significantly differed from the profiles of *in-vitro* aged oocytes, which were characterized by a pronounced diminished metabolic activity. While in the fresh group, 67% of the oocytes developed into expanded blastocysts, none of the *in-vitro* aged oocytes with low metabolism produced a blastocyst ($p < 1x10^{-05}$). Interestingly, MST oocytes that were imaged 1h after nuclear reconstruction, showed a restored metabolic spectrum that resembled the profile from fresh non-manipulated controls. This restoration of the metabolic activity was concomitantly associated with an enhancement of developmental competence, with expanded blastocyst rates (63%) comparable to those of fresh non-manipulated controls ($p = 0.668$). Blastocyst developmental rates of fresh oocytes after hyperspectral imaging were comparable to oocytes from the same cohort that were not imaged and used as controls.

Limitations, reasons for caution: The metabolic profiles obtained from these experimental groups need to be correlated with implantation rates in mouse. The impaired embryo developmental pattern characteristic of poor-quality oocytes may not be exclusively attributed to metabolic deficiencies, so caution must be exerted when generalizing these results to oocytes with other cytoplasmic dysfunctions.

Wider implications of the findings: MST experiments confirm that the developmental decline of these oocytes is primarily attributed to abnormal function of cytoplasmic factors involved in metabolism, while the nuclear genome remains developmentally competent. MST can benefit from

hyperspectral imaging to identify oocytes with impaired developmental potential within a cohort.

Trial registration number: not applicable

SELECTED ORAL COMMUNICATIONS

SESSION 67: IMPROVED TECHNOLOGIES FOR EMBRYO ASSESMENT

Tuesday 27 June 2023 Hall D1 17:00 - 18:00

Abstract citation ID: dead093.255

O-209 The incidence of different ploidy alterations in abnormally fertilized oocytes (AFO)-derived embryos

L. Girardi[1], F. Cogo[1], P. Zambon[1], I. Pergher[1], J.A. Castellòn[2], C. Patassini[1], C. Rubio[3]

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Study question: Which is the incidence of *de novo* ploidy alterations in different abnormally fertilized embryos categories analysed with SNP-array?

Summary answer: Based on the number of pronuclei (PN) identified, 0PN embryos were mostly diploid, while 1PN and >2PN-derived embryos showed increased rates of ploidy abnormalities.

What is known already: Recent development of SNP-array and haplotyping by sequencing approaches are contributing to expanding preimplantation genetic testing (PGT) clinical utility including ploidy level evaluation. When applied to euploid embryos derived from AFO, the identification of abnormal ploidy constitution (i.e., haploidy, triploidy, or tetraploidy) could rescue viable diploid embryos otherwise considered non-transferable in IVF. However, no definitive genetic evidence has been obtained showing the incidence of different ploidy abnormalities in each AFO-derived blastocyst category. Here we present 4 years of experience in the clinical application of a validated SNP-array based protocol and custom-made algorithm to detect ploidy defects in AFO-derived embryos.

Study design, size, duration: Prospective observational study evaluating the incidence of altered ploidy configurations in AFO-derived blastocyst. After PN check, AFO samples were divided as follow: absence of observed pronuclei (0PN), monopronuclear (1PN), more than two pronuclei observed (2.1PN, with one smaller additional PN and 3PN). Genetic classification combining PGT-A and ploidy analysis was performed at Igenomix Italy laboratory between May 2019 and January 2023 on 133 AFO-derived embryos (44 0PN, 59 1PN, 30 >2PN).

Participants/materials, setting, methods: Multi-centre study involving 293 consenting patients of advance maternal age (mean=38.6 ± 3.9) undergoing PGT-A on MDA-WGA using Ion ReproSeq kit and IonTorrent S5 (ThermoFisher). SNP-array-based ploidy test was performed using HumanKaryomap-12 kit and NextSeq550 (Illumina). Proprietary algorithm was based on genome-wide BAF obtained: expected BAFs were 1, 0.5 or 0 for diploids,1 or 0 for haploid and 1, 0.66, 0.33 or 0 for triploids, as determined by the frequency of each allele for 300000 SNPs loci.

Main results and the role of chance: Preclinical validation of SNP-array-based ploidy test included 26 samples with known ploidy status and karyotype: 7 triploid re-biopsies, 7 haploid left-overs and 12 cell lines. Moreover, inter-platform comparison of 72 diploids, 9 triploids, 8 haploids embryos analysed with an alternative targeted-NGS approach, validated by Igenomix, showed 100% (95% CI= 95.94%-100%) concordance. In this study, a total of 318 AFO-derived embryos were collected for PGT-A analysis for different indications. Of these, 58.2% (n = 185/318; 95%CI=52.54-63.66) resulted as aneuploid. The remaining 133 euploid AFO-derived blastocyst where subjected to SNP-array based ploidy assessment. 0PN-derived blastocysts (n = 44/133) were diploid in 93.2% (n = 41/44; 95%CI=81.34-98.57) of cases; only 4.5% (n = 2/44; 95%CI=0.56-15.47) were haploid and 2.3% (n = 1/44; 95%CI=0.06-12.02) were polyploid. 1PN-derived blastocysts showed all possible ploidy configurations in these proportions: 59.3% (n = 35/59; 95%CI=45.74-71.93) haploid, 35.6% (n = 21/59; 95%CI=23.55-49.13) diploid and 5.1% (n = 3/59; 95%CI=1.06-14.15) polyploid. Finally, in the group of AFO where more than 2PN were observed (2.1PN or 3PN), the additional PN resulted in 66.7% (n = 20/30; 95%CI=47.19-82.71) of polyploid configurations, the remaining 33.3% (n = 10/30; 95%CI=17.29-52.81) were diploid. No haploid results were obtained from this category. Chi-square test showed significant overall correlation between PN category and ploidy status configurations ($p < 0.05$). Collectively, ploidy evaluation of 133 AFO-derived embryos, allowed the clinical use of 72 additional euploid/diploid embryos otherwise not considered for transfer.

Limitations, reasons for caution: Ploidy assessment protocol was not tested to distinguish triploids from tetraploids. Parental origin of each chromosomal set could not be determined without analysing parental DNA. The way of performing PN check could have affected AFO classification. In future studies we will focus on evaluating clinical outcomes following euploid/diploid embryo transfer.

Wider implications of the findings: An altered number of pronuclei is predictive of the correspondent altered ploidy status, while 0PN category doesn't represent a clear indication for ploidy evaluation. Nevertheless, a significant proportion of euploid/diploid embryos can be rescued from all types of AFO, potentially increasing the overall chance to achieve a live birth.

Trial registration number: n/a

Abstract citation ID: dead093.256

O-210 Detection of small copy number variations as incidental findings in PGT-A: clinical utility from a multisite experience including 12,157 patients

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Study question: What is the technical accuracy and clinical utility of reporting small copy number variants (CNVs below 3Mb) detected by a targeted next-generation sequencing-based PGT-A platform?

Summary answer: Some pathogenic or likely pathogenic CNVs <3Mb can be accurately detected by this assay, increasing clinical utility of PGT-A for a subset of IVF patients.

What is known already: CNVs are linked to a wide range of phenotypes, spanning from syndromes that include reduced penetrance and variable expressivity to more severe phenotypes. Prenatally, the prevalence of pathogenic CNVs is approximately 1.5%. Most PGT-A platforms that rely on whole genome amplification and shallow sequencing have a resolution limit of 5-10 Mb, preventing the detection of smaller CNVs. Here, we report an innovative PGT-A assay that interrogates thousands of targeted sites in the genome to provide robust copy number analysis allowing for the identification of some small CNVs (incidental findings, IF), outside the primary scope of detecting whole-chromosome aneuploidies in PGT-A.

Study design, size, duration: Retrospective observational study performed between 2020-2022, involving 12,157 patients who underwent PGT-A performed by targeted-NGS (PGTseq-A) for whole chromosome and large segmental aneuploidies. If an IF < 3 Mb was detected in multiple embryos, the couple was advised to undergo follow-up analysis by chromosomal microarray (CMA) to confirm the parental origin of the CNV, define its breakpoints and determine whether it is classified as benign-likely benign (B/LB), variant of uncertain significance (VUS) or pathogenic-likely pathogenic (P/LP).

Participants/materials, setting, methods: The PGTseq-A assay employed amplifies 5,000 amplicons across the genome to evaluate copy number. Validation was performed using 5-cell samples from cell lines with known CNVs, and trophectoderm biopsies from embryos with known parental structural rearrangements. An IF was reported when a gain/loss of at least

three consecutive amplicons appeared in at least two embryos from the same cycle. Transfer data was reviewed to determine which embryos were transferred. This study received IRB approval.

Main results and the role of chance: In 77 out of 12,157 PGT-A patients (0.63%;95%CI:0.5-0.8%), an IF that met reporting criteria was identified. To determine the size and pathogenicity, a CMA follow up was requested and performed on 67 couples. In all cases, one of the partners was confirmed to have the CNV identified in the embryos (100.0%: N 67/67 95%CI:94.6-100). The identified CNV was of maternal origin in 36 cases (53.7%) and of paternal origin in 31 cases (46.2%). A strong correlation was identified between PGT-A-predicted CNVs and the genomic coordinates defined by the CMA on parental DNA ($r = 0.86$). All CNVs intervals predicted by the PGT-A included the CMA genomic region. Twenty-seven (40.2%) were classified as B/LB, 31 (46.2%) as a VUS, and 9 (13.4%) as P/LP. Six of the nine P/LP cases (66.6%) involved imbalances of 16p. The remaining P/LP cases involved deletions of X, 15q and 18p11.32. From a review of transfer data, patients typically transferred embryos negative for IFs as first option regardless of the morphology and pathogenicity classification of CNV-positive sibling embryos. Specifically, in 8 cycles where a detected CNV was classified as B, LB or as VUS, negative embryos were prioritized for transfer despite their poorer morphology.

Limitations, reasons for caution: De novo CNVs were not considered by design because the reported CNVs had to be present in at least two embryos from the same cohort. Furthermore, the detection of small CNVs is not uniform in the genome and the negative predictive value of the assay for non-targeted regions is null.

Wider implications of the findings: This PGT-A assay accurately detects small pathogenic CNVs without prior knowledge of parental inheritance, enhancing clinical utility in a subset of patients. Additionally, reporting of VUS and B/LB findings may impact couples' decision-making concerning embryo selection, reinforcing that scientific rigor is a necessity when evaluating the capabilities of PGT-A.

Trial registration number: not applicable

Abstract citation ID: dead093.257

O-211 A comparative analysis of non-invasive preimplantation genetic testing for aneuploidies using next-generation sequencing on day 5 and day 6 spent culture media versus trophectoderm

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Study question: Purpose of this study was to analyze concordances between trophectoderm and spent culture medium from different embryo culture length (day 5 and day 6).

Summary answer: Material collected from embryos cultured to day 6 are more suitable for non-invasive preimplantation genetic testing for aneuploidies.

What is known already: Ni-PGTA (Non-invasive Preimplantation Genetic Testing for Aneuploidy) is a relatively new testing method that analyzes spent culture medium from in vitro fertilization (IVF) embryos to determine the genetic makeup of the embryos. This non-invasive approach eliminates the need for biopsy and allows for the identification of chromosomal abnormalities such as aneuploidy. The utility of ni-PGTA is to provide an efficient, accurate, and safe method for preimplantation genetic screening. The concordance between ni-PGTA and traditional biopsy-based methods for detecting aneuploidy is high, but still further research is needed to fully evaluate its accuracy and reliability.

Study design, size, duration: The study was conducted in 2021-2022 and approved by the Institution of the Bioethical Committee operating at the Regional Medical Chamber in Krakow (No. 161/KBL/OIL/2021). Informed consent for participation in the study was obtained from all patients. A total of 130 embryos were obtained: 70 from 15 patients after 5 days of culture and 60 from 11 patients after 6 days of culture.

Participants/materials, setting, methods: Whole genome amplification of collected materials was conducted using the SurePlex Kit (Illumina, San Diego, CA, USA). The libraries was prepared using VeriSeq PGS (Illumina, San Diego, CA, USA) according to manufacturer protocol. Sequential analysis was then performed on the miSeq device (Illumina, San Diego, CA, USA). The efficiency of WGA, the concordance of chromosome status between biopsied cells and media were investigated.

Main results and the role of chance: The mean DNA concentration of WGA products from embryos for day 5 from TE was 29,6 ± 2,7 (ng/µl ± SD) for SCM 23,4 ± 4,2 (ng/µl ± SD) and for day 6 from TE was 29,5 ± 2,7 (ng/µl ± SD) for SCM 33,4 ± 6,1 (ng/µl ± SD). The mean DNA concentration of TE between day 5 and day 6 are comparable but the comparison between SCM from day 5 and 6 shows significant difference. 5 of 60 samples considered uninformative based on a Derivative Log Ratio (DLR) - DLR >0.4 (TE day 6: 1 sample and SCM day 6: 4 samples) were excluded from the analysis. All samples form day 5 were informative. The overall concordance rate between the ni-PGTA for day 5 and PGTA was 39/70 (55,7%) and ni-PGTA for day 6 and PGTA was 52/55 (94,5%). Sex chromosome consistence between the ni-PGTA for day 5 and PGTA was 54/70 (55,7%) and ni-PGTA for day 6 and PGTA was 51/55 (92,7%)

Limitations, reasons for caution: Special care was taken while washing embryo before changing culture medium in order to avoid maternal contamination. Special precautions were taken to avoid losing genetic material between consecutive washing steps of TE. When Conducting ni-PGTA analysis, maternal contamination needs to be considered and results interpreted with caution.

Wider implications of the findings: The results of invasive PGT-A using TE are similar to the non-invasive method based on SCM. In addition, the best results were recorded for SCM cultured up to day 6. These results are another piece of evidence confirming utility of SCM analysis for the purpose of aneuploidy evaluation.

Trial registration number: not applicable

Abstract citation ID: dead093.258

O-212 Screening IVF embryos with polygenic risk scores: risk reduction estimates, population impact, and attitudes of patients and clinicians

S. Carmi[1], D. Barlevy[2], L. Klausner[1], R. Furer[3], K.M. Kostick-Quenet[2], M. Mukherjee[4], I. Cenolli[3], T. Lencz[5], G. Lázaro-Muñoz[3], S. Pereira[2]

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[2]Baylor College of Medicine, Center for Medical Ethics and Health Policy, Houston-TX, U.S.A.
[3]Harvard Medical School, Center for Bioethics, Boston- MA, U.S.A.
[4]University of California- Berkeley, Sociology, Berkeley- CA, U.S.A.
[5]Zucker School of Medicine at Hofstra/Northwell, Psychiatry and Molecular Medicine, Hempstead- NY, U.S.A.

Study question: IVF embryos can now be screened for complex diseases. What is the expected risk reduction? What are the attitudes of patients and clinicians?

Summary answer: Substantial risk reductions can be achieved, but only in the best-case scenario. The views of patients and clinicians regarding the technology are not fully aligned.

What is known already: It recently became possible to screen IVF embryos for a variety of complex diseases using polygenic risk scores. Prioritization of embryos for transfer based on these scores may lead to reduced disease risk in offspring. Preliminary risk reduction modeling suggested that substantial risk reductions are possible, particularly when screening for a single disease. However, multiple factors remain unexplored, such as the effect of implantation failure and assortative mating on risk estimates and the impact of embryo

prioritization on population allele frequencies. The attitudes of IVF patients and clinicians towards polygenic embryo screening are also unknown.

Study design, size, duration: We estimated the expected risk reduction under various settings using statistical modeling, based on the liability threshold model and other quantitative and population genetic models.

To learn about attitudes of patients and clinicians, we performed in-depth, semi-structured interviews with 26 USA patients and 27 USA clinicians over a period of about one year.

Participants/materials, setting, methods: We used R to numerically estimate risk reductions and implemented a web app in Shiny.

To recruit patients and clinicians for the interviews, we used flyers (for patients), emails on publicly available lists (for clinicians), snowball sampling, and convenience sampling. Interviews were conducted over Zoom for one hour each by a single interviewer (D.B.). The study was approved by Baylor College of Medicine's IRB committee. We applied deductive and inductive thematic analysis using a team-based approach.

Main results and the role of chance: Statistical genetic modeling suggested that with five viable embryos, risk reductions of up to 50% can be achieved when screening for a single common disease. We developed a risk reduction estimator web app, allowing users to examine the effect of the number of embryos, the accuracy of the score, the selection strategy, and the score percentile or disease status of the parents. Assortative mating and modeling of implantation failures are expected to only slightly decrease risk reductions. Risk allele frequencies are expected to change very little across generations even if embryo selection becomes widespread.

In interviews, patients accepted screening for health conditions, particularly severe diseases. Many were concerned about costs, and some were interested in screening for informative purposes. Most patients did not oppose screening for traits. In contrast, about half of clinicians were unwilling to offer screening due to concerns over utility, implementation, harm to patients, and lack of scientific validity, although all were willing to reconsider it in the future for specific conditions. Many clinicians considered screening for traits a non-medical procedure. About half agreed that screening should be regulated.

Limitations, reasons for caution: The risk reduction estimates may depend on the assumptions of the statistical models. As with any qualitative research, interview findings may not be generalizable. Furthermore, the design of the semi-structured interview guide may have influenced participants' expressed attitudes.

Wider implications of the findings: In the best-case scenario, prioritizing IVF embryos based on polygenic scores may achieve substantial risk reductions. Several initial concerns seem unfounded. IVF patients are broadly interested in the technology but may be unaware of its many limitations. Clinicians have major concerns regarding clinical implementation and harm to patients.

Trial registration number: not applicable

SELECTED ORAL COMMUNICATIONS

SESSION 68: IMPACT OF LIFESTLY AND ENVIRNOMENT ON MAR

Tuesday 27 June 2023 Hall D4 17:00 - 18:00

Abstract citation ID: dead093.259
O-213 Breathing New Life into Air Pollution Research: Examining the Link Between Air Pollution and Adverse Birth Outcomes in the Eastern Mediterranean Region (EMR)

E. Whaibeh[1,2], G. Abi Tayeh[3], W. Farah[4], M. Abboud[4], M. Mrad Nakhlé[1]

[1]*University of Balamand UOB, Public Health Department, Beirut, Lebanon*
[2]*Université Saint-Joseph de Beyrouth USJ, Doctoral School of Health and Sciences-Faculty of Medicine, Beirut, Lebanon*
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[4]*Université Saint-Joseph de Beyrouth USJ, UEGP – Environmental- Genomic and Proteomic Research Unit- Faculty of Sciences, Beirut, Lebanon*

Study question: What is the current understanding of the relationship between air pollution and perinatal health in the EMR?

Summary answer: The literature on exposure to air pollutants and perinatal health in the EMR is scarce, limited in scope, and using simply study designs and methods.

What is known already: Air pollution is a significant public health concern in the Eastern Mediterranean Region, due to many factors such as the arid nature of the region, rapid urbanization, oil production, and the emissions from the transportation, energy productions, and other practices. Not only can the degradation in air quality put the health of infants in jeopardy but it can also increase the risk of adverse pregnancy outcomes. Studies on air pollution and birth outcomes and early life development have been conducted in other parts of the world, but only a small number have been conducted in the EMR.

Study design, size, duration: The authors conducted a comprehensive search of various databases to identify relevant studies and publications on the topic. The findings were then critically evaluated and discussed in relation to the current understanding of the relationship between air pollution and perinatal health in the EMR. The study does not include any new data collection or primary research. The authors used a narrative approach to synthesize the existing literature on the topic.

Participants/materials, setting, methods: Not applicable

Main results and the role of chance: The existing literature is concentrated in specific geographic locations out of the 22 countries and territories that comprise the region, and it is focused on a limited number of exposures and outcomes, namely secondhand smoking and criteria air pollutants. The main gaps include the inconsistent and poorly funded air quality monitoring, inappropriate study designs, imprecise and/or unreliable assessments of exposures and outcomes, and the focus on traditional air pollutants rather than new emerging and increasingly concerning air pollutants. Moreover, even though the studies establish associations between air pollutants and adverse birth outcomes, the mechanisms through which these processes take place are yet to be fully understood. An area of research that holds great potential in explaining these processes is epigenetics; however, due to limited funding, expertise and health and environmental data, epigenetic research on the impact of air pollution in the EMR is still in its infancy.

Limitations, reasons for caution: The review is based on a narrative approach, which is subjective and may not be as rigorous as a systematic review. The reviewed studies also have their own limitations, such as inconsistent air quality monitoring and imprecise assessments of exposures and outcomes. Studies focused on epigenetics in EMR are scarce.

Wider implications of the findings: The review highlights the need for future research examining the epigenetic processes that underlie the adverse birth outcomes, to better understand them and to develop effective recommendations and intervention strategies.

Trial registration number: Not applicable

Abstract citation ID: dead093.260
O-214 interaction of air pollution and meteorological factors on IVF outcomes: a multicenter study in China

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[2]*Tianjin Central Hospital of Obstetrics and Gynecology, Obstetrics and Gynecology, Tianjin, China*
[3]*The Second Hospital of Hebei Medical University, Department of Reproductive Medicine, Shijiazhuang, China*
[4]*The Affiliated Hospital of Inner Mongolia Medical University, Reproductive Medicine Centre, Hohhot, China*
[5]*Shanxi Women and Children's Hospital, Gynecology and Obstetrics Center, Taiyuan, China*
[6]*Jinghua Hospital, none, Shenyang, China*

Study question: Is there an interaction between air pollution and meteorological factors on IVF outcomes?

Summary answer: The correlation between air-pollutant exposure and IVF outcomes was modified by meteorological conditions, especially temperature and wind speed.

What is known already: Previous studies revealed associations between air-pollutant exposure and *in vitro* fertilization (IVF) outcomes. However, modification effects of air pollution on IVF outcomes by meteorological conditions remain elusive.

Study design, size, duration: Multicenter retrospective cohort study, 2015–2020.

Participants/materials, setting, methods: This multicenter retrospective cohort study included 15,217 women from five northern Chinese cities during 2015–2020. Daily average concentrations of air pollutants ($PM_{2.5}$, PM_{10}, O_3, NO_2, SO_2, and CO) and meteorological factors (temperature, relative humidity, wind speed, and sunshine duration) during different exposure windows were calculated as individual approximate exposure. Generalized estimating equations and stratification models were used to assess the associations of air pollution and meteorological conditions with IVF outcomes and estimated potential interactions.

Main results and the role of chance: Exposure to $PM_{2.5}$, SO_2, and O_3 was adversely correlated with pregnancy outcomes in fresh IVF cycles. Positive associations of wind speed and sunshine duration with pregnancy outcomes were detected. In addition, we observed that embryo transfer in spring and summer had a higher likelihood to achieve a live birth compared with winter. In stratified models, pieces of evidence of interaction between meteorological factors and air pollutants on pregnancy outcomes were detected. Negative associations of $PM_{2.5}$ with clinical pregnancy were only significant at lower temperatures and wind speeds. Moreover, the effects of O_3 on live birth were enhanced by higher quartiles of wind speed.

Limitations, reasons for caution: First of all, we used the data from monitoring stations to estimate the individual exposure, which was inaccurate and may introduce bias. Second, due to data limitations, this study did not discuss socio-economic factors, which were reported to be associated with spatial-temporal variations of air pollutants and reproductive outcomes.

Wider implications of the findings: Pregnant women should be advised to reduce outdoor time when the air quality was poor, particularly at lower temperatures.

Trial registration number: not applicable

Abstract citation ID: dead093.261
O-215 Negative impact of the reduction in family income following the SARS CoV 2 Covid 19 pandemic on access to Assisted Procreation : observational study

A. Pacchiarotti[1]

[1]*San Filippo Neri Hospital, IVF Unit, ROMA, Italy*

Study question: What are the consequences of the health emergency and the consequent restrictive measures on the economic accessibility of medically assisted procreation techniques.

Summary answer: Reduction of family income rate and consequentely bias of PMA attempts rate

What is known already: - The birth rate in Italy over the past 10 years has been declining rapidly and continuously

- Fertility income correlation has an inversely proportional trend demonstrated in a study conducted by the Ministry of Economy and Finance and Istat

- Pandemic has further impoverished Italian families

Study design, size, duration: This is a transversal observational study conducted at the Assisted PReproduction Center of the San Filippo Neri hospital in Rome between Jenuary 2021 and December 2022

The results were obtained by administering an online questionnaire consisting of 20 multiple choice questions to which the participants answered anonymously.

Participants/materials, setting, methods: Two hundred and ninety-five patients were enrolled with an overall average age of 37.5 years (minimum age 25, maximum age 47).

Main outcomes measures. Bias of PMA attempts rate. Reduction of family income rate

Main results and the role of chance: Majority of people said they have not seen their desire for offspring reduced since the onset of health emergency. the willingness to use MAP techniques during the pandemic remained unchanged for 58.7% of respondents, increased for 12.5% and decreased for the remaining 28.8%. The individuals who have declared to have momentarily renounced to the procedures of PMA have been 31.7%, of these 24% has renounced because of economic factors. When asked about the cost of the necessary medical procedures, 62.5% of the participants considered the price in public facilities to be "adequate" and 79.8% defined the cost in private clinics as "excessive".

Limitations, reasons for caution: A limitation of the study may be the comprehension of the questionnaire, since it could not be speieged verbally. The administration was done online, so there is no assurance that it was received by everyone. Therefore, the percentage of patients who did not respond may be overestimated

Wider implications of the findings: The study and analysis of these data provides a current overview of the condition of infertile couples in our country, so that all useful interventions can be put in place to restore the interest and economic conditions necessary for the initiation of medically assisted procreation.

Trial registration number: not applicable

Abstract citation ID: dead093.262
O-216 Sex, smoking, age and natural fecundity: results of a survey performed on 2510 puerperal women and their partners

M.I. Limia Pérez[1], **R. Matorras Weining**[1]

[1]*Cruces University Hospital, Gynecology and Obstetrics, Barakaldo, Spain*

Study question: Sex, smoking, age and natural fecundity: results of a survey performed on 2510 puerperal women and their partners.

Summary answer: We reported that the best fertility marker was amongst couples who had 7 intercourses per week.

What is known already: Most reproductive scientific societies recommend that infertility studies begin after 12 months of unprotected intercourse without pregnancy. There is a series of parameters whose influence on assisted reproduction has been extensively studied, but which have received little attention when studying natural fertility. For example, it is well known that human fertility is strongly related to female age and that there is a decline in ART success rates with increasing woman's age. Also smoking has been reported to decrease fertility in women, both in natural fertility and in ART cycles.

Study design, size, duration: The aim of our study was:

1) to analyze the cumulative fecundity rate (CFR), the per month fecundity rate (FR) and the time to pregnancy (TTP).

2) to ascertain the influence of woman and male age, smoking, previous fertility and frequency of sexual intercourse in the aforementioned fecundity markers.

Participants/materials, setting, methods: The study population consisted of 2510 puerperal women and their partners whose labor was performed at our Hospital. During a 12-month period, an anonymous survey was performed during hospital admission, within 36-72 hours postpartum. The survey was offered to all postpartum women, although only those who met the inclusion criteria were analyzed.

Main results and the role of chance: - There was a close correlation between woman's age and male's age (Pearson coefficient= 0.72, $p < 0.00001$). When the age of one partner was considered independently of the age of the other there was no correlation between woman's age and TTP (Person coefficient = 0.04, $p = 0.08$) or between man's age and TTP (Person coefficient = 0.02, $p = 0.38$).

- There was a significant correlation between TTP (time to pregnancy) and man's age (Pearson coefficient= 0.07, p = 0.0006).
- We observed a significantly higher fecundity rate (FR) at the first month and a significantly higher cumulative fecundity rate (CFR) at 3 and 6 months in non smokers. Moreover we found a significant correlation with the number of cigarettes smoked and the TTP. It has to be highlighted that in our study the fertility impact of smoking was remarkably higher in men than in women.
- Pregnancy rates were similar, independently the existence or not of previous pregnancies.
- Regardless of coital frequency and natural fertility, our study revelaed two remarkable facts, one related with infrequent intercourse and the other regarding frequent. The best fertility marker among the couples was reported a 7 per week coital frequency.

Limitations, reasons for caution: - Only couples reaching pregnancy were studied.
- Second hand smoking was not considered.
- Possible inaccuracy because at long-term recall, TTP may sometimes only be estimated roughly by couples completing the questionnaire.
- Assumption that it is only one cycle per month.
- Misscariages were not taken into account.

Wider implications of the findings: Our results challenge the widely accepted idea that among healthy women trying to conceive, nearly all pregnancies can be attributed to intercourse during a 6-day period ending on the day of ovulation. We reported that the best fertility marker was amongst couples who had 7 intercourses per week.

Trial registration number: Not applicable

SELECTED ORAL COMMUNICATIONS

SESSION 69: REGULATION, INNOVATION, AND MORAL RESPONSIBILITY IN MEDICALLY ASSISTED REPRODUCTION

Tuesday 27 June 2023 Auditorium 10-12 17:00 - 18:00

Abstract citation ID: dead093.263
O-217 Balancing 'the desires of individuals' against 'the situation of the world as a whole': (Re)considering planetary responsibilities in the context of assisted reproduction

S. Segers[1]

[1]*Maastricht University, Department of Health- Ethics and Society, Maastricht, The Netherlands*

Study question: How do the moral reasons for offering access to assisted reproductive technology (ART) relate to ethical responsibilities generated by sustainability and planetary health duties?

Summary answer: If one accepts that both governments and medical professionals should yield to environmental sustainability, it is ethically pertinent to balance this against respecting reproductive autonomy.

What is known already: Respecting autonomy is a central ethical value in the context of reproduction, which, in the context of ART, is considered to generate a prima facie obligation to help people conceive a child in line with their personal goals. Arguments in terms of population ethics, use of planetary resources and climate change are traditionally not considered to counter arguments for access to ART relying on the reproductive autonomy of individuals. This may come under tension due to adjusted encouragements to physicians for being committed to sustainability as part of professional role responsibilities.

Study design, size, duration: A literature study of academic literature and institutional codes of conduct was performed to inventory how professional duties of physicians are being directed to encompass not only responsibilities towards individual patients, but also towards environmental sustainability. Consideration of earlier documents has shown that, sporadic objections notwithstanding, such arguments have mostly been morally inconsequential in the regulation and praxis of ART. This was critically evaluated against the backdrop of bioethical literature and ethical principles.

Participants/materials, setting, methods: Literature study; conceptual analysis; normative analysis.

Main results and the role of chance: Climate change and its future impact is on the political agenda as a shared responsibility of governments, industry, and citizens. This is seeping through into communiqués of professional medical bodies and codes of conduct. In the particular context of ART, this has recently led to several calls to recognize the impact of climate change on fertility. This has not (yet) led to an institutionalization of the more provocative claim, sometimes voiced in popular media, that fighting climate change may require restraints in the area of procreation. Nonetheless, claims that physicians have a responsibility to contribute to a sustainable healthcare sector at least require ethical attention in this regard, especially in view of observed shifts compared to canonic positions. Likewise, if we expect governments to take efforts to attain sustainability goals, this may evoke moral questions about possible tensions with moral commitments to offering access to assisted procreation. This contribution addresses (i) the limited critical stance towards beliefs that procreation is problematic from a planetary health perspective, and (ii) the need to scrutinize the ethical balance for reproductive health professionals between accommodating (individual) procreative goals and more global responsibilities towards the world at large.

Limitations, reasons for caution: This is an agenda-setting analysis, and so it is not meant to serve as an exhaustive discussion of these moral concerns. While attention for future impacts of climate change is gaining momentum in healthcare, controversial suggestions to cap procreation is largely still limited to lay contexts.

Wider implications of the findings: This analysis is meant to propel ethical reflection about the balance between global elements that may restrain individuals' reproductive options. This puts into focus that moral considerations about individual and societal (and planetary) interests in the context of ART require continued specification and careful ethical deliberation.

Trial registration number: n/a

Abstract citation ID: dead093.264
O-218 A quantitative investigation into UK public attitudes towards embryo research and the 14-day rule

S. Norcross[1]

[1]*Progress Educational Trust, Director, London, United Kingdom*

Study question: Is there UK public acceptance for increased embryo research and the extension of the 14-day rule?

Summary answer: Respondents broadly supported the use of human embryos in research but believed the 14-day-rule remains 'about right'. However, this might be extended under certain circumstances.

What is known already: UK's Human Fertilisation and Embryology Act (1990 as amended) may be reviewed in the near future. The Act currently allows licensed research on embryos up to the equivalent of the 14th day of development. The former PET Patron, the late Baroness Mary Warnock, was largely responsible for establishing the 14-day rule in UK law. The rule was subsequently adopted by many countries and institutions around the world. Baroness Warnock's work on this issue gave confidence to scientists and the wider public alike.

Study design, size, duration: This is a quantitative piece of research commissioned by PET. The field work was carried out by Ipsos who interviewed a sample of 2,233 adults aged 16-75 in UK using its online i:omnibus. The responses were gathered between 24 and 27 March 2022. The data has been weighted to the known offline population proportions for age, working status and social grade within gender and Government office region.

Participants/materials, setting, methods: The principal material used was an online questionnaire developed by PET and its advisers with the input of Ipsos survey research experts. All research was carried out in accordance

with the requirements of the international quality standard for market research, ISO 20252, and in accordance with the Ipsos Terms and Conditions. All percentage calculations are rounded up to the nearest whole number. Where percentages do not add up to 100%, this is due to rounding.

Main results and the role of chance: =Only 11% of respondents could give a scientifically correct definition of a human embryo.

=More than 40% of those surveyed said they supported the use of human embryos in scientific and medical research.

=44% supported the government funding of such research with 17% being opposed.

=Asked whether the 14-day limit is too long, too short or about right, more than half of those who supported (or neither opposed nor supported) the use of laboratory-created human embryos in research responded that the limit was about right.

=Those not implacably opposed to embryo research were willing to countenance an extension to the '14- day rule', if reasons were presented to them.

=This demonstrates the importance of scientists giving a clear explanation of the benefits that such an extension to the 14-day rule may bring.

=The most supported reasons for an extension were to find new treatments for congenital diseases, improving medical understanding of stillbirth and miscarriage.

=However, with significant numbers remaining neutral or answering, 'Don't know', this situation could change. Public education is vital to help people engage.

=The sample size of 2233 people allowed for statistically significant differences to be identified and reduced the risk of misleading answers.

Limitations, reasons for caution: This research is a snapshot in time and public opinion can evolve with new information. A significant number of respondents gave a 'don't know' answers. If these members of the public were to shift their view to either 'support or opposition' then the landscape for embryo research could markedly alter.

Wider implications of the findings: The study suggests there is limited public understanding of this subject, as illustrated by only 11% of respondents selected the scientifically correct definition of an embryo. This gap in knowledge makes it challenging to have a meaningful national debate on the potential changes to the current law.

Trial registration number: Trial registration number is not applicable. PET are grateful for the generous support of Ferring without which it would not have been possible to conduct this piece of wide-ranging, nationally representative research.

Abstract citation ID: dead093.265
O-219 Surplus Frozen Eggs Lay to Waste Opportunities for Research and Treatment

G. Fuscaldo[1], M. Johnston1[1], S. Hunt[2], C. Mills[1], L. Rombauts[2]

[1]*Monash University, Monash Bioethics Centre, Clayton, Australia*
[2]*Monash University and Monash IVF, Monash Bioethics Centre- Department of Obstetrics & Gynaecology and Monash IVF, Clayton, Australia*

Study question: Is there a moral duty to donate surplus frozen eggs (SFEs)?

Summary answer: SFEs are a valuable resource with the potential to mitigate harm and improve well-being and should be donated where the costs to donors are small.

What is known already: In Victoria, Australia, eggs can be stored for up to 10 years, after which SFEs must be discarded, donated to research or to others. Our research shows that many people do not return to use their frozen eggs, many are left in storage and most SFEs are then discarded. At the same time, there is a serious shortage of donated gametes and a high demand for eggs for both research and reproductive treatments. The recent legalisation of mitochondrial donation in Australia has added to the demand for eggs for training, research, and clinical purposes.

Study design, size, duration: This paper considers whether donation of SFEs can be grounded on 'the duty of easy rescue', broadly defined as a moral duty to help others when the harm that might be averted is sufficiently great, and the costs are sufficiently small.

Participants/materials, setting, methods: We present an ethical analysis of the harms that could be mitigated by egg donation and the costs to donors associated with donation to both research and to others.

Main results and the role of chance: The harms associated with prolonged infertility, long waiting lists for donor eggs, and gamete donation in less regulated settings are well known. In addition, the use of donated eggs for mitochondrial replacement offers the possibility of avoiding the transmission of serious disease. Our research shows that many SFEs are abandoned or discarded but little is known about why people discard SFEs. Among the 'costs' to donors, there are logistical barriers such as the time needed, and the physical and emotional demands associated with donor eligibility screening. There may be additional emotional and ethical costs associated with donation to others; concerns about donor anonymity, the parental status of donors, and the meaning of genetic relationships have been cited as barriers to embryo donation and may also factor in egg donation. We suggest that while donation of SFEs to others may have costs, there are opportunities to facilitate this option, such as offering donor screening at the time of egg freezing, providing education about the current demand for SFEs, and greater discussion and critical reflection about the moral status of donors. We reason that donating to research does not incur the same 'costs' and in most cases, represents an 'easy rescue'.

Limitations, reasons for caution: This analysis considers 'costs' to donors and 'harms' relating to the shortfall of donor eggs. However, donors and recipients are not the only actors involved in egg donation. There may be other morally relevant considerations, such as the experience of donor-conceived people, that complicate assessments of costs, harms, and benefits.

Wider implications of the findings: The large number of SFEs abandoned and discarded suggests the need for more serious consideration about the disposition of SFEs, prior to egg freezing. In addition, more information should be provided to would-be users about the current fate of and demand for eggs.

Trial registration number: n/a

Abstract citation ID: dead093.266
O-220 Artificial intelligence for embryo selection: ethical, social and regulatory issues

C. Mills[1], M. Johnston[1], J. Koplin[1]

[1]*Monash University, Monash Bioethics Centre, Clayton, Australia*

Study question: What are the key ethical, social and regulatory issues raised by artificial intelligence technologies (AI) for embryo selection, and how might these be best managed?

Summary answer: Key issues with AI for embryo selection include deskilling, transparency, accountability, and fairness. These require attention to improve psychosocial and clinical outcomes in ART.

What is known already: Artificial intelligence (AI) technologies play a growing role in the assisted reproductive technology (ART) sector. Machine learning holds enormous promise for improving the selection of human embryos for transfer after IVF. Time-lapse imaging in conjunction with machine learning has the potential to standardise and automate embryo selection, improving clinical outcomes. Promising results have recently been published, and AI tools have reached the market. However, ethical and regulatory analysis has not kept pace with the technology. There has been negligible research on the ethical, social, and regulatory issues raised by the use of machine learning methods for embryo selection.

Study design, size, duration: A terrain-mapping review of the academic literature was performed to identify the key social, ethical and regulatory issues raised by AI for embryo selection. This is part of a multi-method study, collecting qualitative and quantitative data alongside ethical and regulatory analysis.The study is expected to take 1 year.

Participants/materials, setting, methods: A systematic review of the extant literature on the ethical, social, and regulatory issues raised by machine learning for embryo selection, combined with a narrative review of the broader

literature on the use of AI in healthcare was undertaken. This review of ethical and regulatory issues will ultimately combine with data from semi-structured interviews and an online survey of ART patients and practitioners, including fertility specialists and embryologists, and ART regulators in Australia.

Main results and the role of chance: This paper reports on ethical and regulatory analysis of key issues that emerge from the use of AI for embryo selection. The use of AI in healthcare generally has raised serious concerns about deskilling and 'dehumanization'(as roles traditionally performed by humans are displaced by technology), transparency, accountability, and fairness. This paper examines these issues in the ART context, to begin to develop guidance on how to best implement this technology in ways that balances the interests of all stakeholders and is consistent with maintaining public trust in the field of ART.

Limitations, reasons for caution: It is anticipated that much of the analysis of the social, ethical and regulatory issues will be generalisable internationally. However, the study prioritises one context - that of Australia. Some features of the Australian context may limit generalisability.

Wider implications of the findings: There is a need to address the ethical, social and regulatory issues that arise with the emergence of innovative technologies within ART to maintain public trust and ensure that psychosocial and clinical outcomes are improved. Further data collection on stakeholder views of machine learning for embryo selection will be required.

Trial registration number: Not applicable

SELECTED ORAL COMMUNICATIONS

SESSION 70: INNOVATIVE APPROACHES TO TREATING INFERTILITY

Tuesday 27 June 2023 Hall D5 17:00 - 18:00

Abstract citation ID: dead093.267

O-221 Regenerative endometrial PRGF (plasma rich in growth factors) treatment in patients with thin endometrium, recurrent implantation failure, and recurrent miscarriages: a retrospective, self-controlled, cohort study

D. Bodri[1], S. Rogel[1], J.L. Abad[2], R. Paul[2], H. Izquierdo[1], J. Aizpurua[1]

[1]*IVF Life Alicante, Assisted Reproduction, Alicante, Spain*
[2]*IGLS, Reproductive Genetics and Immunology Laboratory, Alicante, Spain*

Study question: What reproductive outcomes are observed in patients submitted to endometrial PRGF therapy due to thin endometrium (ThE), recurrent implantation failure (RIF) and recurrent miscarriages (RM)?

Summary answer: Following endometrial PRGF treatment, success rates were significantly increased in ThE and RIF patients, whereas in the RM group pregnancy loss rates were not affected.

What is known already: Adequate development of a functional endometrium is a prerequisite for successful embryo implantation in ART cycles. In contrast, the management of patients with thin endometrial lining or recurrent implantation failure has been an ongoing challenge with none of the proposed surgical, hormonal, and pharmacological interventions achieving satisfactory results. In recent years, the use of plasma rich in growth factors (PRGF) - successfully applied in other medical fields - has become a novel treatment option in reproductive medicine. It was used experimentally both for ovarian follicle activation and to enhance endometrial receptivity although published studies are small with often contradictory results.

Study design, size, duration: All consecutive patients (n = 107) who underwent endometrial PRGF treatment (n = 150) in a single, private centre between 2016-2022 were included in this retrospective analysis. Patients were recruited into ThE (n = 64), RIF (n = 36) and RM (n = 7) groups, respectively. Live birth / ongoing pregnancy rates per embryo transfer were compared to success rates obtained in previous embryo transfers preceding the endometrial PRGF intervention. External Ethics Committee approval was obtained for a comparative pilot study including above patient groups.

Participants/materials, setting, methods: PRGF was obtained by processing the patients' autologous blood sample in an in-house validated open system. Endometrial PRGF interventions were performed using a thawed PRGF sample with a series of intrauterine instillations (43%) or with a combined approach performing hysteroscopic subendometrial infiltration during early follicular phase (57%). Endometrial preparation was conducted using an artificial hormone replacement protocol with high-dose oral estrogens and vaginal progesterone. Most embryo transfers involved the replacement of a single, vitrified-thawed blastocyst.

Main results and the role of chance: A total of 107 patients underwent 150 endometrial PRGF treatments and 131 subsequent embryo transfers. Altogether 19 (13%) embryo transfers were cancelled, higher in ThE than in the RIF group (16 vs 7.1%). In the ThE group (64 patients, 98 PRGF cycles and 107 controls), positive pregnancy (41 vs 32%, NS), clinical pregnancy (35 vs 22%, $p = 0.049$) and ongoing pregnancy/live birth rates (24 vs 4.7%, $p < 0.0001$) per embryo transfer were significantly higher in the endometrial PRGF treatment group compared to previous embryo transfers. In the RIF group (36 patients, 42 PRGF cycles and 101 controls), positive pregnancy (59 vs 20%, $p < 0.0001$), clinical pregnancy (44 vs 11%, $p < 0.0001$) and ongoing pregnancy/live birth rates (33 vs 7.9%, $p < 0.0001$) per embryo transfer were significantly higher in the endometrial PRGF treatment group compared to previous embryo transfers. In the RM group (7 patients, 10 PRGF cycles and 15 controls), positive pregnancy (50 vs 60%), clinical pregnancy (20 vs 27%) and ongoing pregnancy/live birth rates (0 vs 0%) per embryo transfer were not significantly different and no ongoing pregnancies were achieved. So far, 20 singletons and 1 set of twins have been confirmed to be born from the above PRGF cycles (12 pregnancies still ongoing).

Limitations, reasons for caution: Heterogeneity of clinical severity between included ThE patients could affect observed reproductive outcomes. The self-controlled design of the study might have influenced the comparison between pre-, and post-intervention pregnancy rates, although this also highlights the poor-prognostic nature of included participants. RM patients were too few to evaluate pregnancy loss rates.

Wider implications of the findings: This preliminary, retrospective study has shown that regenerative therapy using autologous PRGF is a safe, affordable, and efficient treatment option for ThE and RIF patients. Further randomized studies are warranted, although they are hampered by patient selection issues and the lack of applicable efficient treatment options in the non-intervention group.

Trial registration number: not applicable

Abstract citation ID: dead093.268

O-222 Functional characterization and clinical implications of patient-specific iPSC-derived endothelial cells to unravel the pathogenesis associated with androgen-mediated endothelial dysfunction in polycystic ovarian syndrome

C.C. Huang[1], M.J. Chen[1], J.H. Yang[1]

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Study question: Whether and how the endothelial cell (EC) dysfunction is involved in the pathogenesis of PCOS revealed by iPSC-based disease modeling?

Summary answer: PCOS-specific iPSC-derived ECs were successfully established and the results showed that EC proliferation and function were impaired in PCOS through the androgen receptor(AR)-mediated signaling pathway.

What is known already: ECs have been shown to play critical biological roles in the regulation of vascular tone and inflammatory process and any injury to ECs or disturbance in their homeostasis is called EC dysfunction, which has been recognized as an early marker of atherosclerosis and cardiovascular diseases. Several studies have also revealed indirect evidence of EC dysfunction among women with PCOS, including elevated circulatory markers

of endothelial damage and impaired vascular structure. But whether and how EC dysfunction is involved in the pathogenesis of PCOS is still unclear.

Study design, size, duration: This is an experimental study at tertiary university hospital. iPSCs were established from skin fibroblasts and peripheral blood mononuclear cells from two patients with PCOS and two control participants.

Participants/materials, setting, methods: iPSCs were differentiated into ECs through monoculture with chemically defined conditions. Single cell RNA sequencing (scRNA-seq) was performed on the iPSCs-derived ECs to compare the differences between PCOS and control. iPSCs-derived ECs were treated with different dosages (1, 10 and 100 nM) of dihydrotestosterone (DHT). Cell cycle phases were analyzed by flow cytometry with BrdU incorporation assay. The expression of AR, cyclin-dependent kinase 1 (CDK1) and vascular endothelial growth factor (VEGF) were analyzed using rtPCR.

Main results and the role of chance: Up to 90% of iPSC-derived ECs successfully expressed EC markers, including CD31, CD144 and von Willebrand Factor in both PCOS and control groups, showing a high differentiation efficiency in the present study. The pathway enrichment analysis of transcriptomic data of iPSC-derived ECs showed functional differences involving cell cycle process, VEGF signaling and apoptotic process between PCOS and control. Analysis using sc-RNA seq revealed decreased cellular proliferation in the PCOS iPSC-derived ECs compared to control group, which was due to cell cycle arrest revealed by flow cytometry. The modulating effects of androgen on the proliferation and gene expression of iPSC-derived ECs were further investigated. DHT at doses ranging from 1 to 100 nM stimulated iPSC-derived ECs proliferation in the control group through upregulation of AR, CDK1 and VEGF gene expression. In contrast, DHT did not promote the cellular proliferation of PCOS iPSC-derived ECs at physiological concentrations of 1 and 10 nM and the expression of AR, CDK1, and VEGF remained unchanged. These data suggest that DHT-induced cellular proliferation and the expression of associated genes were more sensitive and dose-dependent in the control iPSC-derived ECs and blunted in the PCOS iPSC-derived ECs.

Limitations, reasons for caution: One of the major limitations of our study was the limited number of cases during the establishment of iPSCs, which was unable to reflect the heterogeneity and complexity of PCOS.

Wider implications of the findings: Impaired cellular proliferation and differentially expressed genes involved in cell proliferation, immune responses, cardiovascular functions, and apoptosis were observed among PCOS iPSC-derived ECs. Additionally, androgen-induced ECs proliferation was blunted in PCOS through a diminished AR/VEGF/cyclin D1 signaling pathway, which might hinder VEGF-dependent vascular repair and probably increase cardiovascular risks.

Trial registration number: not applicable

Abstract citation ID: dead093.269
O-223 Sperm Genome Editing by CRISPR-Cas9 through an Ooplasm-Mediated Approach

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Study question: Can we edit the sperm genome by CRISPR-Cas9 through an ooplasmic-mediated approach?

Summary answer: The ooplasm-mediated technique induced the decondensation of the male gamete nucleus and allowed CRISPR-Cas9 to access the sperm DNA to carry out successful edits.

What is known already: Previous studies on genome editing using CRISPR-Cas9 have been performed at the S-phase or zygote stage, but challenges were represented by mosaicism and possible off-target edits. To address these issues, gene editing at the gamete level would be ideal. While this may be feasible in oocytes, it appears ambitious in the spermatozoon due to the DNA hypercoiling around the protamine core and the chromatin compaction. In preliminary experiments, permeabilization of sperm membrane allowed penetration of CRISPR-Cas9 to enter the cell, however, genomic editing was proved unsuccessful.

Study design, size, duration: In the past 5 months, a total of 128 oocytes were divided into 2 groups. To edit exclusively the male genome through oocyte-mediated sperm decondensation (OMSD), a single spermatozoon was injected into an enucleated oocyte to produce a haploid androgenetic embryo. The control cohort consisted of embryos generated through standard ICSI with the established heritable genome editing (HGE) approach. Both groups were treated with CRISPR-Cas9 aiming to knockout *Tyr* gene to create an albino phenotype.

Participants/materials, setting, methods: B6D2F1 mice were used to retrieve oocytes and spermatozoa. A cohort of oocytes used for OSMD approach were enucleated. While the intact oocytes were used for HGE control. All oocytes were injected with a single spermatozoon together with CRISPR-Cas9 solution, containing *Tyr* gRNA. All embryos were cultured up to the 8-cell stage. Individual blastomeres were isolated and sequenced to proof editing. DNA was extracted and amplified for T7E1 analysis to validate genome editing efficiency.

Main results and the role of chance: Of the 128 oocytes used for the study, 51 were enucleated for the OMSD experiments, while 77 were used for HGE control. After undergoing ICSI with CRISPR-Cas9 solution, 84.3% (43/51) of the OMSD cohort displayed a single male pronucleus and 79.2% (61/77) control HGE fertilized. In a time-lapse microscopy, 95.3% (41/43) of the OMSD experimental group developed to the 2-cell stage, comparable to the HGE control development at 96.7% (59/61). After 48 hours in culture, the HGE control reached 8-cell development at 88.5% (54/61), while the OMSD cohort cleaved at the lower rate at 60.4% (26/43, $P<0.001$). A 423-bp region around the CRISPR target site was amplified on DNA extracted by isolated blastomere. Gene modification at the target site was confirmed in 33.7% (70/208) of OMSD cohort and 29.7% (88/296) of the HGE control embryos.

Limitations, reasons for caution: While the experimental observations are still limited, it is important to note that these results may be underestimated since embryos with completely uniform modifications may go undetected by T7E1 assay. The technique needs further refinement to optimize targeting efficiency and support embryo development.

Wider implications of the findings: Once the genetic editing method is optimized, individual pseudo-blastomeres generated through this OMSD approach can be utilized as male gametes to fertilize oocyte. The generation of live offspring with the corrected haplotype will serve to prove the safety of genome editing through this technique.

Trial registration number: N/A

Abstract citation ID: dead093.270
O-224 Effect of intraovarian injection of platelet rich plasma on ovarian response in poor responder women

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Study question: Does intraovarian administration of platelet rich-plasma (PRP) increase ovarian response in women with poor ovarian response (POR) undergoing IVF treatment?

Summary answer: In patients with low ovarian response, the number of oocytes and embryos is higher in the 4 cycles after PRP administration compared to pre-PRP cycles.

What is known already: The number of oocytes after controlled ovarian stimulation is a crucial factor in IVF outcomes. For this reason, different approaches have been investigated for the management of patients with POR,

such as the administration of adjuvant treatments, or the change of protocol, dose or type of gonadotropin. However, none of these strategies has proven to be fully effective in improving oocyte yield.

More recently, innovative treatments aimed at activating preantral follicles have emerged, such as intraovarian administration of autologous platelet rich plasma (PRP). Although the evidence is still limited, some studies suggest an improvement in ovarian response following PRP treatment.

Study design, size, duration: Retrospective study of 83 patients diagnosed with low ovarian reserve according to Bologna criteria who underwent intraovarian PRP between July 2021 and December 2022. 111 stimulation cycles after PRP were compared with the cycles immediately prior to PRP administration.

Participants/materials, setting, methods: Patients with POR were presented with an oocyte or embryo preservation strategy, which included the option of administering intraovarian platelet-rich plasma (PRP) either during the initial oocyte retrieval or in the follicular phase of the cycle preceding stimulation. A 2 ml dose of PRP was administered into each ovary. The ovarian response was analyzed in terms of the number of oocytes, MII, and embryos, and was further evaluated based on different age groups.

Main results and the role of chance: 83 women (age: 38,68 ± 3,54, range 28-46 years) underwent a PRP injection. The patients had low ovarian reserve markers (AMH 2,90 ± 2,76 pmol/l, AFC 4,74 ± 2,70).

Of the cycles after PRP, 48 were performed in the luteal phase of the same cycle, 28 in the follicular phase of the first post-PRP cycle and 35 between the 2nd and 4th cycle post PRP.

The overall mean number of oocytes (2,38 ± 2,09 vs. 3,19 ± 2,70; p = 0,002) and MII (1.84 ± 1.77 vs. 2.54 ± 2.25, p = 0.0008) was significantly higher after PRP. There was also a statistically significant difference in the number of blastocysts pre and post PRP (0,85 ± 1,12 vs. 1,77 ± 1,89; p = 0,0005). There were no differences in the blastulation or euploidy rates.

Differences in the number of oocytes were not observed in the luteal phase cycle (2.97 ± 2.35 vs. 3.45 ± 2.66, p = 0.2) compared to the subsequent 1st-4th cycles (1.88 ± 1.77 vs. 2.95 ± 2.73, p = 0.003).

Improvement in response was observed in patients younger than 40 years (2 ± 1.52 vs 3.3 ± 2.62 oocytes; p = 0.0002), but not in patients aged ≥ 40 years (2.9 ± 2.58 vs 3.02 ± 2.77 oocytes; p = 0.7).

Limitations, reasons for caution: The retrospective nature of the study is a significant limitation. As this research is based on real-world clinical practice, the patients stimulation protocols were diverse which may have impacted the results. To verify our findings, further studies with a uniform control group are required.

Wider implications of the findings: We observed improved response to intraovarian platelet-rich plasma administration in patients with POR under 40 years of age. This enhancement was observed from the initial follicular cycle following administration. Our findings suggest that intraovarian PRP may be a valuable supplementary treatment for this patient population.

Trial registration number: not applicable

POSTER DISCUSSION SESSION
SESSION 71: NURSING AND MIDWIFERY
Tuesday 27 June 2023 Hall D2 17:00 - 18:00

Abstract citation ID: dead093.271

P-557 Validating the Dutch polycystic ovary syndrome questionnaire (PCOSQ) and the polycystic ovary syndrome quality of life tool (PCOSQOL) in Dutch and Flemish women with PCOS

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Study question: What are the test-retest reliability and the domain structures of the Dutch version of the PCOSQ and PCOSQOL in Dutch and Flemish women with PCOS?

Summary answer: The Dutch version of the PCOSQ and the PCOSQOL are reliable and can both be used to measure health-related quality of life (HRQoL) in PCOS.

What is known already: The polycystic ovary syndrome questionnaire (PCOSQ) is recommended to measure the quality of life in women with PCOS. The PCOSQ was developed in 1998 to complement generic health-related QoL instruments. The PCOSQ has been validated in Arabic, German, Chinese, and Swedish. Recent research suggested that the PCOSQ focuses on the physical impact of PCOS and that psychological, social, or environmental aspects are less represented. Therefore, the Polycystic Ovary Syndrome Quality Of Life Scale (PCOSQOL) was developed in 2018. Both questionnaires have not been validated in Dutch yet.

Study design, size, duration: A forward and backward translation was performed on the original English PCOSQ and PCOSQOL by two independent translators. PCOS patients were contacted with a request to complete both questionnaires (and some additional demographic questions) online in their home environment (T0). A test-retest design was applied to demonstrate stability over time by having all women complete the same questionnaires a second time (T1). Approval of the clinic's Ethics Committee was obtained in both participating centres.

Participants/materials, setting, methods: Women of at least 18 years old, who were able to speak and write Dutch, and who were diagnosed with PCOS according to the Rotterdam criteria (ESHRE/ASRM, 2003) or according to the international evidence-based guideline for the assessment and management of PCOS (ESHRE, 2018) were eligible for the study. Women who were pregnant at T0 or T1 were excluded from the study. Participants were included between January and December 2021.

Main results and the role of chance: In total, 245 women were included in this study. The median age was 31 (19-54) years. For the PCOSQ, the Cronbach alpha statistic for the five domains ranged from 0.88 to 0.96 demonstrating good to excellent internal consistency. The Intra-class Correlation Coefficient (ICC) for the five domains was high to excellent ranging from 0.88 to 0.96. For the PCOSQOL, the Cronbach alpha ranged from 0.91 to 0.96 demonstrating excellent internal consistency. The ICC ranged from 0.91 to 0.96 for the four domains indicating excellent reliability. The factor analysis presented five domains of the PCOSQ and the original domain structure was partly confirmed. An extra domain for acne was included based on previous studies. Therefore, the Dutch version of the PCOSQ contained six domains: Weight, Body hair, Emotions, Infertility, Menstrual problems, and Acne. Based on the factor analysis, an extra domain was added to the original four domains of the PCOSQOL, which included items related to dealing with PCOS. Most women (70.6%) needed approximately 15 minutes to complete both questionnaires and had no preference for one of the two questionnaires (55.9%).

Limitations, reasons for caution: A limitation of our study could be that women with more PCOS complaints were more motivated to participate in this study. Therefore, potential selection bias should be taken into account.

Wider implications of the findings: The PCOSQ and PCOSQOL are disease-specific QoL measures for women with PCOS. The Dutch versions can be used in studies and in clinical settings (e.g. by nurses and midwives, counsellors, psychologists) to examine the impact of PCOS on QoL in Dutch and Flemish women.

Trial registration number: NA

Abstract citation ID: dead093.272

P-555 Do IVF patients prefer the rectal route to the vaginal for luteal phase progesterone administration? - a cohort study

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[2]Aarhus University, Department of Clinical Medicine-, Aarhus, Denmark

Study question: How do IVF patients perceive rectal administration of progesterone for luteal phase support compared to vaginally administered progesterone?

Summary answer: Rectal administration of progesterone causes less discomfort in IVF patients, undergoing Hormone Replacement Therapy Frozen Embryo Transfer (HRT-FET) and is preferred compared to vaginal administration.

What is known already: Progesterone is essential for implantation and normal development of pregnancy in spontaneous pregnancies as well as in ART pregnancies. In IVF, progesterone is most often administered vaginally in Europe for luteal phase support, although, this route may cause cumbersome discharge, vaginal bleeding, and irritation of the vulva. For many years the vaginal route has been the gold standard, although, progesterone is well absorbed rectally and recent studies have shown that rectally administered micronized progesterone was well accepted among IVF patients. Until now, in IVF very little focus has been on patient convenience as regards luteal phase support.

Study design, size, duration: An interventional cohort study, conducted from January 2020 to November 2022 in a public fertility clinic. A total of 479 patients received a questionnaire (Q1) by e-mail during the period between the embryo transfer and the pregnancy test; A second questionnaire (Q2) was answered at the time of the first ultrasound scan in gestational week 7.

Participants/materials, setting, methods: Patients underwent an HRT-FET protocol, including vaginal progesterone (VP) (400mg/12hours). In patients with serum progesterone levels lower than 11 ng/ml on the 6th day of progesterone, additional progesterone was administered rectally (RP) (400mg/12hours) from that day until the day of pregnancy testing, and in pregnant patients until the first scan in week 7.

Side effects and patient convenience of both routes were reported in a questionnaire, including 27 questions, using a visual analog scale (0-100).

Main results and the role of chance: A total of 73% of HRT-FET patients (349/479) answered Q1 before the pregnancy test and a total of 27 % (93/349) of the cohort received progesterone both vaginally and rectally. The response rate of Q2 in gestational week 7 was 100 % (221/221) and a total of 28 % of patients (62/221) administered progesterone both vaginally and rectally.

In Q1, a total of 60% of patients (54/90) preferred RP over VP in the cohort of patients treated with both administration regimens.

In Q2, a total of 62% of patients (37/60) preferred RP over VP in the cohort of patients treated with both administration regimens.

The overall discomfort was lower in relation to RP in both questionnaires and, especially discharge was lower in RP compared to VP; median interquartile range (IQR) in Q1: 11 (3-23) vs. 50 (25-66) and Q2: 19 (9-28) vs. 47 (22-62), respectively. Changes in defecation pattern after RP were reported in Q1 as well as Q2, 48% (45/92) and 55% (34/61), however, the discomfort was reported as mild. An increased flatulence was reported by 50% (46/92) in Q1 and 63% (39/92) in Q2, however, again the discomfort was reported as mild.

Limitations, reasons for caution: Currently, it is unknown whether the present results apply to progesterone products other than the one used in the present study (Cyclogest®). The questionnaire used, although having been used in another published study with another progesterone product, was not specifically validated for this product.

Wider implications of the findings: The rectal route should be remembered for luteal phase support in IVF, and some patients might choose the rectal route over the vaginal for their luteal phase support due to less discharge.

Trial registration number: EudraCT no.: 2019-001539-29

Abstract citation ID: dead093.273

P-556 Digital platform for remote pre-treatment assessment: Perceived usefulness and quality by patients and doctors

A. De Oliveira Trigo[1], B. De Oliveira Trigo[2], F. Khan Sullivan[3], N. Felix[3], M. Fanti[4]

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Study question: Is a digital platform for remote pre-treatment assessment perceived as useful and able to fulfil the needs of patients and clinicians?

Summary answer: The digital platform was found to be of high quality, useful, and answer the expressed needs of the targeted audiences in cross-cultural samples.

What is known already: Infertility is a life crisis. It takes on average 3.2 years to be diagnosed, 1.6 years to access a specialist, and 2.2 years to complete treatment. The pre-assessment phase is time-consuming, costly, often disruptive to the patient's life, and may require traveling abroad. Patients need organized care where they can schedule/order tests, access information about their specific health conditions, and reach out to professionals. On the other hand, clinicians find themselves trying to assess a large number of patients before initiating treatment abroad without having a unified platform where they can review the patient's clinical history and treatment plan.

Study design, size, duration: Study I explored patients' experience with pre-treatment care and their perceived usefulness of the Patient's Portal. An online questionnaire of 25 questions was used. Study II explored clinicians' perceived usefulness and quality of the Doctor's Portal. A semi-structured interview was conducted and an online questionnaire of 17 items was used. Both questionnaires had multiple options, Likert-scale, and open-ended items. The studies followed a cross-sectional design. The data were analyzed using IBM SPSS Statistics, Version 28.0.

Participants/materials, setting, methods: Study I had a cross-cultural sample of 59 women (M = 37.2, SD = 4.9 years). 41% are trying to conceive for more than 24 months, 89.8% were in a heterosexual relationship, and all were undergoing treatment/pursuing a clinic. The internal consistency of the questionnaire is α=.97. Study II had a cross-cultural sample (N = 8) of clinic leaders, all from different clinics. The interview questions were open-ended to capture unprompted opinions. The internal consistency of the questionnaire is α=.94.

Main results and the role of chance: Study I: People who took >4 weeks to have tests done/get the results scored significantly higher values than people who took less time in terms of how much they had to pay, struggled to find where to have the tests done, had difficulty managing emotions, and would've preferred their clinic had scheduled the testing for them. Overall, 61.02% reported the testing had been disruptive to their lives. Regarding the perceived usefulness of the platform, means >4 and a mode of 5 on a scale of 1-5 were obtained. We also found that regardless of the woman's age or how long she's been trying to conceive, she'd benefit from having this platform available.

Study II: 75% made mention the high-quality of the platform, 50% said it would improve their current practice, covered information comprehensively, and was user-friendly, and 37.5% said it would make the process of diagnosing patients faster. Suggestions were used to adapt the platform. Regarding the quality questionnaire, the means varied between 7.86 and 9.14 (SD between .69 and 1.11) and the mode between 8 and 9 on a scale of 1-10. The inter-rater reliability coefficient was .94. This means the perceived high-quality and usefulness of the platform were consensual.

Limitations, reasons for caution: Small sample sizes condition the extrapolation of the findings. Further studies of the perceived usefulness and quality of the Patients' Portal and Doctors' Portal after the implementation of the digital platform are required.

Wider implications of the findings: The digital platform for remote pre-treatment assessment is currently available and being used by clinicians and patients. In the future it, can be further developed to develop and train an

Artificial Intelligence algorithm to Diagnose & Predict Fertility Treatment Outcomes with Personalised Patient Pathways.

Trial registration number: not applicable

Abstract citation ID: dead093.274
P-560 Stigma related to male infertility in China: a scale development and validation study

F. Zou[1], **Y. Lin**[1], **S. Hang**[1], **G. An**[1], **M. Cao**[1], **J. Liu**[1]

[1]*The Third Affifiliated Hospital of Guangzhou Medical University, Center for Reproductive Medicine, Guangzhou, China*

Study question: Is it possible to develop a trustworthy instrument to evaluate the infertile men's stigma and to document fully all methodological steps, including validation?

Summary answer: The Male Infertility Stigma Scale (MISS) has been developed to evaluate the stigma status of infertile men with a good reliability and validity.

What is known already: Infertile men regard childless as a hidden stigma, which influence all the dimensions of their lives and well-being. Most instruments focus exclusively on women and no questionnaires have been directed at infertile men.

Study design, size, duration: A cross-sectional study was conducted to analyse the validity and reliability of MISS. This was a one-centre study in the Center of Reproductive Medicine of the Third Affiliated Hospital of Guangzhou Medical University between January 2019 to May 2019.

Participants/materials, setting, methods: This study was carried out on men (n = 432) with a diagnosis and treatment for male-factor infertility. Item generation and reduction of MISS was formed by combining literature review, interviews of experts and patients, and related scale. Exploratory factor analysis (n = 216) and confirmatory factor analysis (n = 216) were performed to indicate the scale constructs. In addition, reliability analyses including internal consistency and test–retest analysis (n = 18)were carried out. Validity was analyzed by convergent validity and content validity.

Main results and the role of chance: This study developed a 18-item MISS with three dimensions (social marginalization, self-devaluation, psychological insecurity). Exploratory factor analysis indicated that these three dimensions explained 60.14% of total variances.The confirmatory factor analysis showed that the construct of the MISS fitted well with the hypothetical model. The Cronbach's α,split-half coefficient and test–retest correlation coefficient for the whole scale was 0.951, 0.884,and 0.945, respectively. The associations of the MISS with other measure suggested good convergent validity. The Content Validity Index (CVI) was 0.988.

Limitations, reasons for caution: The reliability and validity of the MISS developed in this study were verified only among the infertility men in Guangzhou and the discriminant validity of dimension 3(psychological insecurity) in this survey was a little poor. The convenience sampling method precluded the validity and generalization of the conclusions.

Wider implications of the findings: This study may create an effective evaluation measurement to understand the stigma status of infertile men. Future studies should focus on using the MISS in larger samples with different characterizations, such as different areas, and then taking some measures to help them .

Trial registration number: None

INVITED SESSION
SESSION 72: NEW MYTHS IN PREIMPLANTATION GENETICS

Wednesday 28 June 2023 Hall A 08:30 - 09:30

Abstract citation ID: dead093.275
O-225 May embryonic cfDNA in the culture media play a role as a non-invasive assessment of aneuploidy in PGT

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Non-invasive preimplantation genetic testing for aneuploidies (niPGT-A) in spent blastocyst media (SBM) is a promising technique that has the potential to revolutionize current preimplantation genetic testing procedures. This technique is based in the analysis of the cell-free DNA (cfDNA) released by the embryo during the latest stages of preimplantation development. It allows for the detection of chromosomal abnormalities in embryos without the need for invasive procedures that can harm the embryo (reviewed in Navarro-Sánchez et al., 2022).

Our group among others has conducted different studies in this field, and the early clinical application of the cfDNA analysis in the culture medium has been proposed as a screening tool, giving a priority score for embryo transfer based on the media results (Rubio et al., 2019, 2020). In the final analysis of our multicenter study in day-6 and day-7 blastocysts, we have been able to compare the chromosomal results of the SBM with trophectoderm (TE) biopsies in 2,187 samples, and with the inner cell mass ICM in 230 blastocysts. In this study, the analysis of the cfDNA showed a concordance rate of 87% with the ICM biopsy, which is currently considered the gold standard for PGT-A. However, it is important to continue evaluating the factors that may affect the informativity and concordance rates of cfDNA-based assays, such as the culture day when medium is collected, contamination with external and/or cumulus cell DNA, and previous manipulation of the embryos. Multivariate analysis has shown that the longer the time in culture, the higher the informativity rate, and female age had the greatest impact on concordance rates with TE biopsies. Regarding technical aspects, the SBMs with higher number of reads after sequencing resulted in higher concordance rates, reflecting the importance of analysing samples with sufficient DNA quantity. Finally, the predictive value in terms of concordance with TE biopsies was higher for aneuploid blastocyst. No significant independent correlation was observed for embryo quality.

The improvements in IVF lab protocols and the use of time-lapse and short incubation protocols for vitrified-thawed blastocysts are also noteworthy, as they may further increase the concordance rates of cfDNA-based assays with blastocysts. In fact, concordance in previously vitrified-thawed blastocysts as result as high as 90%.

Regarding clinical outcomes, several groups have evaluated non-invasive PGT-A with transfer of euploid embryos showing good results. In a pilot study with only 7 SETs in couples with PGT-A or PGT-SR indication, 5 pregnancies were achieved resulting in 5 livebirths (Xu et al., 2016). In a second study, in couples with recurrent spontaneous abortion or repeated implantation failure, 50 transfers of euploid media were performed resulting in a 58% clinical pregnancy rate, with 27 healthy babies born (Fang et al., 2019). Similar results were described in good prognosis patients <38 years of age with ongoing pregnancy rates comparable to PGT-A (61.5%), and higher than conventional IVF or ICSI (48.5%) (Franco et al., 2021).

Overall, the high concordance rates with trophectoderm biopsies and inner cell mass biopsies are encouraging, and the clinical outcomes with transfer of euploid embryos are promising. It is exciting to see the progress in non-invasive preimplantation genetic testing using cfDNA in culture medium, and it will be interesting to see how this technology evolves and its potential role as a screening or diagnostic tool in the future.

Abstract citation ID: dead093.276
O-226 Is there a place for polygenic risk scoring in PGT (PGT-P)?

A.H. Handyside[1]

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Preimplantation genetic testing (PGT), by embryo biopsy and genetic testing for inherited diseases and chromosome abnormalities following IVF, is now well established world-wide. PGT for monogenic conditions (PGT-M) is generally performed for childhood-onset, lethal disorders, but is increasingly accepted for certain adult-onset conditions, conditions with available treatment options or conditions with lower penetrance. Over recent years, machine learning methods applied to large genomic datasets, including genome-wide association studies (GWAS) using single nucleotide polymorphism (SNP) genotyping, has enabled polygenic risk scores (PRSs) to be calculated to identify individuals who are at elevated risk for a broad range of conditions including important common multifactorial diseases, such as coronary artery disease (CAD), diabetes, hypertension and various cancers. It is now possible to perform genome-wide SNP genotyping of embryo biopsy samples with the same accuracy as adult genomic DNA and the first live birth following PGT for polygenic risk scoring (PGT-P) was reported in 2019 (Treff et al (2019) Front Endocrinol 10, 845) . The clinical application of PGT-P is controversial and raises many practical, ethical and societal issues. To examine the question 'is there a place for PRS in PGT', the example of predisposition to breast cancer will be reviewed.

INVITED SESSION

SESSION 73: THE IMPACT OF INFECTION ON HUMAN REPRODUCTION

Wednesday 28 June 2023 Hall D3 08:30 - 09:30

Abstract citation ID: dead093.277
O-227 Viral, bacterial and fungal infections in patients with recurrent miscarriage

J. Laven[1]

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Miscarriages occur in 15% of clinically recognized pregnancies in the general population. Miscarriage has several physical and psychological consequences and might be a risk marker of severe obstetrical complications during the following pregnancies and various long-term pathological conditions, such as venous thromboembolism and cardiovascular diseases. Up to 80% of reproductive losses occur in the first trimester. Miscarriage may be caused by chromosomal abnormalities, antiphospholipid syndrome and other thrombophilia's, immune and endocrine disorders as well as inflammation. However, in up to 50% of cases, the cause of the miscarriage remains unknown.

The microbiome consists of microbes that are both helpful and potentially harmful. Most are symbiotic and some, in smaller numbers, are pathogenic. In a healthy body, pathogenic and symbiotic microbiota coexist without problems. But if there is a disturbance in that balance brought on by infectious illnesses, certain diets, or the prolonged use of antibiotics or other bacteria-destroying medications dysbiosis occurs, stopping these normal interactions. In this presentation the role of the microbiome as well as the virome in recurrent miscarriage will be discussed. Possible mechanism of action will be reviewed and, if available, treatment of a perturbed microbiome will be presented.

Trial registration number: XXXX

Abstract citation ID: dead093.278
O-228 Dysbiosis and reproduction

H. Svarre Nielsen[1]

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Bacteria, viruses, and fungi are collectively known as our microbiome and constitute approximately 1-2% of our body weight. The microbiome is closely associated with our health. Dysbiosis refer to a suboptimal microbiome composition and while gut dysbiosis has been extensively investigated there is far less studies on how the reproductive microbiome is associated with reproductive health. This presentation will cover definitions, methods, and give an overview of the studies that have investigated the association between gut, vaginal and endometrial microbial dysbiosis and the association with implantation and early pregnancy. Possible mechanisms and treatment options will also be adressed.

INVITED SESSION

SESSION 74: GUIDELINES SESSION: PCOS – UPDATE OF THE INTERNATIONAL GUIDELINES

Wednesday 28 June 2023 Hall D1 08:30 - 09:30

Abstract citation ID: dead093.279
O-229 Long term features of PCOS and Medical treatment

T. Piltonen[1]

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Objective: To develop and translate comprehensive evidence-based guidelines for diagnosis, assessment and treatment, to improve the lives of those with polycystic ovary syndrome (PCOS) worldwide.
Participants: Health professional and consumer/ patients informed priorities. International society-nominated panels included consumers, and experts in reproductive endocrinology, obstetrics, gynaecology, paediatrics, endocrinology, primary care, psychology, dietetics, exercise physiology, sleep, bariatric/ metabolic surgery, public health, other co-opted experts, evidence synthesis and translation.
Evidence: Best practice guideline development involved extensive evidence synthesis and the Grading of Recommendations, Assessment, Development and Evaluation (GRADE) framework covered evidence quality, feasibility, acceptability, cost, implementation and recommendation strength.
Process: Governance included international advisory and project, guideline development, consumer and translation committees. The Centre for Research Excellence in Women's Health in Reproductive Life, funded by the Australian National Health and Medical Research Council (NHMRC), and led by Monash University, partnered with the American Society for Reproductive Medicine, Endocrine Society, European Endocrine Society and European Society of Human Reproduction and Embryology. Another 30 collaborating organisations engaged. Overall 52 systematic and three narrative reviews, informed international guideline groups, generating evidence-based and consensus recommendations and practice points for 55 clinical questions. Collaborating/ partnering organisations and NHMRC methodological experts led peer review.
Recommendations: After diagnosing PCOS, assessment and management should include reproductive, metabolic, cardiovascular, dermatologic, sleep and psychological features. A reproductive health plan is recommended including diagnosis and support in adolescence, lifelong healthy lifestyle / prevention of weight gain, fertility optimisation, preconception risk mitigation and recognition of PCOS as a high-risk pregnancy condition requiring identification, screening, monitoring and management. Metabolic risk factors, diabetes, cardiovascular disease and sleep disorders are increased in PCOS, with screening and management recommended. Increased premenopausal endometrial cancer risk should be recognised. Depressive and anxiety symptoms

are significantly increased, with universal screening recommended. Greater awareness of psychological features including eating disorders, body image and quality of life impacts is recommended. Life-style intervention is the first line treatment for PCOS, but in many cases medication is also needed. Combined oral contraceptive pills are first line pharmacological treatment for menstrual irregularity and hyperandrogenism, with no specific recommended preparation, and a preference for lower dose and less side-effects. Metformin is recommended primarily for metabolic features noting greater efficacy than inositol, which offers more limited clinical benefits. Metformin is not routinely recommended for use in pregnancy. Laser therapy is effective and recommended for hair reduction in identified subgroups, whilst anti-androgens have more limited recommendations. Anti-obesity agents and bariatric/ metabolic surgery may be considered based on general population guidelines, balancing benefits and side effects. Overall, evidence was low to moderate quality. Based on high prevalence and significant health impact, greater priority, education, funding and research is strongly recommended.

Abstract citation ID: dead093.280
O-230 Fertility treatment

A. Balen[1]

[1]*The Leeds Centre for Reproductive Medicine, Leeds, United Kingdom*

Abstract citation ID: dead093.281
O-231 Diagnosis, psychological aspects, lifestyle, models of care and translation

H. Teede[1]

[1]*Monash University, Victoria, Australia*

SELECTED ORAL COMMUNICATIONS

SESSION 75: EXTRA HELP WITH GAMETE SELECTION AND EMBRYO DEVELOPMENT

Wednesday 28 June 2023 Hall D4 08:30 - 09:45

Abstract citation ID: dead093.282
O-232 Extracellular vesicles secreted by the maternal endometrium functionally regulate processes related to embryo development and implantation in human blastocysts

M. Segura-Benitez[1], **M.C. Carbajo-Garcia**[1], **A. Quiñonero**[2], **M.J. DeLos Santos**[3], **A. Pellicer**[4], **H. Ferrero**[2]

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[4]*IVIRMA Rome, Reproductive Medicine, Rome, Italy*

Study question: What is the response of human embryos in a transcriptomic level to the uptake of extracellular vesicles (EVs) secreted by the human endometrium?

Summary answer: EVs secreted by the maternal endometrium induce a transcriptomic response in human embryos that modulates molecular mechanisms related to embryo development and implantation.

What is known already: Communication between the maternal endometrium and the embryo is essential for a successful implantation, and EV role in this cross-talk has been recently established. Results of our previous experiments showed that endometrial EVs carry miRNAs and proteins related to endometrial receptivity, embryo implantation process and early embryo development, and that they can be uptaken by human blastocysts. However, there are no studies which address the transcriptomic response of human embryos to the uptake of these EVs, which would demonstrate the functional relevance of this communication system.

Study design, size, duration: Endometrial biopsies were collected from oocyte donors (n = 20) with confirmed fertility, on oocyte retrieval day, and primary human endometrial epithelial cells (pHEECs) were isolated and *in vitro* cultured. Hormonal treatment was added to mimic secretory phase of menstrual cycle. Conditioned medium was collected and EVs were isolated and characterized. Day 5 human blastocysts (n = 24) were divided into two groups (n = 12/group) and cultured *in vitro* with or without these EVs. RNA-sequencing of these embryos was performed.

Participants/materials, setting, methods: Conditioned media from pHEECs was pooled into 4 replicates (n = 5 biopsies/replicate) and EVs were isolated by ultracentrifugation. Characterization was performed by western blot, nanoparticle tracking analysis and transmission electron microscopy (TEM). Human blastocysts were devitrified and cultured in vitro for 24h with or without EVs previously isolated. RNA-sequencing analysis was performed, and DESeq2 was used to identify differentially expressed genes (FDR<0.05). QIAGEN Ingenuity Pathway Analysis (IPA) was used to perform the functional enrichment analysis.

Main results and the role of chance: EVs presented a size range within 100-300nm, and expression of EV protein markers HSP70, TSG101, CD9 and CD81. EV morphology was corroborated by TEM. RNA-sequencing identified 26610 genes, being significant 519 upregulated and 395 downregulated in EV-embryos compared with control. Among top20 most significant upregulated genes, CSF1R (log2FoldChange [logFC]=3.36) has been associated with formation of blastocyst cavity, and PLOD2 (logFC=2) deficiency leads to early embryonic lethality. Genes related to antioxidant defense, as PDK1 (logFC=2.82), or vesicle trafficking, as SEC24D (logFC=2.17), were also upregulated. Regarding top20 downregulated genes, DAB2IP (logFC=-1.19) negative regulation was found beneficial in early pregnancy, and DUSP6 (logFC=-2.07) was associated with cell differentiation and pluripotency. Functional analysis demonstrated positive regulation of biological processes related to embryonic development, cellular invasion and migration, cell cycle, cellular organization, and cell viability in EV-embryos. Contrary, there was a negative regulation of processes related to cell death, apoptosis, and DNA fragmentation. Some pathways upregulated in EV-embryos were NANOG role in embryonic pluripotency (z-score [z]=1.89), unfolded protein response (z = 3.05), involved in cellular homeostasis, VEGF signaling (z = 1.67), that regulates trophoblast adaptation to hypoxia, and integrin signaling (z = 3.36), which regulates trophoblast adhesion and differentiation; PTEN signaling (z=-1.73) was inhibited in EV-embryos, suppression of which prevents apoptosis.

Limitations, reasons for caution: This is an *in vitro* study in which conditions of endometrial cell culture could not mimic the intrauterine environment.

Wider implications of the findings: EVs secreted by the maternal endometrium and uptaken by human blastocysts regulate processes related to embryo development and implantation, suggesting the functional relevance of this endometrial-embryo crosstalk during the implantation process. This study opens further research insight to define potential targets of implantation success and embryo competence.

Trial registration number: Not applicable

Abstract citation ID: dead093.283
O-233 Follicular fluid extracellular vesicles as a potential tool for human spermatozoa selection in vitro

S. Anastasiya[1], **N. Makarova**[1], **O. Nepsha**[1], **B. Zingerenko**[1], **D. Silachev**[2], **Y. Shevtsova**[2], **K. Goryunov**[2], **V. Chagovets**[3], **A. Novoselova**[3], **E. Kalinina**[1]

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Study question: What characteristics and features of follicular fluid (FF) extracellular vesicles (EVs) of women of different ages can affect the functional characteristics of spermatozoa?

Summary answer: The size, lipid composition, and progesterone levels of the FF EVs change with a woman's age and affect the fertilizing ability of spermatozoa.

What is known already: Aging influences on molecular composition of the FF, reducing fertilization processes occurring in the fallopian tubes. FF is an important factor in activating spermatozoa during oocyte fertilization, enhancing the process of sperm hyperactivation and capacitation. FF progesterone is one of the main chemoattractants in the female genital tract, attracting sperm to the oocyte and starting the fertilization processes. Our previous studies have shown a significant effect of FF EVs of young women compared to advanced maternal age women (AMA) on the hyperactivation and motility of spermatozoa. The binding of FF EVs to the acrosoma of the sperm also was detected.

Study design, size, duration: FF EVs were obtained by sequential centrifugation at different rotational speeds. The sperm fraction was isolated, washed by differential centrifugation in a density gradient, suspended in the saline to a concentration of 10*6/ml and incubated with EVs (1:2) at 37 °C in CO2-incubator for 1 h. Next steps: immunofluorescent staining, test for acrosomal reaction of spermatozoa after incubation, NTA analysis, the lipid profile and progesterone measuring of FF EVs of women of different ages.

Participants/materials, setting, methods: All study participants signed a voluntary informed consent for the use of biological samples for research purposes. Sperm samples were isolated from seminal fluid (n = 21) aged 28-36. FF EVs were obtained from young (n = 14) aged 22-30 and AMA patients (n = 14) aged 42-47. The methods used in this work also include immunofluorescence microscopy, nanoparticle tracking analysis, flow cytometry, liquid chromatography–mass spectrometry, statistical data analysis. Lipids were extracted from EVs using a modified Folch extraction protocol.

Main results and the role of chance: Progesterone levels were significantly higher in FF EVs samples from young women compared to FF EVs samples from AMA women and controls (3951000 a.u., 598500 a.u., 6701 a.u.). This may explain the increase in sperm hyperactivation and motility after incubation with FF EVs. The difference in size (144.78 nm ± 9.92 VS 127.03 nm ± 17.17) and lipid composition of young women's FF EVs may explain the more significant functional and therapeutic effect on spermatozoa compared to AMA women FF EVs. A total of 389 vesicle lipids were identified. The level of cholesterol esters, cholesterol, lysophosphatidylcholines, phosphatidylcholines was 1,5 times higher in FF EVs of young women. The level of di- and triglycerides, ceramides, oxidized forms of phosphatidylcholines was higher in FF EVs of AMA women. The level of spontaneous acrosomal reaction was almost 3 times lower in sperm samples after incubation with FF EVs compared to the control (11,21% VS 32,88%). This supports the hypothesis that FF EVs may restrain premature acrosomal reaction in the fallopian tubes. Age-related changes are reflected in reproductive important biological processes, which leads to a change in the composition and functions of FF and affect the functional role of FF EVs.

Limitations, reasons for caution: Small amount of data. To be completely confident in the importance of the participation of FF EVs in the activation of spermatozoa, it is necessary to perform proteomic analyses of FF EVs to discuss the possible mechanisms underlying the effects of FF EVs of different age groups on sperm characteristics.

Wider implications of the findings: Further studies may help not only to complement the fundamental understanding of the processes of fertilization and the impact on spermatozoa morphofunctional characteristics, but also to develop a tool for improving ART outcomes in couples with male infertility. The solutions could be the use of FF EVs from young donors.

Trial registration number: not applicable

Abstract citation ID: dead093.284
O-234 Constant antioxidant supplementation increases blastocyst formation from oocyte donors

J. González Martínez[1], L.B. Ramirez-Dominguez[2], I. Jimenez Medina[2], A. Matamoros-Volante[3], L.G. Villar-Muñoz[2], A. Rambhatla[4], A. Agarwal[5], I. Maldonado[2]

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Study question: Does constant supplementation of antioxidants to the culture media increase blastocyst formation from oocyte donors?

Summary answer: The constant supplementation of antioxidants to the culture media increases blastocyst formation from oocyte donors

What is known already: The use of a combination of various antioxidants in embryo culture media has recently been investigated to explore the potential benefit for embryo development and clinical outcomes. However, the concentration of antioxidants added to the culture has only been determined by its effect. In this study, we performed a repeated antioxidants supplementation to resemble a physiological oxidation reduction environment. Therefore, we investigated the effect of antioxidants added every 12 hours to the culture media on blastocyst formation and expansion in oocyte donors.

Study design, size, duration: This prospective study was conducted at CITMER, Mexico from April 2020 to November 2022.We included a total of 258 recipients from oocyte donors undergoing IVF/ICSI.

Participants/materials, setting, methods: A total of 2403 zygotes were divided into 4 groups and cultured in the following conditions until blastocyst stage: Group 1A: 563 zygotes 20% O2 with antioxidants every 12 hours, Group 1B: 1109 zygotes 20% O2 with antioxidants at the beginning, Group 2A: 339 zygotes 5% O2 with antioxidants every 12 hours, Group 2B: 392 zygotes 5% O2 with antioxidants at the beginning. Embryo development was assessed. Odds ratio and Fisher test were performed. $p < 0.05$=significant

Main results and the role of chance: For both oxygen tensions, we found a significant increase in the total blastocyst formation rate (day 5+day 6) when antioxidants were added repeatedly (1A: 54.7% vs 1B: 45.9%, p = 0.0007*; 2A: 58.7% vs 2A: 46.9%, p = 0.001*). In addition, the rate of expansion at days 5 + 6 was also significantly higher than in the groups where the antioxidants were added only at the beginning of the culture (1A: 35.9% vs 1B: 29.9%, p = 0.01*; 2A: 41.3% vs 2A: 31.6%, p = 0.006*).

Limitations, reasons for caution: Given this is a sibling zygotes study, patients are their own controls.

Wider implications of the findings: Constant supplementation of antioxidants to the culture media increases blastocyst formation from oocyte donors as well as the expansion rate, which may significantly improve clinical outcomes.

Trial registration number: 06162020-aox04

Abstract citation ID: dead093.285
O-235 Antioxidants in culture media improve ICSI fertilisation rate: results from a randomised controlled trial

R. Kelley[1], L. Lee[1], F. Agresta[1], A. Huang[1], J. Sgroi[1], G. Rozen[1], A. Polyakov[1], L. Hale[1], R. Lew[1], C. Stern[1], M. Toledo[2], F. Cattrall[1], D. Gardner[1]

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Study question: Does addition of three antioxidants to G-Series media during gamete collection, insemination, and embryo culture increase the clinical pregnancy rate from fresh blastocyst transfers?

Summary answer: Antioxidants increased the ICSI fertilization rate, and consequently the number of blastocysts utilised, but the clinical pregnancy rate from fresh blastocyst transfers was not affected.

What is known already: Supplementation of IVF media with a combination of three antioxidants known as A3 (10 µmol/l acetyl-L-Carnitine, 5 µmol/l α-Lipoic acid and 10 µmol/l N-acetyl-L-cysteine) is beneficial in mouse IVF, embryo culture and cryopreservation, and improves post-transfer outcomes. Further, in a prospective randomised trial on sibling oocytes, addition of A3 to G-1/G-2 media (Vitrolife, Sweden) resulted in more good quality embryos on day 3 (Gardner et al., 2020). The study was not powered to determine the effect of A3 on pregnancy rate, but an increase in ongoing pregnancy rate was observed in women aged 35-40 years following frozen blastocyst transfer.

Study design, size, duration: Single-centre, prospective randomised controlled trial, superiority study comparing G-series media (G-MOPS, G-IVF and G-TL (Vitrolife)) with or without the addition of A3 from January 2019 to November 2021. 1482 patients were randomized before egg collection. Patients and their doctors were blinded to the treatment group. The primary endpoint was clinical pregnancy per randomised couple from the fresh transfer of a single blastocyst.

Participants/materials, setting, methods: Patients undergoing IVF/ICSI cycles and intending to undergo a fresh transfer of a single blastocyst were recruited. Exclusion criteria were previous participation in the study, freeze-all cycle, or extraction of sperm from testicular biopsy. 743 patients were randomised to the A3 media, 739 to the control.

Main results and the role of chance: Patients randomised to A3 media had significantly higher fertilisation rate per inseminated oocyte (64.4 ± 25.6%) compared to the control (59.2% ± 26.3%, $P < 0.001$). This was more pronounced in patients undergoing ICSI (68.1% ± 24.9% vs 57.9% ± 27.2%, $P < 0.0001$), where the number of cycles with failed fertilisation decreased from 8.0% to 3.9% with A3 media ($P < 0.05$). There was no effect of A3 on fertilisation rate following IVF. Blastocyst development rate was unaffected by A3, but the higher fertilisation rate resulted in more blastocysts available for transfer or cryopreservation per patient in the A3 group (3.06 ± 2.97 vs 2.67 ± 2.60, $P < 0.01$). Clinical pregnancy rate from fresh cycles was not different between the control (26.1%) and A3 media (23.0%; $P > 0.05$; RR 0.88, 95% CI 0.73-1.05). When patients who did not have a fresh transfer were excluded (due to freeze-all or no embryo available), there was also no difference between the control (35.8%) and A3 media (32.6%; $P > 0.05$; RR 0.91; 95% CI 0.77-1.08).

Limitations, reasons for caution: This was a single-centre study, so the effects of A3 supplementation in clinics with different media and protocols are unknown. Detection of fetal heart by ultrasound was the primary endpoint, birth outcomes are currently being investigated. Outcomes of frozen embryo transfers and cumulative pregnancy rates are yet to be determined.

Wider implications of the findings: Addition of antioxidants to media during gamete collection, incubation and ICSI can increase fertilisation rate and reduce the frequency of failed fertilization cycles. This results in more blastocysts available for transfer and cryopreservation per egg collection, potentially leading to higher cumulative pregnancy rates.

Trial registration number: ACTRN12618001479291

Abstract citation ID: dead093.286

O-236 Fatty acid supplementation into warming solutions improves pregnancy outcomes after single vitrified-warmed cleavage stage embryo transfer

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[3]*Kato Ladies Clinic, Gynecology, Tokyo, Japan*

Study question: Does fatty acid (FA) supplementation into warming solutions affect pre-implantation development, outgrowth competence, and clinical outcomes of human vitrified cleavage stage embryos?

Summary answer: FA-supplemented solutions improve morphology and outgrowth of blastocysts derived from vitrified cleavage stage embryos and pregnancy outcomes after single vitrified-warmed cleavage stage embryo transfer (SVCT).

What is known already: Vitrification procedure decreased the intracellular lipid content and subsequent developmental competence. Furthermore, a recent study reported that FA supplementation into warming solutions increased intracellular lipid content and improved developmental competence by stimulating the beta-oxidation pathway in mice and bovines. However, in humans, the effects of FA addition to warming solutions on developmental competence and morphokinetics during the pre-implantation period remain unknown. Additionally, the efficacy of FA supplemented warming solutions in clinical settings has not yet been evaluated.

Study design, size, duration: A total of 217 discarded human vitrified 4-cell stage embryos donated for research by consenting couples were randomly allocated, to be warmed in solutions either with (FA, n = 111) or without FA (control, n = 106). Embryonic development, morphokinetics, and trophoblast migration were experimentally compared between the groups. Furthermore, a total of 701 clinical records of women who underwent SVCT between April and September 2022 were retrospectively analysed (control, April–June; FA, July–September).

Participants/materials, setting, methods: Discarded 4-cell stage embryos were warmed and cultured for 72 h in a time-lapse incubator. Furthermore, the blastocysts produced were plated on fibronectin-coated dishes and cultured to assess blastocyst adhesion and outgrowth. In the clinical study, vitrified cleavage stage embryos were warmed in solutions with or without FA and transferred on day 2 after ovulation in the natural cycle. The rates of implantation, clinical pregnancy, and ongoing pregnancy were compared between the groups.

Main results and the role of chance: The developmental rates were comparable between the groups. However, the rate of morphologically good blastocysts was significantly higher in the FA group than in the control group ($P = 0.0089$). The developmental timings were comparable between the groups during all stages. Additionally, the incidence of abnormal cleavages, amount of fragmentation at the cleavage and morula stages, and incidence of blastomere exclusion or extrusion during peri-compaction were comparable between the groups. Further, the blastocyst adhesion rate after outgrowth culture was also comparable between the groups. However, the outgrowth area was significantly larger in the FA group than in the control group ($P = 0.0438$). In the clinical study, the characteristics of the patients and transferred embryos were comparable between the groups. The implantation, clinical pregnancy, and ongoing pregnancy rates were higher in the FA group than in the control group ($P = 0.0252$, $P = 0.0223$, and $P = 0.0281$, respectively). Multivariate logistic regression analysis demonstrated that the probability of ongoing pregnancy was significantly higher in the FA group than in the control group (adjusted odds ratio, 1.46; 95% confidence interval, 1.02–2.06; $P = 0.0340$). The rate of miscarriage during the first trimester was comparable between the groups.

Limitations, reasons for caution: This study has some limitations owing to its retrospective design. Further prospective studies are required to validate the clinical efficacy of FA-supplemented solutions. Furthermore, the effects of adding FA to warming solutions on maternal and perinatal outcomes should be assessed in the future.

Wider implications of the findings: FA supplementation into warming solutions would improve the clinical outcomes of frozen embryo transfers, leading to a shortened treatment period and reduced patient burden in assisted reproductive technologies.

Trial registration number: not applicable

POSTER DISCUSSION SESSION
SESSION 76: GSCAIF
Wednesday 28 June 2023 Hall D2 08:30 - 09:30

Abstract citation ID: dead093.287
P-439 Intimate hygiene practices and the correlation with abnormal vaginal microbiota in in vitro fertilization (IVF) patients – a cohort study in 1421 patients

S. Ovesen[1], T. Haahr[1], M. Brix[1], J.S. Jensen[2], B. Alsbjerg[1,3], M. Tanderup[1], A. Mikkelsen[1], L. Prætorius[4], A. Pinborg[5], N.L.C. Freiesleben[4], P. Humaidan[1,3]

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Study question: What are the intimate hygiene practices of women in IVF treatment and do they correlate with the vaginal microbiota?

Summary answer: Hygiene practices, in specific douching and use of intimate soap significantly increased the risk of abnormal vaginal microbiota (AVM).

What is known already: The normal vaginal microbiota acts as a defense system against infection. AVM is a molecularly defined vaginal dysbiosis, resembling bacterial vaginosis (BV) and is dominated by anaerobic bacteria such as Gardnerella vaginalis. Importantly, AVM is associated with an increased risk of genital tract infections, poor IVF outcomes, early miscarriage and preterm labor.

The cause of AVM is multifactorial and hygiene practices may interrupt the normal microbiota. Previous small studies suggested vaginal douching to be associated with an increased risk of AVM. Few studies have investigated the possible correlation between intimate hygiene practices, menstrual practices and the vaginal microbiota.

Study design, size, duration: Observational cohort study, including a total of 1421 IVF patients from four Danish fertility centers. The inclusion period was from 2017 to January 2022.

Participants/materials, setting, methods: Patients aged 18-42 years and undergoing their first, second or third IVF stimulation cycle were eligible for inclusion. The intimate hygiene practices in terms of type of soap, menstrual protection, douching and probiotics, were reported in a structured questionnaire including a total of 40 questions. Vaginal swabs were obtained prior to ovarian stimulation and subsequently subjected to quantitative PCR testing, targeting DNA of dysbiotic bacteria.

Main results and the role of chance: AVM was present in a total of 34 % (479/1421) of women, and 20% (272/1384) reported vaginal douching, which significantly correlated with AVM ($p < 0.01$, OR = 1.62 [1.23-2.12]). Intimate soap was used by 39 % (535/1384) of patients, which also significantly increased the risk of AVM ($P = 0.04$, OR = 1.35 [1.08-1.69]). Water only was used by 36% (492/1384), and regular soap was used by 27% (374/1384). No correlation between use of water only and regular soap and AVM was detected. For menstrual protection 40% (433/1078) used pads, followed by tampons alone 25% (265/1078) or a combination 21% (230/1078), and 14 % (148/1078) used a menstrual cup. A trend for an increased risk of AVM when using tampons, OR 1.21 [0.91-1.62] was seen whereas use of a menstrual cup seemed to lower the risk of AVM, OR 0.71 [0.48-1.04]

Active smoking, higher BMI, drinking >7 units per week and previous chlamydia infection significantly correlated with AVM ($p = 0.01$, $p = 0.01$, $p = 0.04$ and $p = 0.01$).

Finally, 84% (1190/1421) reported gynecological symptoms such as vaginal discharge, dyspareunia and fungal infection, but only fishy odor significantly correlated with AVM ($p < 0.01$).

Limitations, reasons for caution: To the best of our knowledge, this is the largest study investigating the intimate hygiene practice and its correlation with AVM in an IVF population. As these results describe the practices of a Scandinavian IVF population, results may differ from other settings and ethnicities.

Wider implications of the findings: Exploration of the cause-and-effect relations between intimate hygiene practices and AVM is needed, requiring intervention-based prospective studies. This would lead to evidence-based advice on intimate hygiene practices and AVM prevention, possibly increasing live birth rates. The unexpected high prevalence of douching in a Danish IVF population needs further exploration.

Trial registration number: NCT03420859

Abstract citation ID: dead093.288
P-460 The Impact of Assisted Reproductive Technologies on Climate Change : The French Case

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Study question: How much tons of carbon dioxide equivalent (tCO_2e) are generated by Assisted Reproductive Technologies in France in a year ?

Summary answer: It is estimated that Assisted Reproductive Technologies generated 55 691 tons of carbon dioxide equivalent in France in 2022.

What is known already: Since the 1800s, human activities have been the main driver of climate change, primarily due to burning fossil fuels (coal, oil and gas). Created in 1988, the Intergovernmental Panel on Climate Change (IPCC) provides regular assessments of the scientific basis of climate change, its impacts and future risks, and options for adaptation and mitigation. On December 12th 2015, 196 Parties adopted The Paris Agreement at COP 21 (Paris), a legally binding international treaty on climate change, aiming to limit global warming to well below 2 degrees Celsius. The French health sector is only beginning to measure it's carbon emission footprint.

Study design, size, duration: This study was conducted in Poissy/Saint-Germain-En-Laye Hospital during the year 2022 in the Department of reproductive medicine and biology. Greenhouse gas emissions were classified into three scopes. Scope 1 emissions are direct emissions divided into four categories : stationary combustion, mobile combustion, fugitive emissions, process emissions. Scope 2 emissions are indirect emissions from the generation of purchased energy. Scope 3 emissions are all indirect emissions, including both upstream and downstream emissions (separated into 15 categories).

Participants/materials, setting, methods: Every couple that required IVF or ICSI (including donors) during the year 2022 was included in this study. Number of personnel, transportation of patients, total medication prescribed, electricity consumption and medical furnitures were considered for the calculus of total emissions. Emission factors to calculate carbon emission were taken on the Ecological Transition Agency (ADEME) database. Extrapolation to all of France's ART clinics was possible using the BioMedical Agency (ABM) 2020 report on ART activities.

Main results and the role of chance: Assisted Reproductive Technologies generated 55 691 tons of carbon dioxide equivalent (tCO2e) in France in 2022 from 30 963 ICSI and 15 674 IVF procedures.

We estimate that every oocyte retrieval procedure generated 1.19 tCO2e (ICSI and IVF).

Department of Reproductive Medicine and Biology of Poissy/Saint-Germain-En-Laye (PSGEL) generated 796 tCO2e from 666 oocyte retrieval procedures (449 ICSI, 217 IVF), representing 1.43% of all IVF and ICSI in France.

Electricity consumption at PGSEL generated 1 051 tCO2e based on a total of 14 200 kw/h (average price of 0.1740 euros) in 2022.

Drugs prescriptions (758 069 euros) and Furniture (194 648 euros) at PGSEL generated respectively 409.584 tCO2e and 61.314 tCO2e in 2022 .

32 members of medical and non-medical at PGSEL staff generated 320 tCO2e in 2022.

Transportation of patients to PGSEL generated 4.44 tCO2e in 2022.

This is the first study to report an estimation of the carbon emission footprint of French ART.

Limitations, reasons for caution: Not all data was available (CO2 emissions created by the manufacture of active pharmaceutical ingredients in Asia) or taken into account (waste management, anesthesia type, hospital construction, etc).

Fertility preservation procedures were not considered in our calculations.

Results are therefore an under-estimation of real CO2 emissions.

Wider implications of the findings: French Healthcare was responsible for 46 MtCO2e (8% of national total) in 2021.

The biggest impact we can have in ART to lower our carbon footprint is optimise the pharmaceutical supply chain of drug manufacturing and use recycled materials for drug packaging (plastic, glass, aluminium, steel) as much as possible.

Trial registration number: not applicable

Abstract citation ID: dead093.289

P-463 Data from 143,251 women indicates that black women access at-home fertility testing services later in life compared to white women in the United Kingdom

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Study question: Does ethnicity play a role in fertility assessment-seeking behaviour of Black women in the United Kingdom (UK)?

Summary answer: Black women approach an at-home reproductive health and fertility assessment service later in their fertility journey than White women.

What is known already: Evidence from the Human Fertilisation and Embryology Authority (HFEA) shows that Black patients access fertility care two years later than the national average in the United Kingdom (UK) (36.4 vs 34.6 years).

Additionally, previous research has shown different prevalences of reproductive health conditions associated with infertility amongst Black women, such as fibroids and tubal occlusion. In combination with delayed access to fertility care, this may contribute to the lower live birth rates seen in Black patients in the UK.

Study design, size, duration: A retrospective observational study conducted on 143,251 Hertility Health users who completed a virtual health assessment between September 2020 and January 2023. The health assessment collected self-reported data on age, ethnicity, pre-existing diagnoses of reproductive health conditions, whether they were actively trying to conceive (TTC) and the length of time they had been actively TTC.

Participants/materials, setting, methods: A total of 25.9% users (n = 37,170) indicated they were TTC. The majority of those TTC self-identified as White (82.9%), followed by Asian (7.4%), Mixed (3.6%), Black (4.6%) and 'Other' (1.5%). Summary statistics (mean ± SD and %) regarding age and prevalence of pre-existing conditions of users TTC have been reported. Additional statistical analysis of the associations of time spent TTC between ethnicities was conducted via Chi-squared test ($\chi 2$); p values <0.05 were considered significant.

Main results and the role of chance: A significant relationship was observed between time spent TTC and ethnicity; the strongest association was observed between Black and White users $\chi 2$ (16, n = 34,706)=120, $p < 0.0001$). Comparison of time spent TTC, and average age showed that fewer Black women undertook the virtual health assessment at < 6 months of TTC than White women (29.3%, n = 469 vs 34.1%, n = 9820) at a moderately older age (31.3 ± 6.5 years vs 29.8 ± 5.7 years).

A higher percentage of Black women were TTC for >5 years compared to White women (14.2%, n = 227 vs 8.8%, n = 2544). Additionally, Black women at this time point were approximately four years older than White women (36.9 years ± 6.0 years vs 33.0 ± 5.1 years).

Secondary analysis of the prevalence of reproductive health conditions associated with infertility indicated that 20.1% (n = 133) and 22.5% (n = 149) of Black women had a preexisting condition at < 6 months and >5 years of TTC, respectively, vs 22.6% (n = 1363) and 19.2% (n = 1158) of White women in the corresponding TTC length groups.

Limitations, reasons for caution: As the virtual health assessment data was self-reported, there is a risk of recall bias and false reporting. Data was stratified via the main ethnicities and did not take the heterogeneity within ethnic groups or mixed ethnicities into account.

Wider implications of the findings: This data suggests that Black women approach fertility testing services later than White women, which may contribute to the disparities between pregnancy outcomes in the UK and cannot fully be accounted for by preexisting diagnoses associated with infertility. Further investigations are needed to outline the underlying reasons for this.

Trial registration number: not applicable

Abstract citation ID: dead093.290

P-465 Effect of male age on the outcome of frozen embryo trnasfer cycle in women over 40 years

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Study question: Does male age affect the outcome of in vitro fertilization in women over 40?

Summary answer: Advanced male partner's age is associated with decreased pregnancy rate, implantation rate and ongoing pregnancy/live birth rate.

What is known already: Fertility is known to have the greatest correlation with female age. Older women have a relatively lower pregnancy rate and higher miscarriage rate. It is also widely known that male partner's age is associated to pregnancy rate with increased sperm DNA fragmentation and decreased sperm motility. Age of marriage is delayed both male and female partners, assisted reproductive techniques are often conducted in women over 40 years. The effect of male age on IVF/ICSI outcomes on women over 40 is poorly explored.

Study design, size, duration: This is a retrospective cohort study analysed 1046 cycles of couples with an age of 40 or more of female partners and their male partners, performed at a single IVF center of university hospital between 2019 and 2022. The primary outcome was ongoing live birth and secondary outcomes were clinical pregnancy and miscarriage.

Participants/materials, setting, methods: Couples of primary/secondary infertility with female partner age over 40 years who underwent frozen-embryo transfer cycles were selected. couples who collected their sperm from TESE/TESA were excluded.

Main results and the role of chance: We decided the male partner's age in three groups, age less than 40, 41-45, and age over 45. There was a negative effect of male age on ongoing live birth and clinical pregnancy rate.(20.0%, 10.3%, 10.9%, $p < 0.001$, 33.7%, 28.4%, 19.9%, $p = 0.017$). And we sub-analyzed ongoing live birth rate and clinical pregnancy rate into female age group(female age of 40, 41-42, and over 43). The ongoing pregnancy/live birth rate and clinical pregnancy rate were negatively associated with male partner's age especially in female age group of over 43 years.

Limitations, reasons for caution: This study is limited to the information on confounding factors. The study may also be limited due to its selection criteria.

Wider implications of the findings: This study provides information regarding IVF/ICSI outcomes on women over 40 years of age

Trial registration number: not applicable

SELECTED ORAL COMMUNICATIONS
SESSION 77: NEWS ON ARTIFICIAL INTELLIGENCE (AI)
Wednesday 28 June 2023 Hall A 10:00 - 11:45

Abstract citation ID: dead093.291
O-237 Metabolic classification of embryos and oocytes based on hyperspectral imaging and machine learning

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Study question: To classify embryos according to their metabolic fingerprint with a robust, non-invasive methodology

Summary answer: Our Hyperspectral (HS) phasor analysis coupled to artificial intelligence simultaneously detects 6+ metabolites to profile and classify competent embryos and oocytes safely and consistently.

What is known already: Metabolism plays a key role in oocyte and embryo developmental competence. However, current methods to measure oocyte and embryo metabolism are either invasive, phototoxic, too slow or based on indirect analysis in cumulus cells or culture media. Some intracellular metabolites are autofluorescent and their concentration can be quantified using HS imaging when using the correct light excitation. HS imaging is an optical technique whereby the full emission spectrum is obtained for each pixel of an image. Thus, raw HS data encodes rich metabolic information of an imaged cell as numerous metabolites are excited simultaneously.

Study design, size, duration: A total of 96 mouse embryos and 115 oocytes were analyzed. *In vivo* fertilized embryo cohorts included control, glucose-starved, pyruvate/lactate-starved, and glucose/pyruvate/lactate-starved. Oocyte cohorts included oocytes obtained from young female mice analyzed blindly either immediately after collection or after overnight culture (*in vitro* aged), and from old female mice. After HS analysis, oocytes were inseminated by ICSI and cultured *in vitro* to correlate their potential to develop up to blastocyst with their metabolic profiles.

Participants/materials, setting, methods: Samples were imaged using multiphoton illumination near the infra-red to avoid phototoxicity. The HS detection covered the whole visible spectrum allowing simultaneous measurement of 6+ relevant metabolites for the embryo/oocyte biology: NADH (free and bound), FAD^{+}, retinol, retinoic acid, and flavins among others. Afterwards, we applied dimensionality reduction (2D phasor image) from which machine learning algorithms learned to classify the different samples. We used Akaike Information Criterion (AIC) model. Cross validation was performed using 80%/20%.

Main results and the role of chance: The Area Under the ROC Curve (AUC) achieved by binary classification as well as the average class-accuracy (computed as the average of the confusion matrix diagonal) were calculated in four-class classification in the case of embryos. [EM1] Brightfield images of the test set of embryos were evaluated by 4 embryologists to look for compromised embryos. Human graders reached an AUC of 51% of the samples using brightfield compared to 93.7% of the AI algorithm using HS-Phasor. A similar approach using the HS-Phasor was conducted on the different cohorts of mouse oocytes. It was able to separate the oocyte classes with an average AUC of 96.2% and make a statistical correlation with their blastulation efficiency with a 82.2% AUC. The viability of embryos and oocytes after imaging was comparable to that of non-imaged controls, indicating that the imaging procedure had no effect on the posterior *in vitro* development of the embryos.

Limitations, reasons for caution: The cohort size is limited although statistically robust. Blastulation efficiency will need to be complemented (ongoing work) with implantation measurements.

Wider implications of the findings: The HS-phasor method has the potential to become a paradigm shift in IVF embryo selection due to the reliable, non-invasive direct measurement of the oocyte/embryo physiology. The adaptation into clinical practice can be straightforward as it offers an additional layer of information for oocyte/embryo assessment without compromising time nor viability.

Trial registration number: NA

Abstract citation ID: dead093.292
O-238 Artificial intelligence algorithms in assisted reproduction: differences in the evaluation of embryos from fresh or vitrified donor oocytes

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Study question: Do the Embryoscope and EmbryoscopePlus systems' built-in software evaluate differently embryos from vitrified or fresh oocytes?

Summary answer: The embryo scores provided by artificial intelligence (AI) algorithms are lower for embryos originated from vitrified/warmed oocytes than for those that come from fresh oocytes.

What is known already: Time-lapse technology has enabled embryologists to develop several AI-based tools that allow the automation and standardization of embryo quality assessment. EmbryoScope and EmbryoScopePlus systems include deep learning-based models using only time-lapse image sequences (iDAScore v1.0 and v.2.0) and the machine learning-based model (KIDScore D5 v3) which classifies embryos in categories based on cleavage time points and blastocyst appearance. It has been reported that fertilization, pregnancy and implantation rates in vitrified oocytes are comparable to those of fresh oocytes. To our knowledge, this is the first study to test whether artificial intelligence tools assess embryos derived from these oocytes differently.

Study design, size, duration: This retrospective study includes the analysis of 34950 videos of embryos from fresh and vitrified donor oocytes. Embryos were cultured on EmbryoScope and EmbryoScopePlus Time Lapse systems and morphological and morphokinetic parameters were automatically detected by EmbryoViewer software. Senior embryologists routinely evaluated the embryos according to the ASEBIR criteria. In addition, embryos were graded using IDAScore v1, IDAScore v2 and KIDScoreD5 algorithms in different scores from low to high quality (1-9.9).

Participants/materials, setting, methods: The scores of embryos from fresh or vitrified donor oocytes obtained with the different algorithms were compared. Specifically, 21533 embryos from fresh and 12710 from vitrified oocytes with the IDAScore v1; 21335 from fresh and 12705 from vitrified with the IDAScore v2; and 13544 from fresh and 6559 from vitrified with the KIDScore v3. In addition, the ASEBIR evaluation performed by embryologists was also compared between embryos from fresh (21968) and vitrified oocytes (12980).

Main results and the role of chance: Mean scores of embryos coming from fresh oocytes from IDAScore v1, IDAScore v2 and KIDScore v3 were 5.34 ± 2.91, 3.48 ± 2.93, 5.13 ± 2.1 respectively. And mean scores of those from vitrified oocytes were 5.09 ± 2.83, 3.1 ± 2.71, 4.99 ± 2.01. Thus, mean embryo scores obtained by the 3 artificial intelligence algorithms were significantly higher* for embryos from fresh oocytes compared to those from vitrified oocytes. However, this lower embryo assessment of those from vitrified oocytes was not detected when comparing embryo quality according

to ASEBIR classification. We hypothesized that it might be because several embryos that had not reached blastocyst stage on D6 were included in this comparison. Comparisons were repeated excluding these embryos, mean scores of embryos from fresh oocytes from IDAScore v1, IDAScore v2 and KIDScore v3 were 7.31 ± 2.12, 5.15 ± 2.79, 5.18 ± 2.07 respectively. Mean scores of those from vitrified oocytes were 7.14 ± 2.12, 4.75 ± 2.70, 5.01 ± 2.01. Again, mean scores of embryos from fresh oocytes were significantly higher* than those from vitrified oocytes. In this analysis, this significant difference* in embryo score was also detected according to the ASEBIR classification.

*p<.05

Limitations, reasons for caution: This project is limited by its retrospective and single-center nature. A multicenter study could be performed to determine whether these minimal but significant differences in the evaluation of embryos from fresh or warmed oocytes have any impact on clinical outcome.

Wider implications of the findings: This study shows that embryos from vitrified oocytes have significantly lower scores than those from fresh oocytes. The embryo assessment performed in this study is based on AI models that allow their instantaneous, non-invasive, accurate and objective evaluation. Thus, the impact of these differences on clinical outcome, should be analyzed.

Trial registration number: not applicable

Abstract citation ID: dead093.293
O-239 A model based on artificial intelligence for the non-invasive prediction of embryo aneuploidy

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Study question: Can the automated artificial intelligence (AI) model iDAScore predict embryo aneuploidy?

Summary answer: The AUC for aneuploidy prediction of iDAScore was AUC 0.640, with optimal cut-off score 7.75, sensitivity 61.0% and specificity 60.9%.

What is known already: Embryo morphokinetics and image analysis using AI have been proposed as alternative approaches for non-invasive embryo aneuploidy prediction. The automated AI model iDAScore, as well as the morphokinetic embryo selection model KIDScoreD5 have been shown to have correlation with embryo ploidy status. The aim was to study the performance of aneuploidy prediction using the iDAScore, KIDScoreD5 and morphology grading (Gardner criteria).

Study design, size, duration: Retrospective study including 382 blastocysts of known ploidy from 92 patients undergoing PGT-A in a single centre during January-December 2022. For statistical analysis, continuous and categorical data were compared using ANOVA and Fisher's exact test/Chi-square, respectively. ROC analysis was used to estimate the discrimination performance of variables to predict aneuploidy. Stepwise logistic regression was used to identify confounding variables and construct a model for aneuploidy prediction.

Participants/materials, setting, methods: Embryos were cultured in Embryoscope, underwent laser assisted hatching on Day3, were biopsied on Day5-Day6 and were vitrified. Whole-genome amplification and next-generation sequencing were used for PGT-A. iDAScore values were calculated using v1.2.0. KIDScoreD5 v.3 was calculated after annotation of the required morphokinetic parameters in Embryoviewer. For Gardner criteria, inner cell mass (ICM) and trophectoderm (TE) were classified into grades A to C. The expansion in all biopsied blastocysts was > =grade 4.

Main results and the role of chance: Out of 382 embryos analysed, 127 (33.2%) were euploid and 255 (66.8%) were aneuploid. Euploid embryos were associated with significantly higher iDAScore (7.69, 95% CI 7.44-7.95 vs 6.86, 95% CI 6.66-7.09, $p < 0.001$), higher KIDScoreD5 (6.41, 95% CI 6.07-6.75 vs 5.74, 95% CI 5.51-5.97, $p = 0.001$) and lower maternal age (37.10 years, 95% 36.39-37.82 vs 39.83 years, 95% CI 39.36-40.30, $p < 0.001$) compared to aneuploid embryos. Significantly more embryos with grade A ICM (A:39.4%, B:26.0%, C:20.0%, $p = 0.013$) and grade A TE (A: 45.9%, B:29.6%, C:23.6%, $p = 0.002$) were euploid compared with B and C grades, respectively. Blastocysts biopsied on Day 5 and Day 6 had similar proportions of euploidy (34.9% vs 27.6%, $p = 0.125$). The AUCs for aneuploidy prediction were: iDAScore: 0.640 (95% CI 0.582-0.699), KIDScoreD5: 0.604 (95% CI 0.542-0.665), ICM grade: 0.581 (95% CI 0.521-0.640), TE grade: 0.596 (0.536-0.657), maternal age: 0.694 (0.640-0.748). The optimal cut-off (Youdex index) for aneuploidy prediction of iDAScore was 7.75 (sensitivity 61.0%, specificity 60.9%). Following stepwise multivariate logistic regression, the variables that entered the model were iDAScore, maternal age and TE grade. The model iDAScore+age+TE grade increased the ability of aneuploidy prediction (AUC 0.747, 95% CI 0.696-0.796). KIDScoreD5, ICM grade and day of biopsy failed to enter the model.

Limitations, reasons for caution: This is a retrospective study and therefore the presence of bias cannot be excluded. iDAScore and KIDScore are models originally developed for the prediction of implantation and not for the diagnosis of embryo aneuploidy.

Wider implications of the findings: iDAScore appears to associate with embryo aneuploidy. Combination of iDAScore+age+TE increased the AUC for aneuploidy prediction. iDAScore can used as a decision-support tool for prioritising embryos for transfer, cryopreservation or biopsy. The analysis of more embryos is needed to confirm the present findings.

Trial registration number: not applicable

Abstract citation ID: dead093.294
O-240 Does embryo categorisation by existing artificial intelligence, morphokinetic, or morphological embryo selection models correlate with blastocyst euploidy rates?

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Study question: Does embryo categorization by existing artificial intelligence, morphokinetic, or morphological embryo selection models correlate with blastocyst euploidy?

Summary answer: Our results show that existing blastocyst scoring models correlate with ploidy status.

What is known already: A previous study suggested that morphokinetic parameters should not be used yet as a surrogate for PGT-A to determine embryo ploidy in vitro. As for predicting pregnancy likelihood, AI models have been proposed for predicting ploidy status. Discrimination performance in terms of AUC has been reported of up to 0.80 when including metadata such as maternal age, and 0.63 when AI predictions were made based on time-lapse images alone. However, as the studies only looked at models trained for ploidy prediction, it is unclear how existing blastocyst scoring systems would perform at discriminating euploids and aneuploids.

Study design, size, duration: A total of 834 patients, 3,573 blastocysts were retrospectively analyzed. The cycles were stratified into five maternal age groups according to the Society for Assisted Reproductive Technology (SART) age groups (< 35, 35–37, 38–40, 41–42, and >42 years). The quality and scoring of embryos were assessed by iDAScore v1.0 (iDA, Vitrolife, Sweden), KIDScoreTM D5 v3 (KS; Vitrolife), and Gardner grading (GG).

Participants/materials, setting, methods: Embryos were cultured in the EmbryoScope+ and EmbryoScopeFlex (Vitrolife). iDA was automatically calculated using the iDAScore model running on the EmbryoViewer (Vitrolife). KS was calculated in EmbryoViewer after annotation of the required parameters. ICM and TE were annotated according to the Gardner grading. The degree of expansion in all blastocysts was Grade 4 due to our freezing policy. Furthermore, Gardner's scores were stratified into four grades (A: AA, B: AB BA, C: BB, D: others).

Main results and the role of chance: At first, the correlation between all assessment methods and euploid rates were analyzed using a trend-test. Euploidy rates significantly correlated with iDA in each age group ($p = 0.035$ for age <35 years, $p < 0.001$ for all other age groups) and decreased

progressively with increasing maternal age in each score group. Furthermore, euploidy rates were significantly correlated with KS ($P < 0.0001$), except in the youngest age group ($p = 0.07$). They decreased progressively with increasing maternal age in each score group. Similarly, euploidy rates were significantly correlated with GG ($P < 0.0001$), except in the youngest age group ($p = 0.06$). They decreased progressively with increasing maternal age in each score group. Secondly, the performance of the euploidy prediction for each embryo scoring model was compared using the area under the curve (AUC) of the receiver operating characteristic curve. The AUCs for euploid prediction of iDA, KS, and GG for all ages were 0.666, 0.655, and 0.642, respectively. iDA and KS were significantly higher than Gardner grading (iDA vs. GG: $p = 0.004$; KS vs. GG: $p = 0.02$). Additionally, there was no significant difference between iDA and KS.

Limitations, reasons for caution: It was based on minimal stimulation and the use of natural cycle for IVF treatment, which involved only insemination by ICSI. Furthermore, the blastocyst assessment models that we tested were initially developed for pregnancy prediction and not for the prediction of the chromosomal status of an embryo.

Wider implications of the findings: Existing blastocyst assessment models that reflect blastocyst viability in view of implantation potential to some extent correspond with ploidy status. Therefore, existing blastocyst scoring models can be used to support the decision for embryo biopsy and/or to reduce the patient's cost by deciding on which embryo(s) to biopsy.

Trial registration number: not applicable

Abstract citation ID: dead093.295

O-241 Association of a deep learning-based scoring system with morphokinetics and morphological alterations in human embryos

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Study question: How is the deep learning-based scoring system, iDAScore, associated with biological events during the pre-implantation period?

Summary answer: The morphokinetic analysis revealed that during the transformation to blastocyst stage, morphokinetic and morphological events were strongly associated with iDAScore.

What is known already: A recent study reported that the commercially available deep learning model for automatic scoring, iDAScore, performed as well as, or even better than, the more traditional embryo assessment-based model on blastocyst morphology or annotation-dependent ranking tools. Furthermore, a correlation between iDAScore and the incidence of rapid cleavage, time duration until the blastocyst stage, and morphological grade of the inner cell mass and trophectoderm was reported. However, the association of iDAScore with other key morphokinetic parameters remains undetermined.

Study design, size, duration: This retrospective observational study included 925 patients who underwent oocyte retrieval in a clomiphene citrate-based minimal stimulation cycle and obtained expanded blastocysts in ICSI cycles between October 2019 and December 2020. Oocytes of patients with different diagnoses of infertility were included in the analysis, while cases involving cryopreserved gametes or surgically retrieved sperm were excluded. The association of iDAScore with morphokinetics and morphological alteration during fertilisation, cleavage stage, compaction, and blastocyst stage were analysed.

Participants/materials, setting, methods: Microinjected oocytes were assessed using a time-lapse monitoring (TLM) culture system (Embryoscope). Oocytes not suitable for TLM assessment, due to excess of residual corona cells or inadequate orientation for correct observation, were not analysed. Phenomena, relevant to meiotic resumption, pronuclear dynamics, cytoplasmic/cortical modifications, cleavage pattern, and embryo quality, were annotated. Multiple linear regression analysis was used to assess the relative importance of the possible predictor variables in explaining the iDAScore.

Main results and the role of chance: The duration of the cytoplasmic halo was significantly prolonged in the low scoring blastocysts. The timing of female and male pronuclei breakdown was significantly delayed in the low scoring blastocysts compared with the high scoring blastocysts. Embryos with either trichotomous, multi-chotomous, rapid, or reverse cleavage or asymmetric division exhibited a lower score than embryos with normal cleavage. The cell number and amount of blastomere fragmentation on days 2 and 3 were significantly associated with iDAScore. Delayed division, compaction, blastulation, and blastocyst expansion were observed in the low scoring embryos. The incidence of blastomere exclusion and extrusion during embryonic compaction was significantly higher in low scoring embryos than in high scoring embryos. Blastocyst morphology was significantly associated with iDAScore. Multiple linear regression analysis revealed that morphokinetic and morphological events were strongly associated with iDAScore especially during the transformation to blastocyst stage, which has been considered important in embryo assessment. It was also revealed that some morphological parameters and time durations during the cleavage stage were also correlated with the iDAScore.

Limitations, reasons for caution: The study data derive from treatments carried out in a single centre. The study findings therefore require independent verification from other research groups.

Wider implications of the findings: We found that iDAScore was significantly correlated with morphokinetics and morphological alterations of pre-implantation embryos, especially during the late pre-implantation period. Our findings may contribute to the literature on deep learning model-based embryo selection, which may provide patients with a compelling explanation regarding blastocyst selection.

Trial registration number: not applicable

Abstract citation ID: dead093.296

O-242 Comparing the performance of an artificial intelligence model for predicting embryo implantation between clinics with patient cohorts of different maternal age distributions

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Study question: Do differences in maternal age distributions contribute to the variability in the discrimination performance of an embryo viability model based on artificial intelligence (AI)?

Summary answer: Using a common reference age distribution, enables a less biased and thus more standardized comparison of embryo implantation prediction between clinics.

What is known already: Studies from different clinics have shown a quite large variation in the performance of the same AI model. The most common method of comparing performance between clinics is to use simple clinic-specific performance statistics while ignoring dissimilarities between clinics in patient cohorts, culture conditions etc. A more appropriate method is meta-analysis of performance across clinics rather than using overall performance, however, this does also not account for dissimilarities between clinics. Assessing the performance in subgroups, although common practice, reduces the sample size significantly and limits interpretation to each separate subgroup.

Study design, size, duration: This is a retrospective observational multi-site study with data from four clinics collected over varying time periods. In all participating clinics embryos were cultured in EmbryoScope time-lapse incubators (ES-D, ES+, ES-Flex; Vitrolife). Embryo implantation likelihood was

evaluated with the iDAScore v1.0 AI decision support tool. Data from 4,805 single embryo transfers on day 5 and 6 (fresh and frozen) with known fetal heartbeat outcome were included. Donor egg cycles were strictly excluded.

Participants/materials, setting, methods: Performance of embryo implantation prediction was measured in terms of areas under the ROC curve (AUC). To account for age differences, we performed a standardization of the AUCs using a weighted version of the ROC curves with weights defined as the ratio between the age densities in a reference population and in the specific clinic. The reference population was comprised of data that was held out from the original model development.

Main results and the role of chance: Different age distributions with medians ranging from 32 to 40 years were observed in the four clinics. The unweighted overall AUC was 0.65 (95% CI: 0.64 to 0.69), whereas the AUC in the reference population was 0.67 (95% CI: 0.63 to 0.71). The predictive performance of the iDAScore model varied between clinics with the unweighted AUCs ranging from 0.58 to 0.69. Meta-analysis across all four clinics showed a summary average of 0.65 (95% CI: 0.59 to 0.70).

We then standardized to the age distribution in a reference population in which the maternal age ranged from 21 to 44 years with a median of 38. The values of the AUCs after standardization ranged from 0.60 to 0.71 between clinics with a summary average of 0.67 (95% CI: 0.62 to 0.72). The standard error of the summary average was 5% lower when standardization was used and the estimated variation between clinics in the meta-analysis was 16% lower.

In conclusion, we have shown with our standardized analysis method that the variability in embryo performance prediction between clinics can be partially explained by the difference in maternal age distributions.

Limitations, reasons for caution: The summary average of the AUC is highly dependent on the age distribution in the chosen reference population. Therefore, the exact value of this estimate should be interpreted with caution. However, comparing AUCs between clinics using this standardization approach is less biased by differences in age distribution.

Wider implications of the findings: Simple comparisons of the performance of AI-based embryo implantation prediction in different clinics may be influenced by factors such as age. Using a standardization approach, this influence can be mitigated, enabling a less biased comparison. Differences in other important factors apart from age can potentially also be considered.

Trial registration number: not applicable

Abstract citation ID: dead093.297
O-243 Personalized pregnancy odds estimation can be obtained using AI embryo evaluation and individual patient characteristics

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Study question: Can EMA™ (AIVF, Israel) artificial intelligence (AI) platform provide personalized success estimates based on the patient's metadata and embryonic development?

Summary answer: Individual patients can be given an accurate estimation of their chances for a clinical pregnancy using AI-based embryo evaluation and patient metadata.

What is known already: AI models for embryo evaluation are trained on diverse datasets using data from multiple clinics and from patients with varying ages and clinical history. Precision medicine can be attained by AI models that provide individual patients with personalized success rates per embryo transfer based on their characteristics.

Study design, size, duration: A large dataset (9,812 embryos) from 3 geographically diverse IVF Clinics (Israel, Spain, USA) with known clinical pregnancy (fetal heartbeat) and patient characteristics obtained from Electronic Medical Records (EMR) were used to evaluate the importance of individual features contributing to pregnancy.

Participants/materials, setting, methods: Machine learning models were trained to predict clinical pregnancy on a large training and feature set which combines AI scores (EMA™, AIVF) with additional information obtained from EMR systems and intrinsic features that can be derived from the embryo cohort. The importance of each one of the features for the prediction ability of the models was evaluated.

Main results and the role of chance: AI embryo score, maternal age and cohort features were found to be the major contributing factors for the prediction models, thus improving the accuracy of the estimates for pregnancy probability. Significant contributing factors were: cohort size, number of viable embryos, patient age, number of past treatments, BMI and EMA score. The AUC of the AI embryo score model was 0.7, when factoring in the patient metadata and the cohort features, the AUC of the combined model was 0.75.

Limitations, reasons for caution: This study is limited by its retrospective design. A prospective study is needed to validate the results.

Wider implications of the findings: This study validates the use of AI-based scores for embryo evaluation, for relative grading of the embryos within the cohort, and also to estimate the true pregnancy odds of each embryo based on individual patient features. This can provide a superior decision support tool for doctors, embryologists, and patients.

Trial registration number: not applicable

SELECTED ORAL COMMUNICATIONS
SESSION 78: NEW CONCEPTS: MECHANISMS OF FERTILITY AND INFERTILITY

Wednesday 28 June 2023 Hall D3 10:00 - 11:45

Abstract citation ID: dead093.298
O-244 Mitochondrial fission factor (MFF) is required for female fertility and oocyte development

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Study question: To explore the role of Mitochondrial fission factor (MFF) in female fertility and oocyte development using a *Mff* knockout mouse model.

Summary answer: *Mff* deficiency influences mitochondrial dynamics in oocytes at germinal vesicle stage (GV), and results in premature ovarian failure (POF) and female infertility.

What is known already: *Mff* regulates mitochondrial quality control (MQC) by influencing mitochondrial dynamics. MQC has critical effects on oocyte development and POF. However, little is known about the role of *Mff* in oocyte development and female fertility.

Study design, size, duration: The *Mff* knockout ($Mff^{-/-}$) mice were generated via the CRISPR-Cas9 system. Adult $Mff^{-/-}$ female mice were compared to wildtype (WT) female mice. Female mice at 2-month old from each group ($Mff^{-/-}$ or WT, n = 5) were used to assess the fecundity for 10 months. The follicle development (n = 4 mice), the number of GV oocytes (n = 5 mice) and metaphase II (MII) oocytes (n = 5 mice) were determined.

Participants/materials, setting, methods: Female mice from each group were mated with adult WT males to assess the fecundity. Follicle development was evaluated in ovaries after fixation, paraffin embedding, and sectioning, followed by hematoxylin and eosin staining. MFF and DRP1 protein expression was evaluated by western blot. Oocyte mitochondria were stained with the Mito-Tracker Red CMXRos.

Main results and the role of chance: The *Mff* deficient ovaries had significantly lower levels of MFF (0.056 vs. 1, $p < 0.01$) than the WT ovaries. $Mff^{-/-}$ female mice were infertile (0 vs. 39, $p < 0.05$). Mature (8-week-old) *Mff* deficient ovaries had significantly lower weight (0.0043 vs. 0.011, $p < 0.05$) compared to the WT. The 2-month-old female $Mff^{-/-}$ mice had a lower number of primordial (220 vs. 775, $p< 0.05$), primary (128 vs. 580, $p< 0.05$),

secondary (29 vs. 82, $p< 0.05$) and antral follicles (5 vs. 23, $p< 0.05$). The numbers of GV oocytes (18 vs. 37, $p< 0.05$) and MII oocytes (2 vs. 16, $p< 0.001$) were lower in the *Mff*$^{-/-}$ mice than the WT mice. In addition, the *Mff*$^{-/-}$ ovaries expressed significantly less mitochondrial fission key regulator DRP1 (0.54 vs. 1, $p< 0.05$). *Mff*$^{-/-}$ oocytes contained elongated and aggregated mitochondria compared to the discrete and evenly distributed mitochondria in the ooplasm of WT oocytes. Herein, we found that the *Mff* played a concerted role in oocyte and follicle development.

Limitations, reasons for caution: The present observations in mice may not be applicable to humans. The results are derived global knockout of *Mff*. It is not known which kind of cell plays a vital role in fertility. Further investigation is needed to explore the regulatory mechanism of *Mff* in oocyte development and fertility regulation.

Wider implications of the findings: This study provides important insights into the relationship between mitochondrial dynamics and female fertility.

Trial registration number: not applicable

Abstract citation ID: dead093.299
O-245 Monitoring oxytocin release in vivo during female sexual behavior using a genetically encoded sensor

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Study question: To investigate if the release of oxytocin (OT) in the medial prefrontal cortex (mPFC) fluctuates during discrete phases of female sexual behavior.

Summary answer: Using a highly sensitive OT-specific genetically encoded sensor, we have recorded behavioral phase-specific OT release in mPFC in freely moving mice.

What is known already: OT has been reported to participate in the regulation of sexual behavior of both male and female mice. OT activated mPFC neurons by increasing their firing activity. Loss of function studies indicated that pharmacological or genetic ablation of OT signaling in mPFC largely disrupts female sociosexual interest in male mice. OT action is indispensable in female sociosexual behavior, however, its release dynamics have never been recorded before due to technical difficulties. We have developed a highly sensitive OT-specific G-protein-coupled receptor activation-based sensor (OT1.0), and recorded intracellular OT release in behaving mice on the millisecond time-scale.

Study design, size, duration: Eight C57BJ/6N mice were divided into two groups (n = 4 per group). Intracellular viral transduction with OT1.0 or OT1.0mut vectors was administrated to each group. After viral transduction, 3 weeks were waited to allow sufficient viral expression.

Participants/materials, setting, methods: After anesthetization, AAVs encoding hSyn-OT1.0 or hSyn-OT1.0mut were injected into the left mPFC of adult female wild-type C57BJ/6N mice; Optical fibers were implanted into the mPFC 3 weeks after AAV injection. The animal's sexual behaviors were recorded and annotated, while a fiber photometry system was used to record fluorescence signals in the mPFC, using a 470-nm laser at 50 μW for OT1.0 or OTmut.

Main results and the role of chance: To investigate the dynamics of OT release in mPFC during female sexual behavior, we recorded OT1.0 fluorescence in behaving female mice using fiber photometry. In mPFC of female mice, increases in OT1.0 fluorescence were first observed during the sniffing stage (with rise and decay time constants (T1/2) of 0.4 s and 1.4 s, respectively). After ejaculation of male mice, mPFC OT1.0 signals of the female mice increased significantly with rise and decay time constants (T1/2) of 0.5 s and 1.5 s, respectively. As a negative control, fluctuation of fluorescence was not observed during any phase of sexual behavior in mice expressing OT1.0mut, suggesting the specificity of the signals measured in mice expressing OT1.0.

Limitations, reasons for caution: The OT dynamics in mPCF during female sexual behaviors are only recorded in mice. Clinical trials and imaging studies are required to substantiate the therapeutic role of OT in human subjects.

Wider implications of the findings: This study is likely to deepen our understanding of the role of OT in modulating female sociosexual behavior, which provides more evidence suggesting OT's potential therapeutic potential in treating sexual dysfunctions.

Trial registration number: not applicable

Abstract citation ID: dead093.300
O-246 The molecular mechanism of miR-96-5p in the pathogenesis and treatment of polycystic ovary syndrome

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Study question: Polycystic ovary syndrome, characterized by the androgen excess and arrest of antral follicles, is lacking novel specific diagnostic biomarkers and therapeutic targets.

Summary answer: Study of the molecular mechanisms of microRNAs in the etiology and its potential applications in PCOS provides the insight.

What is known already: Some of molecular mechanisms of microRNAs have been demonstrated involved in the etiology of PCOS

Study design, size, duration: Clinically, we collected the samples of serum, follicular fluid (FF) and primary human granulosa cells (hGCs) of PCOS patients (n = 70) vs. non-PCOS women (n = 60). Experimentally, we carried out studies with 3-types of induced PCOS-like mice.

Participants/materials, setting, methods: By analysing the expression levels of miR-96-5p in serum, follicular fluid (FF) and primary human granulosa cells (hGCs) of PCOS patients (n = 70) vs. non-PCOS women (n = 60). as well as in ovaries from 3-types of induced PCOS-like mice.

Main results and the role of chance: Clinically, we demonstrated that the elevated circulating miR-96-5p levels were significantly correlated with the PCOS disordered endocrine clinical features, and the area under the curve of receiver operating characteristic was 0.8344, with 75.71% specificity and 80% sensitivity. Mechanically, we identified miR-96-5p as an androgen-regulated miRNA that directly targets the forkhead transcription factor FOXO1. Inhibition of miR-96-5p decreased estrogen synthesis, while decreasing the cell proliferation index of KGN via regulating the expression of FOXO1 and its downstream genes. Inversely, inhibition of FOXO1 abrogated the effect of miR-96-5p on estrogen synthesis and proliferation index. Of note, ovarian intra-bursal injection of miR-96-5p agomir rescued the phenotypes of dehydroepiandrosterone-induced PCOS like mice.

Limitations, reasons for caution: This study needs a large-scale clinical investigation to confirm the specificity and sensitivity of miR-96-5p as a clinical biomaker.

Wider implications of the findings: In conclusion, our results clarified a vital role of miR-96-5p in the pathogenesis of PCOS and might serve as a novel diagnostic biomarker and therapeutic target for PCOS.

Trial registration number: no

Abstract citation ID: dead093.301
O-247 The intrafollicular inflammatory response during ovulation in women is most likely controlled by high concentrations of active cortisol being synthesized shortly before follicular rupture

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Study question: How is the anti-inflammatory response in human follicles controlled during the ovulatory process leading to the expulsion of the oocyte?

Summary answer: Intrafollicular concentrations of cortisol becomes high close to ovulation concomitant with an exceptionally high biological activity securing a timely and efficient termination of inflammatory processes.

What is known already: Ovulation has been described as a local controlled inflammatory process resulting in the degeneration of the follicle wall to facilitate oocyte extrusion. Ovulation also affects glucocorticoid metabolism of granulosa cells (GC) and although *de novo* synthesis of cortisol only occurs in the adrenal cortex the mid-cycle surge induces a change from high expression of HSD11b2, inactivating cortisol to cortisone, to high expression of HSD11b1 which reversibly catalyses cortisol production from cortisone. Furthermore, the high concentrations of progesterone and 17-OH-progesterone within follicles cause dislodging of cortisol from cortisol binding protein (CBP) thereby activating the biological activity of cortisol.

Study design, size, duration: This prospective study included 50 women undergoing fertility treatment according to a standard antagonist protocol at the fertility clinic at the university hospital of Koege, Denmark. Women donated one follicle for research purpose aspirated at one of four time points during the process of final maturation of follicles; T = 0h, T = 12h; T = 17h; T = 32h and then one additional follicle at OPU at T = 36h

Participants/materials, setting, methods: The concentration of cortisol and cortisone together with sex steroids were measured by LC-MS/MS in follicular fluid (FF) collected at the above five time points. Whole genome microarray data, validated by q-PCT analysis, was used to evaluate gene expression of *CYP11b1, CYP21A2, HSD11b1, HSD11b2* and *NR3C1* in GC at the five different time points.

Main results and the role of chance: The expression of *HSD11b1* is strongly upregulated during the ovulatory process. From 0-12h expression is increased 690 times reaching more than 20.000 times higher than at T = 0 during the remaining period. In contrast, expression of *HSD11b2* is quickly downregulated by 15-20 times through the ovulatory process.

The concentration of cortisol is different from the gene expression but increases significantly from a few ng/ml at T = 0h to around 15 ng/ml at T = 12h—17h and peaks at 40-50 ng/ml at T = 32h—36h. In contrast, cortisone is almost constant from 0 to 17h at a concentration of 30-40 ng/ml being significantly reduced to 10-20 ng/ml at T = 32—36h.

Concentrations of progesterone and 17-OH-progesterone increased during the ovulatory process to high levels which in essence displaces cortisol from its binding protein CPB, due to similar binding affinities. This will contribute to high levels of biologically active cortisol close to ovulation, which allows the inflammatory reaction to act on the follicle wall and secure extrusion of the oocyte to oviduct before becoming inhibited by cortisol.

Furthermore, a significant decrease in 11-deoxycortisol expression was seen but *CYP11B1* expression was below detection limit in GCs. This study provides new information on human ovulation.

Limitations, reasons for caution: Although 50 women were included more observations at each specific time point would have strengthened the conclusions.

Wider implications of the findings: For the first time, this study collectively evaluates the temporal effect of cortisol and cortisone concentrations in connection with gene expression profiles of enzymes involved in regulation the inflammatory response during human ovulation which now forms a physiological framework for understanding potential dysregulations in the involved processes.

Trial registration number: NA

Abstract citation ID: dead093.302

O-248 Treatment with antifibrotic targets decreases accumulation of pro-fibrotic myofibroblasts and fibrotic collagen in cultured cryopreserved-thawed human cortical tissue

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Study question: Could the excess deposition of fibrotic collagen and the presence of myofibroblast be reversed by *in vitro* culture of human cortical tissue with antifibrotic targets?

Summary answer: The *in vitro* treatment of fibrotic ovarian stroma with Metformin, Pirfenidone or Mitoquinone proved effective in decreasing collagen accumulation and the presence of myofibroblasts.

What is known already: Tissue fibrosis is characterized by an excessive accumulation of extracellular matrix proteins, leading to organ dysfunction. The ovary is the first organ to show fibrosis related to ageing, creating permissive conditions for ovarian cancer development. Due to this, there is an urgent need for ovarian fibrosis treatments. The clinically approved treatment with Metformin has shown efficacy in preventing fibrosis progression from postmenopausal ovaries obtained from diabetic patients, while Pirfenidone is used to treat pulmonary fibrosis. Transmasculine people exposed to prolonged androgen therapy show early signs of ovarian fibrosis, providing a suitable model to study ovarian fibrosis management strategies

Study design, size, duration: duration Fresh and cryopreserved-thawed human ovarian samples obtained from 9 cisgender women (cOVA) and 9 transgender and gender diverse people (tOVA) were included for histological analysis of collagen content and fiber organization. Cryopreserved-thawed cortical fragments from 6 tOVAs were cultured for 8 days with or without antifibrotic treatments (Metformin, Pirfenidone and Mitoquinone). Cryopreserved-thawed cortical fragments from 9 tOVAs were used for *in vitro* culture, followed by metabolic assays.

Participants/materials, setting, methods: Human ovarian samples were obtained from 9 cisgender women (29,1,3 ± 7,9years) scheduled for gonadotoxic therapy undergoing oophorectomy for fertility preservation and from 24 transgender and gender diverse people (22,7 ± 2,4 years) undergoing gender affirming oophorectomy. Collagen content and organization were visualized by second harmonic generation and polarized light imaging. Collagen type 1 and 4, ACTA2+ myofibroblasts and TUNEL+ apoptotic cells were detected and quantified using immunofluorescence. Mitochondrial respiration and glycolysis were measured using the Seahorse XF analyzer.

Main results and the role of chance: Histological analysis of collagen content and organization confirmed that tOVA from subjects in fertile age presents characteristics of ovarian fibrosis, such as a higher accumulation and anisotropic (linearized) organization of collagen fibers within the stroma, indicating that long exposure to androgen therapy affects the extracellular matrix composition of the ovary. Furthermore, both tOVA and cOVA cortical tissue showed an increase in anisotropic collagen disposition after being cryopreserved-thawed when compared to its fresh counterpart, indicating cryopreservation could favor fibrotic progression. Additionally, standard *in vitro* culture of cryopreserved-thawed ovarian cortical fragments promotes an accumulation of collagen 1 and 4 as well as an increase in the number of myofibroblasts and apoptotic cells after 8 days of culture. Treatment with antifibrotics targets such as Pirfenidone, Metformin and Mitoquinone proved to be efficient in reducing both collagen accumulation ($P < 0.05$), pro-fibrotic myofibroblasts ($P < 0.05$) and TUNEL+ apoptotic cells ($P < 0.05$). These results support the evidence that ovarian fibrosis can be prevented or reverted *in vitro* with anti-oxidants and drugs targeting inflammatory response. As such, these

treatments should be considered as therapeutic approaches for women of advancing age and metabolic disorders to prevent pro-tumorigenic fibrotic ovarian stroma.

Limitations, reasons for caution: Small sample size. There is variation in the type and duration of the androgen treatment in transgender and gender diverse people included in the study. Furthermore, the collagen analysis from the cOVA samples was performed on 1cm^2 biopsies that might not represent the general collagen distribution in the complete ovary.

Wider implications of the findings: Medical treatments affecting the ovarian metabolic cell function, such as androgen therapy for transgender people and gender diverse or lab procedures to cryopreserved human ovarian tissue, can trigger fibrosis progression. Nevertheless, human ovarian fibrosis seems reversible and preventable using drugs targeting mitochondrial metabolism and inflammatory response.

Trial registration number: not applicable

Abstract citation ID: dead093.303
O-249 Calcium directly influences pituitary function through the Calcium Sensing Receptor (CaSR)

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Study question: Does calcium have a direct effect on pituitary function through CaSR?

Summary answer: CaSR is important for Hypothalamus-pituitary-gonadal (HPG) axis signaling, and lack of CaSR in the anterior pituitary leads to smaller reproductive organs in female mice.

What is known already: In a randomized clinical trial, prepubertal children were given calcium supplementation or placebo and girls in the intervention group went into puberty 5 months earlier than the placebo group. This effect was not detected in the boys who participated. CaSR is the main receptor for systemic calcium homeostasis, and its presence has been established in the anterior pituitary.

Study design, size, duration: We performed a mouse study comparing knock-out mice with a deletion of the CaSR in the anterior pituitary to wild-type mice. 13/13 female and 6/13 male knock-out/controls mice were included in the study.

Participants/materials, setting, methods: Female and male knock-out and control mice were sacrificed at 8 weeks. We obtained blood samples and reproductive organs was dissected, weighted and stored at -80 °C or stored in formalin before being paraffin embedded.

Main results and the role of chance: Female reproductive organs showed significantly lower weight when comparing CaSR knock-out to control mice. This includes ovary weight (3.7 mg vs. 6.4 mg; $p = 0.0004$) and uterus weight (27.9 mg vs. 67.0 mg; $p = 0.0008$). The weight difference was still significant after correcting for total body weight (organ weight/total body weight%): (0.02176 vs. 0,03534; $p = 0.0035$ and 0.1788 vs. 0.3826; $p = 0.0023$ respectively).

We did not observe a significant difference in male reproductive organs, nor any difference in pituitary weight in either sex.

Limitations, reasons for caution: The difference in reproductive organ weights is interesting, but it is also an early finding. Further analysis of the collected organs and blood samples will be conducted to further investigate the phenotype of the mouse model. We are also looking into correcting weight differences for estrous cycle effects.

Wider implications of the findings: If CaSR proves to be important for the regulation of the HPG-axis, it has a clinical perspective since CaSR can be modulated by the drug Cinacalcet, an allosteric agonist of CaSR, already on the market where it is used to treat kidney patients.

Trial registration number: Not applicable

Abstract citation ID: dead093.304
O-250 Increased association of eating disorders in women with polycystic ovary syndrome

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Study question: Is an increased association of eating disorders (ED) in women with polycystic ovary syndrome (PCOS) than in women without PCOS?

Summary answer: Women with PCOS have an increased association of ED than women without PCOS.

What is known already: PCOS is one of the most common endocrinopathies in women. It has been reported to be associated with psychological distress and dissatisfaction with self-appearance due to clinical features of the syndrome, such as hyperandrogenism, metabolic disorders, obesity, and infertility, leading to lower self-esteem and an increased risk of developing ED. This association presents a clinical dilemma, as the primary treatment for PCOS emphasizes the importance of weight loss, leading to potential distress in patients and worsening ED behaviors.

Study design, size, duration: This was a cross-sectional study. The sample size was calculated with the formula for comparison of proportions in two independent groups, with a confidence level of 95% and power of 80%, obtaining a minimum sample size of 116 participants per group. We included a total of 643 women between July to August 2022.

Participants/materials, setting, methods: 643 women who attended a gynecological consultation in Murcia, Spain were included, 238 with PCOS and 450 controls. The information was collected online. The score of the EDE-Q questionnaire and the clinical epidemiological characteristics were compared between the groups. Pearson Chi-square was used for categorical variables, and Mann Whitney U test for quantitative variables. Statistical significance was considered with a P-value <0.05. The data were analyzed with the SPSS version 20.

Main results and the role of chance: We found an increased risk of presenting ED in women with PCOS than in women not reporting PCOS (27% vs 13.7%). The odds of presenting an abnormal EDE-Q score (>4) were greater in women with PCOS (odds ratio [OR] 2.325, 95% confidence interval [CI], 1.55, 3.47, $P < 0.001$) than in women without PCOS. Clinically significant elevated scores were observed in the PCOS group for all four subscales of ED, but with a more significant impact on the eating concern scale (OR 2.49, 95% CI, 1.47, 4.21, $P < 0.001$). We found more prevalence of binge episodes in the PCOS group (OR 1.8, 95% IC, 1.3, 2.5, $P < 0.001$). When evaluating the characteristic of women with diagnosis of PCOS, we observed a higher body mass index (30.4 vs 23.4 [95% CI, 0.89-1.4, $P < 0.001$]) and more prevalence of amenorrhea (100% vs 87%, $P < 0.001$) in women with ED diagnosis than in women without ED.

Limitations, reasons for caution: The limitations observed in our study were that the information was based on an online questionnaire, so there is a possible reporting bias or misunderstanding of the questions.

Wider implications of the findings: The present study shows that women with diagnosis of PCOS have an increased risk of presenting ED than women without PCOS. This is more prevalent in women with a longer period of amenorrhea and a higher body mass index.

Trial registration number: Not applicable

SELECTED ORAL COMMUNICATIONS

SESSION 79: MOLECULAR MECHANISMS IN SPERMATOGENESIS DEFECT

Wednesday 28 June 2023 Hall D1 10:00 - 11:45

Abstract citation ID: dead093.305

O-251 LIN28B gene expression is downregulated in spermatozoa of oligozoospermic men and associates with genetic variants previously linked to pubertal onset

M. Lardone[1], C. Espinoza[1], E. Ortiz[1], M. Diaz-Fontdevila[1], P. Inostroza[1], G. Iniguez[1], M. Ebensperger[1], K. Almstrup[2], A. Castro[1]

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Study question: Is there an association between genetic polymorphisms in *LIN28B* with *LIN28B* expression and semen quality in spermatozoa from oligozoospermic men?

Summary answer: *LIN28B* is downregulated in spermatozoa of oligozoospermic men and explains 39% of sperm count variability. One SNP (rs314280) was associated with low *LIN28B* mRNA expression.

What is known already: Large-scale genomic studies have identified genetic variants in or near *LIN28B* to be robustly associated with pubertal traits such as age at menarche and age at voice break. The LIN28 family of genes are known to control cell division, growth and differentiation. The LIN28B orthologue is exclusively expressed at high levels in the testis and placenta; however, we have limited knowledge about the role of LIN28B in relationship to testicular function. Interestingly, other studies have showed that later onset of puberty is associated with poor semen quality, suggesting that pubertal development may influence adult testicular function.

Study design, size, duration: Twenty-nine oligozoospermic (cases) and fifty-four men with normal sperm concentration (controls) were recruited from patients seeking andrological work-up for male/couple infertility at a University Hospital. Subjects with abnormal karyotype, Yq-microdeletions, varicocele GIII-IV, hypogonadotropic hypogonadism, seminal infection, diabetes, BMI>35, excessive consumption of alcohol/drugs, testicular cancer, chemotherapy/radiotherapy were excluded. Semen samples were obtained for analysis (WHO, 2010) and sperm isolation by density gradient (PureSperm40®). Peripheral blood samples were analysed for reproductive hormones and used for DNA extraction.

Participants/materials, setting, methods: *LIN28B* and *GAPDH-S* mRNA abundance were measured by qRT-PCR ($2^{-\Delta CT}$ method) in RNA isolated from purified sperm using SYBR® MasterMix (Takyon). Five SNPs in linkage disequilibrium (rs7759938, rs395962, rs314268, rs314277 and rs314280) were genotyped (TaqMan™ SNP Genotyping Assay). Statistical differences between groups were assessed by Mann-Whitney test and genetic association was analysed using "SNPassoc" package in R. Values are showed as median, Q1-Q3.

Main results and the role of chance: Cases and controls had similar age and BMI but as expected sperm concentration, morphology and vitality were differed between groups (9.9, 4.9-13.1 vs 82.8, 46.0-146.5 mill/ml; 1, 1-2 vs 3, 1-4% normal forms and 83%, 76-89 vs 86%, 82-90, respectively) ($p < 0.05$). No differences were observed in progressive motility, semen volume and days of abstinence. FSH and LH serum levels were higher in cases compared with controls (4.6, 2.6-7.8 vs 3.2, 2.5-4 mIU/ml and 4.2, 2.9-5.2 vs 3.1, 2.1-4.2 mIU/ml, respectively; $p = 0.019$ and $p = 0.017$). Relative expression of *LIN28B* was significantly diminished in cases compared with controls (0.0034, 0.0015-0.0103 vs 0.0446, 0.0122-0.1026; $p < 0.001$), while expression of the internal housekeeping control *GAPDH-S* was similar between groups. Linear regression model showed significant association of sperm concentration and total sperm count with *LIN28B* relative expression adjusted by age, abstinence time, % progressive motility and % normal morphology (beta=0.408, R^2=0.241, $p < 0.001$ and beta=0.461, R^2=0.393, $p < 0.001$). *LIN28B* rs314280 was associated with mRNA expression in the dominant model ($p = 0.025$) and two other SNPs (rs7759938, rs314268) showed the same trend ($p = 0.09$). Men with at least one minor allele of these 3 SNPs had a lower *LIN28B* mRNA abundance ($p = 0.006$; $p = 0.07$; $p = 0.055$).

Limitations, reasons for caution: Genotype frequency comparisons between cases and control were not conducted because of the small sample size. A larger cohort will be studied to address this assumption and association of genotypes with parameters of semen quality.

Wider implications of the findings: To our knowledge this study represents the first report on *LIN28B* expression and human testicular function. The significant direct association of *LIN28B* mRNA expression with sperm concentration suggests that *LIN28B* may be involved in spermatogonial proliferation.

Trial registration number: not applicable

Abstract citation ID: dead093.306

O-252 Genetic characterization of human sperm centrosome defects in infertile couples with complex embryo aneuploidy

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Study question: Can we characterize the genetic aspects related to sperm centrosome dysfunction in couples with high embryo aneuploidy that cannot exclusively be attributed to the oocyte?

Summary answer: We characterized structural and genomic abnormalities responsible for sperm centrosome dysfunction in infertile couples with complex embryo aneuploidy not exclusively related to the female partner.

What is known already: Conventional semen analysis provides only limited information on the proper function and embryo developmental competence of the male gamete. Therefore, an ancillary test, such as sperm chromatin fragmentation (SCF) and particularly double-stranded DNA breaks, has been claimed responsible for structural chromosomal abnormalities. It remains puzzling the source of complex embryo aneuploidy and chaotic chromosomal mosaicism that cannot solely be attributed to the female partner. The availability of genomic analysis may shed light on the integrity and function of the sperm centrosome responsible for ordaining chromosomal distribution at the first mitotic division.

Study design, size, duration: In the past 4 years, we identified 13 couples with unexpected high poor embryo cleavage resulting in a higher number of embryos with complex aneuploidy not solely attributable to the female gamete, resulting in extremely poor embryo implantation. Therefore, ancillary testing on the spermatozoa of the male partners was carried out to determine if the couple's reproductive failure was eventually due to a stealth male gamete deficiency.

Participants/materials, setting, methods: Thirteen couples with poor clinical outcomes, characterized by poor embryo cleavage, high and complex embryo aneuploidy not exclusively due to the oocyte, with poor embryo implantation in multiple IVF/ICSI cycles, were offered adjuvant male partner testing including centrosome assessment through immunofluorescence staining on centrin (normal $\geq$ 60%), sperm chromatin fragmentation testing through terminal deoxynucleotide dUTP transferase nick-end labeling (TUNEL, normal $\leq$ 15%), transmission electron microscopy (TEM), and whole exome sequencing (WES) on spermatozoal DNA.

Main results and the role of chance: In 13 couples with an average paternal age of 38.1 ± 2 years, semen analysis revealed a volume of 22.2 ± 1.4mL, concentration of 58.2 ± 37x106/mL, motility of 35.2 ± 18% and normal morphology of 2.6 ± 1%. They underwent with female partners (maternal age of 37.2 ± 2 years) a total of 16 ICSI cycles with aneuploidy testing. Only 11 cycles yielded embryos for transfer due to poor embryo development with high aneuploidy rate, and none of them achieved a pregnancy.

Centrosome staining revealed that only 45.8 ± 22% of the spermatozoa displayed this key organelle. An overall SCF was observed at 16.7 ± 10%. TEM confirmed that approximately 70% of the spermatozoa had irregular proximal centrioles. Moreover, genomic analysis by WES revealed mutations on PLK4 gene responsible for centriole duplication, BRSK1 gene involved in centrosome duplication, TUBGCP6 gene responsible for centrosomal microtubule nucleation, MARK4 for centrosome localization, MAP1S for microtubule anchoring and DNA binding, RIC8A for chromosome distribution, and CALM3 for cytokinesis.

Of this cohort, 3 couples that subsequently underwent 3 ICSI cycles had their spermatozoa processed by microfluidics, aiming at selecting male gametes with higher genomic integrity. A fertilization rate of 78.3% (18/23) was achieved generating euploid embryos that resulted in 3 successful pregnancies.

Limitations, reasons for caution: We identified sperm centrosome dysfunction responsible for the peculiar and recurrent embryo aneuploidy. Although we identified shared gene mutations in those couples, these data are still preliminary. With exception of improving spermatozoa selection, there is a lack of a single effective treatment option to address sperm centrosome abnormality.

Wider implications of the findings: Ancillary tests with semen analysis were able to assess the function and competence of the male gamete. Corroborative genomic study can help provide insights into the reason for poor embryo implantation particularly those with high complex aneuploidy.

Trial registration number: Not applicable

Abstract citation ID: dead093.307

O-253 Novel biallelic variants in DNAH1 cause multiple morphological abnormalities of sperm flagella with favorable outcomes of fertility after ICSI in Han Chinese males

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Study question: Is there any novel variants and mutant hotspot in *DNAH1* gene related to multiple morphological abnormalities of sperm flagella (MMAF) and male infertility in humans?

Summary answer: We identified eighteen variants of *DNAH1* gene in eleven MMAF-affected Chinese men, including fourteen novel variants, four previously reported variants, and a candidate mutant hotspot.

What is known already: As a specific type of asthenoteratozoospermia, MMAF is characterized by composite flagellar defects including absent, short, coiled, angulation, and irregular-caliber, and usually presents ultrastructural abnormalities of axonemal and peri-axonemal structures. Genetic defects are the major cause of MMAF and biallelic variants of *DNAH1* have been found to account for approximately 30% of studied human MMAF cohorts. Males with *DNAH1* variants usually present with primary infertility owing to abnormal sperm morphology and motility. Intracytoplasmic sperm injection (ICSI) is the main strategy applied for patients with *DNAH1* variants to conceive.

Study design, size, duration: A total of thirty-five patients with MMAF phenotype were recruited from the center for reproductive medicine from August 2019 to June 2022. A next-generation sequencing (NGS) panel of 22 MMAF-related genes was applied for genetic testing and the hereditary pattern was confirmed with Sanger sequencing. Sperm morphological analysis, immunostaining assays, and assisted reproductive therapy were performed in 2021 and 2022.

Participants/materials, setting, methods: The *DNAH1* variants were identified by NGS and confirmed by Sanger sequencing. Pedigree analysis and *in silico* analysis further confirmed the pathogenicity or likely pathogenicity of these variants. Papanicolaou-staining, scanning and transmission electronic microscopy, and immunostaining were used to characterize the morphology and ultrastructure of spermatozoa. ICSI was applied for the assisted reproductive therapy of *DNAH1*-mutant patients and the outcomes of fertilization, embryo cleavage, and delivery were analyzed.

Main results and the role of chance: We totally identified 18 different *DNAH1* variants (NM_015512.5) in 11 of 35 MMAF-affected males, including 9 missense variants (c.7690G>A, p.A2564T; c.10970C>G, p.T3657R; c.5584G>A, p.G1862R; c.6887T>C, p.L2296P; c.12122C>T, p.T4041I; c.1832T>C, p.L611P; c.2738C>A, p.A913D; c.5795G>A, p.R1932Q; c.7066C>T, p.R2356W) and 9 loss-of-function variants (c.11726_11727delCT, p.P3909Rfs*33; c.12172C>T, p.Q4058*; c.5104C>T, p.R1702*; c.12210del, p.V4071Cfs*54; c.8533del, p.D2845Mfs*2; c.12178_12190del, p.E4060Pfs*61; c.2301-1G>T; c.4552C>T, p.Q1518*; c.12118_12119delCA, p.Q4040Dfs*33). Moreover, 77.8% (14/18) of the identified variants were novel. The results of Papanicolaou-staining, scanning electronic microscopy, and immunofluorescence demonstrated the typical characteristics of MMAF, consisting of absent, short, coiled, angulation, and irregular-caliber flagella, of the spermatozoa affected by *DNAH1* variants. Transmission electronic microscopy further revealed the compound changes of flagella ultrastructure, including loss of central pair and disorganization of microtubule doublets and outer dense fibers. Six of eleven underwent assisted reproductive therapy with ICSI in our clinic. The rates of fertilization, usable embryos and clinical pregnancy for patients with *DNAH1* variants following ICSI were 81.5%, 52.8% and 66.7%, respectively. To date, three couples have given birth to a total of five healthy children.

Limitations, reasons for caution: Because of distance and inconvenience, five of the eleven *DNAH1*-affected men didn't provide additional semen samples for functional analysis. In addition, as the absence of specific antibody of DNAH1, immunostaining for DNAH1 was not performed in this study.

Wider implications of the findings: This study significantly expands the variant spectrum of *DNAH1* gene related to MMAF and male infertility in humans. The systematic description of assisted reproduction therapies will provide clinicians with new insights into molecular diagnosis and genetic counseling.

Trial registration number: not applicable

Abstract citation ID: dead093.308

O-254 ACTRT2 deficiency increases spermatogonia vulnerability to ferroptosis

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Study question: How does the actin-related proteins T2, ACTRT2, which is specifically expressed in spermatogonia, regulate spermatogonia death?

Summary answer: ACTRT2 deficiency in spermatogonia leads to intracellular iron overload and damage to mitochondria, ultimately increasing spermatogonia vulnerability to ferroptosis.

What is known already: ACTRT2 is tightly related to spermatogenesis. On the one hand, ACTRT2 is involved in regulating iron uptake in spermatogonia. ACTRT2 deficiency leads to intracellular iron overload and mitochondrial damage, ultimately increasing the vulnerability of spermatogonia to ferroptosis. On the other hand, for ACTRT2-related spermatogenic impairment, the survival of spermatogonia could be maintained by inhibiting ferroptosis, and spermatogenesis of the testis could be improved.

Study design, size, duration: The expression of ACTRT2 was knocked down in GC-1 cells (spermatogonial cell line). Busulfan was used to induce GC-1 cells death. And then to explore which type of cell death ACTRT2 was involved in, GC-1 cells were treated with 50 μM Z-VAD-FMK (apoptosis inhibitor), 50 μM necrostin-1 (necroptosis inhibitor), or 0.1 μM ferrostatin-1 (ferroptosis inhibitor).The mechanism by which ACTRT2 regulates ferroptosis in spermatogonocytes was investigated in ACTRT2 knockout mice.

Participants/materials, setting, methods: ACTRT2 expression was knocked down in vitro in GC-1 cells using siRNA. Then, GC-1 cell death was

induced using 150 μM busulfan. ROS levels, morphological changes in mitochondria, and the ferroptosis marker GPX4 were detected. ACTRT2 knockout mice were constructed in vivo using CRISPR/Cas9. The spermatogenesis of the mice was measured with H&E staining. After treatment with busulfan, ferrostatin-1 was used to inhibit ferroptosis in order to protect spermatogenesis.

Main results and the role of chance: ACTRT2 was specifically expressed in testicular tissue and was associated with spermatogenesis. In vitro, when the cells were treated with busulfan, the proportion of GC-1 cell death in the low-ACTRT2 group increased significantly compared with the si-NC group. Furthermore, ferrostatin-1 significantly improved the survival of busulfan-treated cells. In low-ACTRT2 GC-1 cells, ROS accumulation and typical mitochondrial changes associated with ferroptosis occurred, and GPX4 was downregulated. In vivo, the seminiferous tubules in ACTRT2-/- mice were significantly shrunken. In addition, after being treated with busulfan, spermatogenesis in ACTRT2+/- mice decreased significantly compared to that in wild-type mice. In ACTRT2+/- testes, the expression levels of ACSL4 and ALOX15 were upregulated, while the expression levels of SLC7A11 and GPX4 were downregulated. Then, ferrostatin-1 was administered, which resulted in improved spermatogenesis. Finally, we found that the expression of SLC11A2, IREB2, and TFRC increased significantly in the low-ACTRT2 group, which transports iron into the cell to increase the intracellular unstable iron pool. Meanwhile, the expression of SLC40A1, which transports excess iron out of the cell, significantly decreased.

Limitations, reasons for caution: We found that ferrostatin-1 can inhibit ferroptosis in ACTRT2-deficient spermatogonia, which is expected to be a new therapeutic strategy for male infertility. However, its safety and efficacy still need to be further evaluated, and the therapeutic schedule still needs to be further explored and optimized.

Wider implications of the findings: Ferrostatin-1 can inhibit ferroptosis in ACTRT2-deficient spermatogonia, which is expected to be a new therapeutic strategy for male infertility.

Trial registration number: not applicable

Abstract citation ID: dead093.309
O-255 microRNA-23a/b-3p regulate expression levels of testis-specific transcripts in men with impaired fecundity

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Study question: To investigate whether microRNA-23a/b-3p targets spermatogenesis-related transcripts and whether this targeting impacts their expression contents in patients with subfertility.

Summary answer: The higher expression of microRNA-23a/b-3p and lower expression of 11 tested target genes are associated with men subfertility

What is known already: Spermatogenesis is a complex and highly regulated process, many genes are involved, the expression levels of which are strongly or partially coordinated by microRNAs (miRNAs). MiRNAs are small, non-coding RNAs that are involved in the post-transcriptional regulation of gene expression. Transcriptome analysis shows that hundreds of genes are expressed exclusively or predominantly in male germ cells including *CEP41, G2E3, GOLGA6B, GOLGA6C, LMLN, NOL4, PAPOLB, PCDHA9, RGPD1, SOX6*, and *ZNF695* genes, which play a crucial role during spermatogenesis and/or sperm function. However, the expression regulation of these genes is still unclear.

Study design, size, duration: Reverse transcription-quantitative PCR (RT-qPCR), dual luciferase assay, Western blot, and bioinformatics analysis were used to validate the lower expression of 11 target genes as a result of the known higher expression of microRNA-23a/b-3p in men with subfertility. A total of 82 men were included for RT-qPCR, consisting of 41 oligoasthenozoospermic subfertile men who attended the IVF center for infertility treatment and 41 age-matched normozoospermic volunteers who served as controls.

Participants/materials, setting, methods: In *silico* prediction and dual-luciferase assays were performed to evaluate the potential links between the higher expression of microRNA-23a/b-3p and the lower expression of 11 genes. Total RNA, including miRNA, was isolated from the sperm of oligoasthenozoospermic (n = 41) and normozoospermic men (n = 41). RT-qPCR was used to detect the expression levels of 11 target genes. Correlation analyses between the mRNA expression levels and basic semen parameters were carried out.

Main results and the role of chance: The expression levels of microRNA-23a/b-3p were significantly up-regulated and 11 genes were significantly down-regulated in oligoasthenozoospermic men compared with age-matched normozoospermic men as determined by RT-qPCR. Using dual-luciferase assays, 9 genes including *CEP41, G2E3, GOLGA6C, NOL4, PAPOLB, PCDHA9, RGPD1, SOX6*, and *ZNF695* were identified as direct targets of miR-23a-3p and 4 genes including *GOLGA6C, PAPOLB, SOX6*, and *ZNF695* were identified as direct targets of miR-23b-3p. Mutations in the miR-23a/b-3p binding site within the 3′untranslated regions (3′UTRs) of the 9 target genes, which target either miR-23a-3p and/or miR-23b-3p, resulted in abrogated responsiveness to microRNA-23a/b-3p and confirmed that *CEP41, GOLGA6C, NOL4, PCDHA9*, and *SOX6* as direct targets for miR-23a-3p and *NOL4, SOX6* and *PCDHA9* as direct targets for miR-23b-3p. Correlation analysis highlighted sperm count, motility, and morphology were positively correlated with the lower expression level of these validated genes.

Limitations, reasons for caution: Despite the correlation between the higher expression of microRNA-23a/b-3p and the lower expression of the validated genes, further validation by Western blotting in human sperm and testicular tissues is needed.

Wider implications of the findings: Findings suggest that the higher expression of microRNA-23a/b-3p or the lower expression of validated target genes are associated with male subfertility, probably through influencing the basic semen parameters. This study lay the groundwork for future studies focused on investigating therapies for male infertility.

Trial registration number: Hedwig-Stalter foundation (2016)

Abstract citation ID: dead093.310
O-256 Evaluation of the fertility potential of semen using Proton NMR

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Study question: Is Proton NMR seminal plasma assessment a valid method for identifying biomarkers allowing to predict top-quality (TQ) embryo development?

Summary answer: Biomarkers important in the context of male fertility can be assessed using proton NMR

What is known already: Currently, the quality of sperm is assessed predominantly by examining its concentration, motility, and morphology. These parameters are insufficient in diagnosing male infertility and predicting treatment outcomes with assisted reproductive technology (ART). Metabolomics techniques have been shown to be potentially useful in identifying biomarkers related to infertility. It has been proven, using proton NMR, that there are a number of substances in the semen plasma that correlate with various physiological states of patients and may potentially indicate impaired functioning of various metabolic pathways. However, the relationship between biomarkers and embryo development has not yet been investigated

Study design, size, duration: This was a prospective case-control study and consisted of 30 semen samples from 13 patients who underwent IVF

treatment between January – March 2022 at INVICTA Fertility Centre, Poland. Sperm motility, concentration, morphology, and DNA fragmentation of the samples were assessed. Before sperm preparation for fertilization, semen was frozen to perform 1H NMR analyses, which were preceded by centrifugation in order to separate the plasma. NMR analysis results were compared to the embryological results.

Participants/materials, setting, methods: It was possible to identify preselected biomarkers in semen plasma through the use of reference spectra and to quantify their concentrations using the internal standard addition method. Their relative concentrations (separately and in pairs) were collated with semen parameters, embryo development data and pregnancy outcomes. A Machine Learning approach was employed to identify the most important variables, which were then used for further modelling with the use of LGBM models.

Main results and the role of chance: NMR allows for the identification and measurement of biomarkers important in the assessment of the fertility potential of semen. The presence of amino acids, organic acids, sugar residues and fatty acid derivatives was confirmed by applying this technique. It was found that the concentration of these biomarkers is an individual feature - probably dependent on the physiological state of the organism. The results indicate that the ratios of biomarker concentrations to each other are more important than their individual concentrations. Machine learning modelling showed a strong correlation of several compounds with the chance of obtaining a TQ blastocyst. The amount of taurine, glutamic acid and choline together with its derivatives seem to be the most important factors in blastocyst development. The results indicate that if the amount of glutamic acid significantly surpasses glycerophosphocholine, choline exceeds taurine, the amount of taurine is higher than that of citrate, or the amount of choline is significantly higher than its phosphorus analogue the likelihood of obtaining a TQ blastocyst is higher. This would suggest that several metabolic pathways as the Krebs cycle, the one-carbon cycle or choline metabolism were modified signalling pathological conditions which the standard assessment of semen parameters cannot detect.

Limitations, reasons for caution: The NMR technique allows a fast determination of the content of many biomarkers, but results are in the mmol/l range. The results should be confirmed in a larger group of patients with different semen disorders

Wider implications of the findings: NMR allows for the rapid creation of unique metabolic profiles of patients, which can ensure the creation of personalized treatments for male infertility and the selection of sperm with the highest chance of obtaining a TQ blastocyst

Trial registration number: The studies were approved by the Ethics Committee at the Gdansk Regional Medical Board (No KB-3/22).

Abstract citation ID: dead093.311
O-257 IGF2BP3 induces apoptosis of spermatogenic cells by regulating the stability of Tcf4 mRNA in an m6A-dependent manner, thereby affecting the occurrence of azoospermia

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Study question: Does IGF2BP3 regulate *Tcf4* mRNA stability in an N6-methyladenosine (m6A)-dependent manner, affect spermatogenesis, and lead to idiopathic non-obstructive azoospermia (iNOA)?

Summary answer: IGF2BP3 induces apoptosis of spermatogenic cells by regulating the stability of Tcf4 mRNA in an m6A-dependent manner, which in turn affects spermatogenesis leading to iNOA.

What is known already: Azoospermia is the most serious phenotype of male infertility, affecting approximately 10-15% of male infertility patients. iNOA refers to a class of non-obstructive azoospermia of unknown etiology for which there is no effective treatment. The etiology of iNOA is poorly understood, and its genetic and molecular mechanisms are poorly studied. IGF2BP3 was found to be highly expressed in tumor tissues and to be a post-transcriptional regulator in cancers. The study found that IGF2BP3 is an m6A reading protein that recognizes mRNA m6A modification and enhances mRNA stability and translation.

Study design, size, duration: We collected testicular tissue from 120 iNOA and 120 patients with obstructive azoospermia through prospective studies to analyze differentially expressed genes by high-throughput RNA sequencing.

Participants/materials, setting, methods: IGF2BP3 knockout and overexpression mouse models were constructed. Spermatogenesis was assessed by immunostaining, HE staining, spermatogenic specific gene expression, and computer-assisted sperm analysis (CASA). The stability of target mRNA was conducted by ActD. Merip-qRCR was used to detect IGF2BP3 binds to the target gene in an m6A-dependent manner; the m6A modification site of Tcf4 was predicted by SRAMP and verified by double luciferase reporting experiments. Overexpression Tcf4 to analyze its effect on spermatogenesis.

Main results and the role of chance: RNA-seq showed that IGF2BP3 was lowly expressed in iNOA testicular tissue which was verified by qRT-PCR and immunohistochemistry. After knocking down IGF2BP3, the spermatogenesis process is impaired, HE showed that the arrangement of spermatogenic cells in the seminiferous tubule was disordered and reduced, the spermatogonia and spermatoblasts were significantly reduced, and there were very few sperm in the seminiferous tubule. CASA showed that sperm density was significantly lower than that of the control group ($p < 0.05$). Western blot and qRT-PCR showed that spermatogenic cells-specific genes Nanos3, Sycp3, Tnp1, proliferation indexes PLZF and PCNA were all reduced compared with the control group. Overexpression of IGF2BP3 can reverse spermatogenesis in azoospermia mice; after knocking down IGF2BP3, the fertility of mice in vivo and in vitro was impaired; apoptosis of spermatogenic cells increased in testicular tissues of mice after knocking down IGF2BP3 and decreased in mouse testicular tissue after knocking down IGF2BP3. IGF2BP3 induces apoptosis of spermatogenic cells by regulating the stability of *Tcf4* mRNA in an m6A-dependent manner, resulting in impaired spermatogenesis; overexpression of Tcf4 in GC1 cells knocked down IGF2BP3 reduces apoptosis and increases cell proliferation.

Limitations, reasons for caution: In this study, we constructed knockdown and overexpression mice models by in situ injection of IGF2BP3 lentivirus in the testes, and the results were more accurate if conditional knockout mice model was used.

Wider implications of the findings: We found direct clinical and in vivo animal model evidence for the causes of male infertility in patients with idiopathic non-obstructive azoospermia, and for the first time found that IGF2BP3 affects spermatogenesis by regulating the stability of target genes in an m6A-dependent manner.

Trial registration number: not applicable

SELECTED ORAL COMMUNICATIONS
SESSION 80: THE RIGHT BUG IN THE RIGHT PLACE
Wednesday 28 June 2023 Hall D4 10:00 - 11:45

Abstract citation ID: dead093.312
O-258 Characterization of vaginal microbiota during IVF fresh embryo transfer (IVF-ET) and in early pregnancy

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Study question: Does the composition of vaginal microbiota affect the success of IVF-ET? Does vaginal microbiota profile change intra-individually from IVF-ET to early pregnancy?

Summary answer: *L. crispatus* is associated with successful IVF outcome. The individual composition of vaginal microbiota tends to shift towards *Lactobacillus*-dominance between IVF-ET and early pregnancy.

What is known already: *Lactobacilli* are the most abundant species in healthy female reproductive tract microbiota and especially *Lactobacillus crispatus* is associated with good gynecological health. The composition of vaginal microbiota may also play an important role in infertility and the success of IVF. Women with *Lactobacillus*-dominated vaginal microbiota may have higher pregnancy rates after IVF-ET compared to women with non-*Lactobacillus* dominance, but the results have been controversial. In healthy pregnancy, vaginal microbiota is usually *lactobacilli*-dominated, whereas miscarriages are associated to a non-lactobacillus dominance. The intra-individual shift on the vaginal microbiota from ET to early pregnancy has not been studied earlier.

Study design, size, duration: This observational and longitudinal study investigates the effect of vaginal microbiota on the success of fresh IVF-ET. Another aim was to study the intra-individual changes in the microbiota profile between IVF-ET and early pregnancy. We recruited 76 subfertile women undergoing IVF with their own gametes between November 2019 and September 2021 in the Reproductive Medicine Unit, Helsinki University Hospital and in Oulu University Hospital. Main outcomes were clinical pregnancy and live birth.

Participants/materials, setting, methods: Study population composed of 76 subfertile women under 40 years of age. One IVF cycle including fresh embryo transfer was studied. Vaginal swabs were taken at the time of IVF-ET and at 8th gestational week. Samples were frozen in − 20 °C and moved to − 80 °C within four weeks. Microbiota analysis was performed with next-generation sequencing of 16S rRNA gene using Illumina MiSeq technology. Clinical data were collected from the patient registries and background questionnaires.

Main results and the role of chance: Altogether 30 (39.5%) of the 76 women achieved pregnancy after IVF-ET and 26 (34.2%) had live birth. The relative abundance of *L. crispatus* among women who achieved clinical pregnancy (46.9% vs. 19.1%, q = 0.003) and live birth (43.3% vs. 19.1%, q = 0.006) was significantly higher compared to women who did not. After adjusting for age, parity and gravidity, the results remained similar when comparing clinical pregnancy (q = 0.039), but in live birth, the difference lost significance (q = 0.14)

Vaginal samples were taken at the 8th gestational week from 21 of 30 women who got pregnant. All samples were dominated by *Lactobacillus* species, mainly by *L. crispatus* (n = 16, 76.2%). Only two (9.2%) women had *L. iners* dominated microbiota at the early pregnancy. When comparing samples taken during fresh IVF-ET and early pregnancy, *L. crispatus* had significantly higher relative abundance in early pregnancy samples compared to the samples taken at the ET (71.5% vs. 43.4%, q = 0.001), whereas the relative abundance of *L. iners* (10.1% vs. 24.1%, q = 0.004), and *G. vaginalis* (0.8% vs. 14.7%, q = 0.004) decreased. Altogether 13 women had an intra-individual shift in their microbiota profiles.

Limitations, reasons for caution: The small sample size was the main limitation of our study. Larger studies are needed to confirm our findings and their relationship with the success of IVF-ET.

Wider implications of the findings: Vaginal microbiota at the time of fresh IVF-ET has an impact of the success of IVF treatment. Also, the intra-individual composition of vaginal microbiota between IVF-ET and early pregnancy changes in some women. The vaginal microbiota modification through probiotics in order to optimize the result of IVF warrants more studies.

Trial registration number: Not applicable

Abstract citation ID: dead093.313

O-259 1.\tComparison of endometrial versus vaginal microbiota in 71 infertile patients

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Study question: 1.Mostly, endometrial microbiome probes are acquired by trans-cervical sampling using a "pipelle". However, it is unclear, whether this approach "contaminates" the endometrial material.

Summary answer: 1.Vaginal and endometrial microbiome samples are significantly different from each other with respect to (alpha-)diversity showing the endometrial community to be more polymicrobial.

What is known already: 1. The female genital tract is colonized by a continuum of microbiota, ranging from the typically more diverse, but lower-biomass, bacterial communities in the endometrium to the typically less diverse, but higher-biomass, communities in the vagina (Chen et al., 2017). In reproductive-age women, vaginal bacterial communities are mainly dominated by lactobacilli (Ravel, 2011). By contrast, the composition of the endometrial microbiome has remained less well-characterized. With the more recent introduction of next generation sequencing a more quantitative and comprehensive picture of the composition of endometrial bacterial communities has begun to emerge (Baker et al., 2018).

Study design, size, duration: 1. The study was conducted at "novum, Zentrum für Reproduktionsmedizin", Essen / Duisburg, Germany. Of 76 enrolled patients undergoing treatment,73 provided both vaginal swab and endometrial biopsy samples from the same sequencing run between February 2020 and March 2021. All analyses were carried out retrospectively. Information about pregnancy or delivery were extracted from medical records or obtained by telephone or email interviews conducted through June 2022.

Participants/materials, setting, methods: 1.Amplification of V1-V2 16S rDNA regions was carried out at "dus.ana, Düsseldorf Analytik", Germany, using the QIAseq 16S screening and regional panels. CLC Microbial Genomic Workbench was used to assess bacterial community composition, alpha diversity, and microbial community structure (PCoA). Pairs of complete corresponding vaginal and endometrial microbiota data were available for 71 patients (two patient was excluded due to too low read counts in the vaginal or the endometrial probe, respectively).

Main results and the role of chance: 1.Patients exhibiting a mean age of 35 years (range 26 – 42) and a mean body mass index (BMI) of 24,5 (range 18,8 to 38,7). 20 (28,2 %) patients were diagnosed with chronic endometritis (more than 1 plasmacyte per HPF) and 15 (21,1 %) with endometriosis. 40 study participants were found to have undergone past miscarriages, and 11 study participants were found to have given birth to 15 children.

2.Abundance analyses showed differences between vaginal and endometrial samples for the Lactobacillus sp., most pronounced for L. jensenii and L. gasserie. The group of non-lactobacilli was only slightly enlarged in the endometrial group, encompassing mainly G. vaginalis. With regard to diversity (Shannon entropy), both microbiota are significantly different (Kruskal-Wallis test). In addition, distribution of the genital microbial communities illustrated by PCoA indicated that the vaginal samples were clustered together showing variance only in one dimension. However, the endometrial samples were far away from each other and grouped very disperse.

Limitations, reasons for caution: 1.Group of infertile patients of an IVF center with different underlying clinical diagnosis studied in a retrospective setting.

Wider implications of the findings: 1.Trans-cervical sampling with a "pipelle" provides an endometrial microbiome which is obviously a more polymicrobial community - with a higher diversity, that may harbor more biological heterogeneity, and hence functionality – than the corresponding vaginal

microbiome. Further analyses studying the correlation between sequencing and clinical data are ongoing.

Trial registration number: 2021-1448

Abstract citation ID: dead093.314

O-260 The influence of the vaginal microbiome on clinical outcomes in patients undergoing a frozen embryo transfer: a prospective pilot study

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Study question: Are Lactobacillus-dominant vaginal microbial compositions advantageous for clinical outcomes and is the specific Lactobacillus species relevant?

Summary answer: Favourable clinical outcomes are increased in patients with a Lactobacillus-dominant vaginal microbiome, particularly in those patients with microbial communities dominated by L.crispatus and L.jensenii.

What is known already: The clinical relevance of the urogenital microbiome is emerging as a topic in female reproductive health. Recent evidence suggests that the composition of the vaginal microbiome may have an impact on clinical outcomes in patients undergoing assisted reproductive treatment. A Lactobacillus-dominant microbiome (LDM), typically composed of L.crispatus, L.gasseri, L.iners or L.jensenii, is considered to be associated with favourable outcomes. In contrast, a non-Lactobacillus-dominated microbiome (NLDM), consisting predominantly of anaerobe species, may be linked to poor outcomes. The evidence surrounding this relationship is controversial and the importance of relative Lactobacillus abundance in the vagina in relation to clinical outcomes remains unclear.

Study design, size, duration: A single-centre prospective pilot study included 81 patients (mean age: 38.2) undergoing frozen embryo transfer using their own (n = 52) or donated (n = 29) oocytes. A vaginal swab was taken on the embryo transfer day and the microbiome analysed. Implantation, ongoing pregnancy and early miscarriage rates were compared between different microbiome compositions. To minimise the confounding effect of aneuploidy on clinical outcomes, a patient sub-group (n = 54) receiving donor oocytes or a euploid embryo transfer was also considered.

Participants/materials, setting, methods: Quantitative PCR was utilised to determine the relative abundance of the four predominant Lactobacillus species as well as 15 species associated with vaginal dysbiosis. Microbiome compositions were grouped according to the relative Lactobacillus abundance and patients divided into an LDM (>80%) or NLDM (<80%) group. Patients were further sub-divided into community state types (CSTs) according to the dominant species present in their sample (CST-I: L.crispatus, CST-II: L.gasseri, CST-III: L.iners, CST-V: L.jensenii and CST-IV: dysbiotic species).

Main results and the role of chance: Relative abundance of Lactobacillus was significantly higher in samples from patients achieving an ongoing pregnancy (80.7% vs. 61.7%; p = 0.05). Implantation rates were comparable between LDM and NLDM patients (66.0% and 64.3%), but the ongoing pregnancy rate showed an apparent increase in LDM patients (58.5% vs. 39.3%; p = 0.11), concomitant with a significant decrease in the miscarriage rate (11.4% vs. 38.9%; p = 0.03). Limiting the analysis to patients at low risk of aneuploidy (young donor and PGT-A cycles), Lactobacillus abundance appeared higher in patients with ongoing pregnancies (74.8% vs. 58.0%; p = 0.34). Likewise, LDM patients showed higher ongoing pregnancy (61.3% vs. 43.5%; p = 0.27) and lower miscarriage (5.0% vs. 33.3%; p = 0.06) rates, although significance was not achieved in this restricted sample. Comparison of CST groups indicated better outcomes in CST-I and CST-V compared to other groups. The ongoing pregnancy rate was significantly higher (65.7% vs. 41.3%; p = 0.04), alongside a lower miscarriage rate (8.0% vs. 32.1%; p = 0.04). When only considering patients with low aneuploidy risk, similar results were obtained (implantation rate: 78.9% vs. 57.1%, p = 0.14; ongoing pregnancy rate: 73.7% vs. 42.9%, p = 0.04; miscarriage rate: 6.7% vs. 25.0%, p = 0.21). Of note, there were no differences in baseline characteristics (age, ethnicity & BMI) or embryo morphologies between groups.

Limitations, reasons for caution: All patients were from a single fertility clinic and the study population was predominantly composed of white women. Consequently, results may not be applicable to women of different ethnicities. Patients with a low risk of aneuploid embryo transfer represented a small subgroup where meaningful statistical analysis was not always possible.

Wider implications of the findings: This study identifies a correlation between Lactobacillus species colonisation in the vagina and successful clinical outcomes, suggesting that the vaginal microbiome modulates the chances of IVF success. The results are promising, providing motivation for further, larger studies, involving more diverse populations in order to draw definitive conclusions.

Trial registration number: not applicable

Abstract citation ID: dead093.315

O-261 The relationship of abnormal vaginal microbiome (dysbiosis of vaginal microbiome) and proinflammatory cytokines in recurrent implantation failure patients

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Study question: Is there any correlation between serum cytokine levels and the composition of the vaginal microbiome in recurrent implantation failure (RIF) patients?

Summary answer: The levels of IFN-γ/IL-10 and TNF-α/IL-6 ratios are higher in patients with dysbiotic microbiome which associates with an imbalance towards inflammation between Th1/Th2 lymphocytes.

What is known already: In recent years, the development of sequencing-based technologies has enabled the evaluation of the vaginal microbiome. Studies have shown that a vaginal microbiota with less than 90% of Lactobacillus is predictive of IVF treatment outcome and suggests that dysbiosis in the reproductive tract negatively affects the reproductive outcome compared to women with a Lactobacillus-dominated microbiome.

Furthermore, it is known that pregnancy is characterized by a Th2-dominant cytokine pattern, and, in contrast, an altered Th1/Th2 ratio, and altered NK cell and macrophage numbers are more prevalent in women with RIF.

Study design, size, duration: Twenty patients were included in a retrospective study between December 2018 and May 2022. Thirteen had normal microbiome and seven had dysbiotic microbiome. In all of them, a vaginal microbiome sample was analyzed and the levels of cytokines IL-2, IL-4, IL- 6, IL-8, IL-10, VEGF, IFNγ, TNFα, IL-1α, IL-1β, MCP-1 and EGF in serum were measured. Patients with uterine malformations, uterine surgery or other known factors which may influence RIF were excluded from the analysis.

Participants/materials, setting, methods: To measure the levels of the different cytokines, a sandwich immunoassay with specific antibodies for the cytokines IL-2, IL-4, IL-6, IL-8, IL-10, VEGF, IFNγ, TNFα, IL -1α, IL-1β, MCP-1 and EGF were used. Vaginal microbiome was analyzed by mass sequencing of the V3V4 region of 16S rRNA. The statistical analysis was performed with R Statistical Software, version 4.0.3 and the bioinformatic analysis of vaginal microbiome was performed using QIIME2 and MicrobiomeAnalyst packages.

Main results and the role of chance: For the ratios between cytokines produced by Th1 lymphocytes and Th2 lymphocytes, we observe that levels of IFN-γ/IL-10 (p = 0,043) and TNF-α/IL-6 (p = 0,021) ratios are higher in patients with dysbiotic microbiome, which have an imbalance towards inflammation.

Comparing levels of cytokines with respect to alpha diversity, no differences are observed either individually or in the ratios between Th1/Th2 cytokines. However, the multivariate model that predicts alpha diversity (p = 0.037; AIC=20.734) does consider the IFN-γ/IL-10 and TNF-α/IL-6 aforementioned ratios. Alpha diversity, in turn, is higher in vaginal samples

from patients with dysbiotic microbiome ($p = 0.003$). Regarding beta diversity, it is also higher in patients with altered microbiome ($p = 0.007$).

Considering the relative abundance of each bacteria, we can predict the levels of the aforementioned ratios: for TNF-α/IL-6 ratio, the multivariate model reaches 91.37% of prediction including the relative abundance of *Burkholderia, Chryseobacterium, Enterococcus, Escherichia, Gemella, Herbaspirillum, Negativicoccus* and *Staphylococcus*. For IFN-γ/IL-10 ratio, the multivariate model reaches 87,65% of prediction including *Aerococcus, Burkholderia, Escherichia, Herbaspirillum, Megasphera, Pseudomonas, Ralstonia* and *Staphylococcus*. It is worth noting the absence of *Lactobacillus* in these models, which suggests that the relative abundance of some pathogenic bacteria is what alter the production of cytokines, not the lower relative abundance of *Lactobacillus*.

Limitations, reasons for caution: The inherent limitations of a retrospective study design and a limited sample size. In addition, cytokine levels were measured in serum and not in vaginal fluid. Additional studies including a metagenomic approach might be able to determine functional groups of bacteria for analysis.

Wider implications of the findings: These results indicate that pathogenic bacteria, such as Burkholderia and Staphilococcus, which modify cytokine levels and cause inflammation, may also induce an imbalance in the Th1/Th2 ratio. When the vaginal microbiome is altered, the administration of antibiotics or probiotics may improve the immunologic environment, thus, avoiding the use of immunosuppressants.

Trial registration number: -

Abstract citation ID: dead093.316

O-262 The vaginal and faecal microbiome in women with recurrent pregnancy loss (RPL) before pregnancy according to the reproductive outcome after referral

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Study question: Is the vaginal or faecal microbiome different between primary RPL (pRPL) and secondary RPL (sRPL) patients, and is it related to reproductive outcome after referral?

Summary answer: Before pregnancy, the vaginal microbiome differed between pRPL and sRPL, and the faecal microbiome was altered in those who did not achieve pregnancy after follow-up.

What is known already: RPL is a heterogeneous condition leaving 50% of the couples without any known risk factors after the initial diagnostic workup. The microbiome of the reproductive tract seems to be an essential factor in women's health, including pregnancy loss. Only a few studies have investigated the vaginal microbiome in women with RPL and found dysbiosis with a decrease in *Lactobacillus crispatus*. To our knowledge, the faecal microbiome in women with RPL has never been investigated.

Study design, size, duration: A prospective cohort study including 106 women referred with unexplained RPL between 04/2018 and 12/2019. Patients were routinely screened for established risk factors as recommended in the ESHRE RPL guideline. Exclusion criteria were >40 years, >1 shared child, the use of antibiotics, antimycotics and antiviral medication within the past two weeks, known chromosomal aberrations and major uterine malformations. Follow-up ranged between 12-31 months.

Participants/materials, setting, methods: The women were referred with a minimum of three unexplained consecutive pregnancy losses (64 pRPL and 42 sRPL) to the tertiary Recurrent Pregnancy Loss Unit at Copenhagen University Hospital (Rigshospitalet and Hvidovre Hospital), Denmark. Vaginal and faecal samples were collected before pregnancy and shot-gun sequenced on a DNBSEQ-G400 sequencer (MGI) using the high-throughput sequencing set (PE150 1000016952; MGI).

Main results and the role of chance: The beta diversity in the vaginal samples was significantly different ($p = 0.001$) between pRPL and sRPL, with *Lactobacillus crispatus* dominating more women with pRPL compared to sRPL who were dominated by *Lactobacillus iners*. Overall, twenty-nine patients (27.3% of the cohort) had vaginal dysbiosis defined as < 60% Lactobacillus spp. with no significant difference between pRPL and sRPL (vaginal dysbiosis in 21.8% vs. 35.7%, $p = 0.118$). During follow-up, 93 (87.7%) patients achieved pregnancy of which 50 (53.8%) resulted in a live birth and 43 (46.2%) in another pregnancy loss. There were no differences in age, BMI, alpha or beta diversity in the vaginal samples between the live birth group and pregnancy loss group. In the live birth group, 11 patients (22.0%) had vaginal dysbiosis compared with 32.1% of the rest of the cohort, $p = 0.242$. Ninety-three of 106 women collected a faecal sample at home after the first consultation and there was a significant difference in both alpha diversity ($p = 0.014$), beta diversity ($p = 0.05$) and richness ($p = 0.001$) between women who achieved pregnancy compared with those who did not conceive after follow-up.

Limitations, reasons for caution: Patients with recurrent pregnancy loss constitute a heterogenic population, which underlines the importance of subgroup comparisons such as pRPL and sRPL.

Wider implications of the findings: These findings underline the role of an altered vaginal and faecal microbiome as a potential risk factor for RPL. In-depth knowledge about the altered microbiome compositions in these patients can contribute to the generation of future treatment strategies and potentially improve patient care.

Trial registration number: not applicable

Abstract citation ID: dead093.317

O-263 Effect of clindamycin and a live biotherapeutic containing lactobacilli on the reproductive outcomes of IVF patients with abnormal vaginal microbiota: a double-blind, placebo-controlled multicentre trial

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Study question: Does treatment of abnormal vaginal microbiota improve the reproductive outcomes of IVF patients?

Summary answer: Data is being analysed and will be presented at ESHRE 2023.

What is known already: An increasing number of studies reported an association between abnormal genital tract microbiota and adverse reproductive outcomes in IVF patients. One hypothesis could be that vaginal microbiota ascend to the endometrium where the microbiota hampers implantation. To the best of our knowledge, this is the first study powered to investigate causality between abnormal genital tract microbiota - in this study defined by a bacterial vaginosis like vaginal microbiota - and clinical pregnancy rate in IVF patients.

Study design, size, duration: Double-blind, placebo-controlled multicentre trial in IVF patients diagnosed with abnormal vaginal microbiota and subsequently randomised into three parallel groups 1:1:1. The first group received clindamycin 300 mg ×2 daily for 7 days followed by vaginal *Lactobacillus crispatus* until the clinical pregnancy scan. The second group received clindamycin and placebo, whereas the third group received placebo/placebo. A total of 1518 patients were screened, and 338 patients were randomised. The study duration was from 2017 to 2023.

Participants/materials, setting, methods: IVF patients with any cause of infertility embarking on their first, second or third IVF stimulation cycle or embryo transfer were approached for inclusion. At a minimum 12 days prior to embryo transfer, patients were screened for abnormal vaginal microbiota defined by a qPCR assay, targeting high quantitative loads of *Fannyhessea vaginae* and *Gardnerella spp*. Patients were excluded if they had intrauterine malformations like polyps, septum, and fibroma.

Main results and the role of chance: Data and safety monitoring board has decided not to open the randomization code yet. The final dataset is being completed. Data is being analysed and will be presented at ESHRE 2023.

Limitations, reasons for caution: Data is being analysed and will be presented at ESHRE 2023.

Wider implications of the findings: The present drug intervention study used clindamycin and a live biotherapeutic containing *Lactobacillus crispatus*. The results of the study could have a worldwide important clinical impact on daily IVF practice as currently there is no evidence that screening and treating IVF patients with abnormal vaginal microbiota improves the reproductive outcome.

Trial registration number: EudraCT 2016-002385-31

Abstract citation ID: dead093.318

O-264 Vaginal Microbiota Transplantation (VMT) for treatment of vaginal dysbiosis without the use of antibiotics – A Randomized Controlled Trial in healthy women with vaginal dysbiosis

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Study question: Can vaginal microbiota transplantation with shotgun-verified eubiotic vaginal microbiome over three menstrual cycles, convert vaginal dysbiosis to eubiosis in healthy women?

Summary answer: The RCT is ongoing until it is by March 2023. Results will be ready for presentation at EHSRE conference.

What is known already: Vaginal dysbiosis covers imbalances in the vaginal flora, caused by the composition of bacteria, viruses, and vaginal fungi. A large proportion of women who have vaginal dysbiosis do not experience any symptoms. Dysbiosis occurs in about 16-20% of all women. Vaginal dysbiosis is associated with infertility, euploid pregnancy loss, preterm labour or bacterial vaginosis. Treatment of vaginal dysbiosis consists of antibiotic treatment, and/or probiotics. Vaginal transplantation with eubiotic vaginal bacterial flora in combination with antibiotics has successfully been performed in four out of five recipients in an earlier study, but no study has been performed without use of antibiotic pretreatment.

Study design, size, duration: Randomized, controlled, double-blinded trial with a randomization ratio 3:1 to receive either a eubiotic microbiome transplant or placebo. 320 healthy women between 18 and 40 years, was enrolled for screening of vaginal dysbiosis. 30 donors with a eubiotic vaginal microbiome and 48 recipients with a dysbiotic microbiome were identified. The trial began in June 2021 and will end in March 2023

Participants/materials, setting, methods: Vaginal microbiome composition was assed by next-generation ShotGun sequencing. To qualify as donor bacterial DNA from a vaginal swab had to show a bacterial composition of at least 80% lactobacilli and less than 5% pathogenic bacteria. To qualify as recipient vaginal microbiome composition should be with at least 20% pathogenic bacteria and no more than 10% lactobacilli. The recipient could have up to three attempts of VMT treatments with a follow-up period of 6 months.

Main results and the role of chance: We expect the RCT to end in March 2023, and the results of this trial will be presented at the ESHRE annual meeting . If we can show engraftment of a eubiotic microbiome transplant without the use of pre-treatment with antibiotics it could be a potential treatment of vaginal dysbiosis.

Limitations, reasons for caution: As this is the first VMT without antibiotic pretreatment we did not have reliable data for our power calculation and a negative result may be due to lack of statistical power.

Wider implications of the findings: Effective treatments for vaginal dysbiosis are urgently needed and VMT may be one such treatment strategy.

Trial registration number: NCT04855006

SELECTED ORAL COMMUNICATIONS

SESSION 81: MANAGEMENT OF ADENOMYOSIS: CURRENT STRATEGIES AND FUTURE PERSPECTIVES

Wednesday 28 June 2023 Auditorium 10-12 10:00 - 11:45

Abstract citation ID: dead093.319

O-265 Increased CD8+ T-cell exhaustion due to high NKG2A/HLA-E engagement and IL-15 induction in adenomyosis

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Study question: Is there dysregulation of immune cells in the local uterine microenvironment of patients with adenomyosis? If any, what's the underlying mediators and mechanisms?

Summary answer: Increased $CD8^+$ T-cell exhaustion due to enhanced HLA-E/NKG2A engagement and IL-15 induction in ectopic adenomyotic lesions existed in human patients and mice models with adenomyosis.

What is known already: The immunological dysfunction has long been considered in the pathogenesis of adenomyosis. However, high heterogeneity in immune cell alterations and contradictory results were reported due to the technique limitation of immunohistochemistry mainly used in previous studies. A comprehensive picture of the dysregulation of immune cells in the local uterine microenvironment of patients with adenomyosis is still lacking. Specially, increased number of $CD8^+$ T cells have been reported in adenomyotic patients, whether functional deficiency of $CD8^+$ T cells may contribute to the abnormal accumulation and residency of ectopic endometrial cells at pathological sites remains unknown.

Study design, size, duration: The paired ectopic lesions and eutopic endometrium from 80 patients with adenomyosis, and normal endometrium and myometrium from 62 patients with uterine myoma (controls) were collected between 2021 to 2022. Mice models with adenomyosis by neonatal tamoxifen treatment were established for experiments in 2022.

Participants/materials, setting, methods: The alteration of immune subsets, NKG2A expression, exhausted phenotypes and $CD8^+$ T-cell dysfunction by flow cytometry, the expression/localization of HLA-E and IL-15 by

immunohistochemistry were investigated in uterine tissues of patients and mice models with adenomyosis. $CD8^+$ T cells were sorted, and in-vitro cultured with recombinant human transforming growth factor-β (rhTGF-β) or rhIL-15 to determine the NKG2A expression. Correlation analyses were conducted for association between immune disturbances and disease severity (dysmenorrhea, local/diffuse lesion, CA125 level).

Main results and the role of chance: We found that an increase in $CD8^+$ T cell number was the predominant alteration in ectopic lesions in patients with adenomyosis, and it was significantly associated with the severity of adenomyosis. For $CD8^+$ T cells in adenomyotic lesions, the expression of NKG2A was positively correlated with CD94 expression, and CD94/NKG2A mainly enriched on $CD103^+$ tissue resident $CD8^+$ T subsets. The $NKG2A^+CD8^+$ T cells showed exhausted phenotypes characterized by co-expression of multiple immune inhibitory molecules (PD1, LAG3, TIM3, and TOX) and decreased degranulation capability demonstrated by reduced expression of perforin, granzyme B, and CD107. Importantly, the exhausted $NKG2A^+CD8^+$ T-cell subset was associated with disease severity and was significantly increased in ectopic uterine tissues of human patients and mice models with adenomyosis. The expression of NKG2A on $CD8^+$ T cells was markedly upregulated by exogenous rhIL-15 but not rhTGF-β treatment. Additionally, we also found increased co-expression of IL-15 and the NKG2A ligand HLA-E on the glandular epithelial cells, where the $NKG2A^+CD8^+$ T cells were closely distributed around the gland, in ectopic adenomyotic microenvironment of patients and mice models with adenomyosis.

Limitations, reasons for caution: The control uterine samples were collected from patients with uterine myoma. Although they were pathologically confirmed as normal, the potential effects are unknown and cannot be excluded. Further studies in larger independent populations and exploration on the functional significance of $CD8^+$ T-cell exhaustion in adenomyosis pathogenesis is warranted.

Wider implications of the findings: Our study reveals a previously unrecognized role for $CD8^+$ T-cell exhaustion in the pathogenesis of adenomyosis and suggests that therapeutic interventions targeting and reinvigorating exhausted $CD8^+$ T cells by NKG2A blockade may be beneficial for patients with adenomyosis.

Trial registration number: not applicable

Abstract citation ID: dead093.320
O-266 Anti-angiogenic therapy as treatment for adenomyosis in mice

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Study question: What is the effect of anti-angiogenic therapy on adenomyosis in mice

Summary answer: Commencing treatment at six weeks with angiogenesis inhibitor axitinib successfully reduced the severity of adenomyosis by approximately 50% at 9 weeks.

What is known already: Adenomyosis is a gynecological disorder characterized by abnormal uterine bleeding, dysmenorrhea, pelvic pain and subfertility. Increased expression of angiogenic markers, as well as increased microvascular density (MVD) in adenomyosis suggest a pivotal role for angiogenesis in the pathophysiology of adenomyosis, which presents an opportunity for treatment.

Study design, size, duration: The effect of angiogenesis inhibition was studied in a tamoxifen-induced adenomyosis mouse model. 102 Mice received oral treatment with axitinib 3 mg/kg ('dose I/AX3 treatment group', n = 34), axitinib 25 mg/kg ('dose II/AX25 treatment group' n = 34), or with vehicle-only ('placebo group', n = 34) from week 6 until week 9. After termination of the mice at week 9, all uteri were analyzed for the presence of adenomyosis.

Participants/materials, setting, methods: The prevalence and severity of adenomyosis were assessed by; (i) grade of adenomyosis (0/1/2/3), based on the depth of endometrial gland infiltration into the myometrium in H&E-stained sections, (ii) percentage of adenomyosis-affected tissue in vimentin-stained sections and (iii) degree of myometrium that is affected in α-SMA-stained sections. The adenomyosis severity index was calculated by multiplying mean grade/mouse with the percentage affected surface area. Changes in angiogenesis-related gene expression were evaluated using real-time quantitative PCR.

Main results and the role of chance: 101 mice completed adenomyosis induction and could be analyzed. The prevalence of adenomyosis was 30/33 (90.0%) in dose I (3mg/kg), 29/34 (85.3%) in dose II (25mg/kg) and 30/34 (88.2%) in placebo treated mice (p=0.78). The prevalence of high grade (2/3) adenomyosis was significantly lower in mice treated with axitinib dose II (N = 19, 55.9%) than in the placebo group (N = 27, 79.4%, $p<0.05$). The grade of adenomyosis per mouse after treatment was 0.7 point lower in the groups treated with axitinib dose I and dose II, than in the placebo treated group (dose I and II both median of 1.0 compared to 1.7, $p < 0.05$). The adenomyosis severity index was reduced by 48% in the axitinib-treated groups (dose I $p<0.05$). In the placebo-treated group, the myometrium adjacent to the affected site (as a marker for hypertrophy) was thicker in the placebo- than in the axitinib-treated groups. There were no signs of fibrosis. MVD was reduced in myometrium of dose I treated mice by 14% compared with Ax25-treated mice ($p<0.05$), as well as compared to 6-week-old tamoxifen-treated mice ($p<0.05$). Expression of angiogenic growth factors and their receptors was markedly reduced in the dose I and II axitinib-treated groups compared to the placebo-treated group.

Limitations, reasons for caution: Limitations of this study include the uncertainty on the optimal timing to start treatment, and the assessment of the presence and severity of adenomyosis. Further research should focus on commonality among different angiostatic drugs, as well as the method and timing of application.

Wider implications of the findings: Since there is currently no treatment option for premenopausal women who wish to retain their uterus and their fertility, treatment with an angiogenesis inhibitor might provide a solution. The major requirement in further development of anti-angiogenesis therapy is a safety and side-effect profile tolerable for this population.

Trial registration number: Not applicable

Abstract citation ID: dead093.321
O-267 Uterine contractility in women with adenomyosis differs significantly from healthy control women - the waves study

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Study question: Description of uterine contractility characteristics in adenomyosis patients compared to healthy controls and according to degree of dysmenorrhoea measured by quantitative 2D transvaginal ultrasound (TVUS).

Summary answer: Women with adenomyosis show differences in uterine contraction patterns (frequency, amplitude, velocity, and contraction coordination) compared to healthy controls and depending on degree of dysmenorrhoea.

What is known already: The natural contraction pattern of the uterus changes throughout the menstrual cycle in response to cyclic changes in hormones. In abnormal uteri, such as adenomyotic uteri, this response may be different. Adenomyosis can cause dysmenorrhoea, menorrhagia, dyspareunia, and subfertility. These symptoms could be explained by different contraction patterns of the uterus, however, this has not yet been objectively quantified due to the absence of a suitable measurement tool. Recently, speckle-tracking

and strain analysis of 2D TVUS was used to assess differences in contraction coordination, frequency, velocity, and direction, depending on the phase of the menstrual cycle in healthy women.

Study design, size, duration: This study is part of an ongoing multi-centre prospective observational cohort study investigating uterine contractility in TVUS recordings. Our study includes the TVUS recordings of 31 women with adenomyosis and 106 women with healthy uteri. Patients were included in 3 centres from 2017 to 2023 (Netherlands, Greece, Italy).

Participants/materials, setting, methods: 31 women with sonographic suspicion of adenomyosis and 106 women with healthy uteri with natural menstrual cycles were included. Uterine contraction frequency, amplitude, velocity, and coordination were assessed by applying a dedicated speckle tracking and strain analysis to 2-4-minute TVUS recordings in midsagittal section. Degree of dysmenorrhoea was measured according to visual analogue score (VAS). Women with suspicion of adenomyosis were compared to women with healthy uteri according to the phase of their menstrual cycle.

Main results and the role of chance: Age, BMI, parity and uterus volume were significantly higher in the women with adenomyosis compared to the healthy controls ($p < 0.05$). Uterine contractility differed between women with adenomyosis and healthy controls during the periovulatory phase, revealing lower frequency (1.50 ± 0.25 vs. 1.75 ± 0.35, $p = 0.031$), higher amplitude (0.076 ± 0.039 vs. 0.046 ± 0.018, $p = 0.001$), and lower velocity (0.64 ± 0.18 vs. 0.84 ± 0.22, $p = 0.020$) of uterine contractions in the group with adenomyosis patients. In the late luteal phase, women with adenomyosis showed higher amplitude (0.050 ± 0.02 vs. 0.035 ± 0.01, $p = 0.039$) and lower velocity (0.51 ± 0.11 vs. 0.72 ± 0.16, $p = 0.032$) than the healthy controls. During menstruation, women with adenomyosis showed a trend towards higher contraction amplitude (0.044 ± 0.01 vs. 0.037 ± 0.01, $p = 0.051$) compared to healthy controls. During the menstrual, periovulatory and late luteal phase, women with adenomyosis showed reduced contraction coordination ($p = 0.047$, $p = 0.015$ and $p = 0.011$, respectively) compared to healthy controls. Increased dysmenorrhoea (VAS score) in overall adenomyosis patients was associated with lower contraction velocity ($p = 0.043$) and a tendency towards lower frequency ($p = 0.160$) and higher amplitude ($p = 0.154$).

Limitations, reasons for caution: No sub-analysis was done to assess effects of additional adenomyosis characteristics (i.e. adenomyosis severity, adenomyosis type, adenomyosis location). Women with extensive adenomyosis were not included due to impossibility to perform analysis of ultrasound images. Women with adenomyosis were older, had higher BMI and larger uterus volumes than healthy controls.

Wider implications of the findings: Uterine contractility differs between patients with adenomyosis versus healthy women throughout the menstrual cycle. More dysmenorrhoea in adenomyosis patients was also associated with a different contractility pattern. This suggests an aetiological mechanism for the clinical presentation of adenomyosis (i.e. dysmenorrhoea and subfertility) and presents potential therapeutic markers.

Trial registration number: NL52466.100.15

Abstract citation ID: dead093.322
O-268 Presence of adenomyosis impairs clinical outcomes in women undergoing frozen embryo transfer

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Study question: Does the presence of adenomyosis impact the clinical outcomes of patients undergoing frozen embryo transfer (FET)?

Summary answer: Presence of adenomyosis is associated with higher miscarriage and lower clinical pregnancy and live birth rates. GnRH agonist pretreatment does not increase clinical outcomes.

What is known already: Presence of adenomyosis among pregnant patients has been associated with a higher incidence of miscarriage and pregnancy complications. Although the role of adenomyosis in women undergoing in vitro fertilization (IVF) has been investigated in several studies demonstrating a potential detrimental effect on live birth rates following IVF, most of them were small studies in which adenomyosis diagnosis was not confirmed based on solid ultrasonographic criteria. Considering the fragmented evidence up to date, we decided to perform a large retrospective study including women with adenomyosis undergoing FET which has been confirmed based on solid ultrasonographic criteria.

Study design, size, duration: Retrospective cohort study of 3503 patients undergoing their first cycle of blastocyst frozen transfer in a university-affiliated fertility center between January 2017 and December 2021.

Participants/materials, setting, methods: Overall, 3503 patients undergoing their first blastocyst frozen transfer through a hormonal replacement (HRT) FET cycle. Patients were categorized as adenomyosis when fulfilling to the Morphological Uterus Sonographic Assessment (MUSA) criteria and as non-adenomyosis when no-adenomyosis was identified. Among them 140 women had a confirmed diagnosis of adenomyosis based on MUSA criteria. Clinical information was retrospectively collected from medical records.

Main results and the role of chance: Comparisons between adenomyosis and non-adenomyosis groups revealed similar baseline characteristics in terms of patients' weight, parity, smoking status, and age. Similarly, endometrial thickness and mean serum progesterone levels the day before FET as well as the number of embryos transferred were comparable between groups.

Adenomyosis patients were more likely to proceed with a deferred FET (freeze-all protocol) as compared with no-adenomyosis women ($p = 0.002$) and were significantly more likely to be treated with GnRH agonist pre-treatment (2 injections) ($P < 0.001$).

Multivariable logistic regression, adjusting for age, autologous or donor oocytes, PGT-A, deferred FET, serum progesterone levels the day before FET, GnRH agonist pre-treatment, number of embryos transferred, and adenomyosis, demonstrated that the use of GnRH agonist protocol did not affect miscarriage, clinical pregnancy rate or live birth rate.

However, the presence of adenomyosis significantly decreased clinical pregnancy rates (OR 0.62, 95% CI: 0.39-0.98, $P = 0.040$) and live birth rates (OR 0.46, 95% CI: 0.27-0.75, $P = 0.003$) and significantly increased miscarriage rates (OR 2.13, 95% CI 0.98-4.37, $p = 0.045$). PGT-A was the only factor associated with a significative reduction in miscarriage rate.

Limitations, reasons for caution: The main limitation of this study is the single-center retrospective approach. Furthermore, due to the limited number of cases with adenomyosis focal and diffuse adenomyosis were analyzed together although this may be clinically distinct entities.

Wider implications of the findings: The results of this study demonstrate that presence of adenomyosis has significant negative impact on the clinical outcomes of patients undergoing FET. Future prospective studies are needed to create a consensus on the optimal ET technique for these patients.

Trial registration number: Not Applicable

Abstract citation ID: dead093.323
O-269 Reproductive and Neonatal Outcomes in Women with Adenomyosis: A Population-based Study

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Abstract under embargo

Abstract citation ID: dead093.324

O-270 Does the 'freeze all' strategy improve the chances of birth in the presence of adenomyosis?

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Study question: Does the freeze all strategy improve cumulative live birth rates in infertile women affected with adenomyosis?

Summary answer: The freeze all strategy in adenomyosis-affected women is associated with significantly higher cumulative live birth rates.

What is known already: Controlled ovarian stimulation enhances the efficacy of assisted reproductive technology (ART) by permitting multiple-oocyte yields, but also may alter endometrial receptivity by an earlier endometrial development which could in turn contribute to diminished pregnancy chances. Technical improvements in vitrification made deferred frozen-thawed embryo transfer (freeze –all strategy) a feasible alternative to fresh embryo transfer (ET). In adenomyosis, the eutopic endometrium is abnormal and its functional alterations are seen as likely to alter the quality of endometrial receptivity. One question in the adenomyosis ART-management is to know whether a freeze all strategy could lead to an increase in reproductive outcomes.

Study design, size, duration: This cohort study conducted in a tertiary care university hospital included adenomyosis-affected women undergoing blastocyst embryo transfer following in vitro fertilization / intracytoplasmic sperm injection (IVF/ICSI) between 01/01/2018 to 31/11/2021. The diagnosis of adenomyosis was based on imaging criteria (e.g. transvaginal ultrasound and/or magnetic resonance imaging).

Participants/materials, setting, methods: Women who underwent a freeze all strategy were compared to those who underwent a fresh ET strategy. Statistical analyses were conducted using univariate and multivariate logistic regression models. The primary outcome was the cumulative live birth rate (LBR).

Main results and the role of chance: A total of 306 women were included in the analysis: 111 in the fresh ET group and 195 in the freeze all group. The phenotype of adenomyosis (internal diffuse adenomyosis, external focal adenomyosis and adenomyomas) was not significantly different between the two groups. The cumulative live birth rate was significantly increased in the freeze all group compared to the fresh ET group [86 (44.1%) vs. 34 (30.6%), $p = 0.020$]. The cumulative OPR [89 (45.6%) versus 37 (33.3%), $p = 0.035$] and the cumulative CPR [122 (62.6%) vs. 53 (47.7%), $p = 0.011$] were significantly higher in freeze all group compared to the fresh group, whereas the early miscarriage rate was not significantly different between the two groups. After multivariate logistic regression analysis, the freeze all strategy in women with adenomyosis was associated with a significant increase in the live birth rate as compared to fresh ET (OR = 1.85; 95% CI = 1.06 – 3.24; $p = 0.031$).

Limitations, reasons for caution: This analysis consists in a retrospective cohort study. The inclusion of patients from a referral center specialized in the management of adenomyosis and endometriosis could constitute a selection bias, as these women may have had particularly severe forms of adenomyosis.

Wider implications of the findings: The freeze all strategy could be an attractive option to increase ART success rates, in adenomyosis-affected womenundergoing IVF/ICSI.

Trial registration number: NA

Abstract citation ID: dead093.325

O-271 Application of HyFoSy in the assessment of fallopian tube patency compared to HyCoSy and HS; results of a systematic review and meta-analysis

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Study question: To assess whether Hystero-salpingo-Foam Sonography (HyFoSy) is an effective and feasible alternative to X-ray Hystero-Salpingo-Graphy (HSG) and Hystero-salpingo-Contrast Sonography (HyCoSy) in fallopian tube patency assessment.

Summary answer: HyFoSy demonstrated significantly superior diagnostic sensitivity and specificity compared to HyCoSy, however only demonstrated minimal diagnostic agreement with HSG.

What is known already: Tubal pathology is a contributing factor in up to 35% of infertility cases, a fact which renders accurate and clinically applicable tubal patency assessment a vital component of the infertility workup. Currently, the most widely utilized methods of assessment are HyCoSy and HSG, which, while effective, do possess certain disadvantages. HyFoSy is an alternative to the aforementioned assessment test, utilizing a specially developed contrast agent, which may offer comparable diagnostic efficacy without the associated disadvantages of the other two methods.

Study design, size, duration: Relevant studies were systematically searched for in the Scopus, Pubmed and Web of Science online peer-reviewed databases. The resulting studies were systematically assessed based on pre-established inclusion criteria (PICOS format) according to the PRISMA algorithm for systematic reviews. The included studies were assessed for risk of bias using a modified version of the QUADAS-2 tool and relevant data was extracted and meta-analysis of diagnostic performance data was conducted.

Participants/materials, setting, methods: This analysis included data from 5 studies and 1433 patients, with 2336 tubes being eligible for inclusion. Data extracted regarding primary outcomes were true positive, false positive, false negative and true negative number of patients for studies that used a reference test and sensitivity and specificity could be calculated. For studies that assessed inter-method agreement, raw data on the number of patients with method agreement and disagreement was extracted and Cohen's k values were calculated.

Main results and the role of chance: With regard to HyFoSy and HyCoSy comparison, pooled sensitivity was 87% and 69% respectively, with the difference being statistically significant ($p = 0.037$), while pooled specificity was 95% and 85% respectively, with the difference being statistically significant ($p < 0.001$). Regarding the HyFoSy and HSG comparison, the pooled Cohen's k statistic resulting from the meta-analysis was 0.39 ; this is interpreted as fair to minimal overall diagnostic agreement between HyFoSy and HSG. Regarding procedure-associated patient pain, HSG was 6.5 times more painful (OR = 6.57, CI 95% 3.11–13.89) compared to HyFoSy and the former was graded at 5.4 ± 2.5 on a 10-point scale compared to the latter that was graded at 3.1 ± 2.2 ($p < 0.001$), a difference that was statistically significant.

Limitations, reasons for caution: The small number of available comparative studies on HyFoSy, the lack of studies that used a reference standard for the comparison of HyFoSy to HSG and the observed statistical heterogeneity, may have introduced a degree of bias in the results if this analysis.

Wider implications of the findings: HyFoSy, being less painful than HSG and more reliable in diagnosing patency, may be applicable as a first line diagnostic test, with HSG reserving its value in the evaluation of abnormal and/or unclear results as a second line test.

Trial registration number: not applicable

Abstract citation ID: dead093.326
O-272 Oral administration of antioxidants can support follicle survival in xenografted human ovarian tissue

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Study question: Can oral administration of antioxidants improve follicle survival in human ovarian tissue through reduction of oxidative stress in a xenotransplantation model?

Summary answer: Daily oral administration of antioxidants for 7 days post-transplantation had a positive effect on follicle survival in grafted human ovarian tissue.

What is known already: Today cryopreservation and autotransplantation of ovarian tissue is an established fertility preservation method in many centres. Hundreds of live births have been achieved but the efficacy of this procedure is hampered by a critical loss of follicles following transplantation. The ovarian tissue is subjected to hypoxia and ischemia postoperative which leads to oxidative damage in the tissue. Postoperative supplementation with antioxidants has been shown to decrease oxidative stress in transplanted ovarian tissue and increase follicle survival, however, these results were mainly obtained through intraperitoneal injections to host animals. Oral administration of antioxidants could be a better alternative for clinical application.

Study design, size, duration: Pieces of frozen-thawed ovarian cortex tissue (total of 56) from 12 women were transplanted to immunodeficient mice in a short- and long-term xenograft study, for 7 and 28 days respectively. In each study the mice were administered daily doses of hazelnut cream (control group) for 7 days post-transplantation or hazelnut cream containing either 150 mg/kg N-acetylcysteine (NAC) or FERTILIX (containing Vitamin E, D and C, Mixed Tocopherols and Tocotrienols, Lycopene, and Coenzyme Q10).

Participants/materials, setting, methods: Cryopreserved ovarian tissue was donated by 12 women undergoing ovarian tissue cryopreservation at Copenhagen University Hospital. Grafts were retrieved after 7 and 28 days of xenografting and histologically processed for follicle counts, immunohistochemistry, and qPCR analysis. The number of surviving follicles and ovarian graft volume were evaluated histologically after 7 and 28 days xenografting. Gene expression analysis of antioxidant defence markers (*Sod1*, *Hmox* and *Catalase*) and angiogenic factor *Vegfa* were performed after 7 days xenografting.

Main results and the role of chance: After 7 days xenografting, the follicle density was similar for all groups (control group: 33.03 ± 14.85 (mean ± SEM) follicles/mm^3; NAC-group: 26.34 ± 10.54 follicles/mm^3, FERTILIX-group: 35.12 ± 10.88 follicles/mm^3). However, the NAC- and FERTILIX-group had a 3- and 3.7-fold increase in follicle density respectively compared to the control group. However, after 28 days of xenografting there was a higher density of surviving follicles in both antioxidant groups, compared to control (control group: 9.51 ± 2.9 follicles/mm^3; NAC-group: 14.9 ± 6.02 follicles/mm^3, FERTILIX-group: 14.43 ± 5.83 follicles/mm^3), which resulted in a 3.2-fold increase in follicle density in the NAC-group and 2.4-fold increase in the FERTILIX-group compared to the control group. The findings were not statistically significantly different in either the 7 days or 28 days study ($p = 0.7072$; $p = 0.5691$). The relative gene expression of the antioxidant defence genes *Hmox* and *Catalase* was overall like the control for all treatment groups after 7 days xenografting, but for *Sod1* the expression was significantly lower for the NAC- and FERTILIX-group compared to the control group ($p = 0.0407$; $p = 0.0255$). For *Vegfa* the expression was similar overall and not significantly different from the control group ($p > 0.05$).

Limitations, reasons for caution: The distribution of follicles in human ovarian cortex is highly heterogeneous and can vary tremendously between cortex pieces from the same woman and between patients. Statistically significant results were not obtained for the follicle data in the current study; thus, the conclusions should be interpreted with caution.

Wider implications of the findings: Oral administration of antioxidants had a positive effect on follicle survival in xenotransplanted human ovarian tissue. These findings provide a step toward potential clinical administration of antioxidants like NAC or FERTILIX postoperative of ovarian tissue transplantation, which could improve follicle outcomes in a feasible way for the patient.

Trial registration number: Not applicable

SELECTED ORAL COMMUNICATIONS

SESSION 82: FERTILITY PRESERVATION: IMPROVING OUTCOME AND PROGNOSTIC FACTORS

Wednesday 28 June 2023 Hall D5 10:00 - 11:45

Abstract citation ID: dead093.327

O-273 Impact of ovarian tissue transplantation on extracellulaire matrix remodelling

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Study question: Does ovarian tissue transplantation affect ovarian extracellular matrix (ECM) components?

Summary answer: Ovarian tissue transplantation affects ECM components in a time- and spatial-dependent manner

What is known already: Human ovarian ECM is subjected to a specific pattern of remodeling during woman's life. Such changes have a crucial influence on folliculogenesis. Ischemia-reperfusion injury after ovarian tissue transplantation also leads to changes in the ECM, such as the occurrence of fibrotic areas. However, there is no in-depth characterization of this remodeling yet or any study on how this change could affect the preantral follicle population, notably primordial follicle activation.

Study design, size, duration: Frozen-thawed human ovarian tissue was collected from reproductive- age women (N = 6). In each case, ovarian tissue was divided into 4 fragments: one for non-grafted controls (D0) and three for grafting to immunodeficient mice for 3, 7 and 21 days (D3, D7 and D21, respectively). After grafting, ovarian ECM components were investigated and compared.

Participants/materials, setting, methods: To assess the impact of ovarian tissue transplantation on ECM remodeling, collagen, elastin, fibrilin-1, EMILIN1, and glycosamoniglycans (GAGs) were investigated at different time points (D0, D3, D7, and D21). Both global (ECM from the entire ovarian tissue fragment) and perifollicular (ECM immediately surrounding preantral follicles) ECM were examined by histological staining and immunofluorescence. Computer-assisted quantification of histological staining and immunolabeling was carried out.

Main results and the role of chance: Collagen content from global ECM significantly decreased from D0 to D7 ($p < 0.05$) and D21 ($p < 0.01$). However, no difference was found in the perifollicular collagen concentration. Compared to D0, in the global ECM, thin collagen fibers decreased significantly on D3 ($p < 0.05$). On the other hand, thick collagen fibers tended to increase from D0 to D3. In the perifollicular area of primordial follicles, thin collagen fibers decreased significantly from D0 to D7 ($p < 0.01$) and D21 ($p < 0.05$), while thick fibers slightly increased from D0 to D3 and decreased significantly up to D21 ($p < 0.01$). In the global ECM, GAGs' content did not change significantly after transplantantion. However, they significantly increased around primordial and primary follicles from D0 to D3 and D7 ($p < 0.001$) and decreased from D7 to D21 ($p < 0.001$). While elastin content did not change after transplantation in the whole tissue, it increased significantly surrounding primary follicles on D3 ($p < 0.05$) and then diminished around primordial and primary follicles from D3 to D7 and D21 ($p < 0.001$). Fibrilin-1 concentration significantly decreased at D21 ($p < 0.05$) in the whole tissue and surrounding primordial and primary follicles. EMILIN1 increased significantly at D3 ($p < 0.01$) and D7 ($p < 0.001$) and decreased significantly from D7 ($p < 0.001$) and D21 ($p < 0.01$) around preantral follicles.

Limitations, reasons for caution: While there is an evident remodeling of the ECM, including the one immediately around primordial and primary follicles, due to the cryopreservation and/or transplantation procedure, we cannot ascertain if it could influence folliculogenesis. For this, the assessment of follicle activation parameters (e.g., mTOR, FOXO-1, YAP) is ongoing.

Wider implications of the findings: At the best of our knowledge this is the first study to describe ovarian ECM remodeling after ovarian tissue transplantation. These data may open new insights for a better understanding of the phenomena occurring in the ovarian follicle microenvironment and develop new strategies to prevent primordial follicle loss after transplantation.

Trial registration number: Not applicable

Abstract citation ID: dead093.328

O-274 Influence of breast cancer prognostic factors on ovarian reserve and response to ovarian stimulation in fertility preservation

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Study question: Do breast cancer (BC) prognostic factors influence ovarian reserve and response to controlled ovarian hyperstimulation (COH) in the context of fertility preservation (FP)?

Summary answer: BC prognostic factors do not influence ovarian reserve and response to COH in the context of FP.

What is known already: Tumor Grade, triple-negative status, overexpression of HER2, as well as high Ki67 expression are all established prognostic factors for BC, influencing patients' therapeutic management. Yet, there are still concerns about the potential impact of cancer status on ovarian reserve and response to COH. Previous studies analyzing the results of COH in BC patients have shown conflicting findings. However, there is limited data on the potential impact of BC status and prognostic factors on ovarian reserve and response to ovarian stimulation in women undergoing urgent FP.

Study design, size, duration: Observational, bicentric retrospective study including all BC patients who had vitrified oocytes after COH using a random start GnRH antagonist protocol after measurement of serum AMH levels and antral follicle count (AFC) between November 2013 and August 2021.

Participants/materials, setting, methods: Three-hundred-fifty-two BC patients having undergone one cycle of COH were analyzed. Number of oocytes recovered, maturation rate (number of mature oocytes/number of oocytes recovered), and Oocyte Retrieval Rate (number of retrieved oocytes/AFC) were studied according to the patients' general characteristics, ovarian reserve markers, as well as BC diagnostic and prognostic factors: histological type, uni/multifocal character, TNM or UICC stage, hormonal receptors expression, HER2 status, Ki67, Grade, presence of vascular emboli, lymph node and *BRCA1/2* status.

Main results and the role of chance: Overall, mean patients' age was 33.6 ± 4 years. Mean AFC and serum AMH level were 20.5 ± 13.0 follicles and 2.7 ± 4.6 ng/mL, respectively. After ovarian stimulation, 12.5 ± 8.5 oocytes were recovered and 9.3 ± 6.9 were mature and further vitrified. Mean oocyte maturation rate was 74 ± 23%. As expected, patient's age was significantly related to the number of mature oocytes recovered. Indeed, the risk of yielding less than 8 oocytes increased significantly after the age of 35 years. In addition, the oocyte maturation rate was also significantly higher before the age of 30 years, with an OR of 0.64 (0.43-0.96) to obtain a maturation rate <60%. Follicular responsiveness to FSH assessed by the follicular output rate (FORT index) as well as OOR were 30 ± 24% and 61 ± 27%, respectively, and both indexes were not influenced by tumor characteristics. However, maturation rate <60% was significantly related to SBR grade (OR = 0.73 (0.54-0.97), $p<0.03$) and the presence of a *BRCA1/2*-mutation (OR = 0.64 (0.42-0.99), $p<0.03$).

Limitations, reasons for caution: The main weakness is the retrospective nature of the study. Furthermore, the lack of data on reutilization of oocytes

after FP prevents drawing reliable conclusions on the fate of these frozen gametes.

Wider implications of the findings: Our findings indicate that BC prognostic factors do not influence ovarian reserve markers nor the response to ovarian stimulation in the context of FP. Therefore, tumor grade, triple-negative status, overexpression of HER2, and high Ki67 should not modify the FP strategy when COH for oocyte vitrification is considered.

Trial registration number: N/A

Abstract citation ID: dead093.329

O-275 Becoming parents after hematopoietic stem cells transplantation: obstetrics outcomes of spontaneous pregnancies

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Study question: Are spontaneous pregnancies after hematopoietic stem cells transplantation (HSCT) of one of the two partners burdened with an increased risk of obstetric complications?

Summary answer: We found a higher risk of Intrauterine growth restriction (IUGR) and preterm delivery after both maternal and paternal HSCT and of pre-eclampsia after maternal HSCT.

What is known already: Conditioning therapies for HSCT are highly gonadotoxic and result in permanent amenorrhea or azoospermia in 70% of patients. Small cohorts of pregnancies after HSCT show an increased risk of complications such as dysfunctional placentation, IUGR or preterm birth. With no more than 9% of survivors reporting a spontaneous pregnancy and the others using of cryopreserved material or gametes donation, it is difficult to untangle the effect of assisted reproduction techniques, gametes donation, radiation on the uterus, and HSCT itself.

Study design, size, duration: This is a retrospective analysis of all spontaneous pregnancies among patients who underwent HSCT for haematological malignancies in a single national referral centre between 1990 and 2016. Obstetric and neonatal outcomes are reported to be compared to the expected incidence in the general population. All patients signed a general informed consent for the use of their anonymized data for research and we obtained the local ethics committee approval.

Participants/materials, setting, methods: We collected the outcome of 53 pregnancies in 30 women after HSCT and 33 pregnancies from 22 men after HSCT. The malignancies treated with HSCT were leukaemia (15 females and 14 males), severe aplastic anaemia (12 and 10), Hodgkin's lymphoma (2 and 1) and myelofibrosis (1 and 1). The conditioning regimen included total body irradiation (TBI) in 20 women (66.7%) and 8 men (36.4%). All patients we included conceived spontaneously after HSCT.

Main results and the role of chance: We observed 53 pregnancies (52 singletons and 1 monochorionic diamniotic twin pregnancy) in women, 12 ± 5.3 years after HSCT. Nine (16.9%) resulted in an early miscarriage. Of the other 44, 15 (34.1%) were uneventful and resulted in a healthy baby born at term. We observed 10 (22.7%) preterm deliveries: 4 very preterm births (1 at 29 and 3 at 31 weeks; 2 spontaneous 2 iatrogenic for pre-eclampsia); 5 moderate preterm births (32 weeks; 4 spontaneous and 1 iatrogenic for pre-eclampsia); 1 late preterm birth (36 weeks, iatrogenic for pre-eclampsia). The incidence of hypertensive disorders in all pregnancies was high (10, 22.7%). In 9 pregnancies (20.5%) there was evidence of IUGR, one of which resulted in an intrauterine foetal death at 35 weeks of gestation. Half of the patients delivered through caesarean section (20, 45.5%). We also reported 33 pregnancies fathered by 22 men who underwent HSCT 7.3 ± 4.3 years earlier. Two pregnancies (6.1%) ended in an early miscarriage. Sixteen of the other 31 pregnancies (51.6%) were uneventful. In 12 cases (38.7%) there was evidence of IUGR, leading to 5 iatrogenic preterm births (16.1%). No maternal hypertensive disorder of pregnancy was reported.

Limitations, reasons for caution: While this is, as far as we know, the largest cohort of spontaneous pregnancies after HSCT present in the literature, the numerosity is still low and we cannot exclude larger numbers would have produced different results.

Wider implications of the findings: HSCT has consequences on reproduction, behind infertility. Pregnancies after HSCT should be followed in a high-risk setting, collecting data collaboratively. The next research effort should be focused on understanding the specific action of the different conditioning therapies used in females and males, to unveil the biological rationale behind these findings.

Trial registration number: not applicable

Abstract citation ID: dead093.330

O-276 Fertility preservation and sperm quality in adolescent transgender patients prior to hormonal treatment

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Study question: This study investigated the take-up, hormonal profile and sperm quality in adolescents undergoing fertility preservation via different methods, prior to medical intervention for gender dysphoria.

Summary answer: Fertility preservation via surgical sperm retrieval (SSR) or masturbation, is possible in this group, however long-term data is required to check gamete health.

What is known already: As increasing numbers of adolescents with gender dysphoria (GD) start gonadotrophin-releasing hormone agonists (GnRHa) to delay puberty to minimise psychological distress. However, the uncertainty of the long-term effects of these medications highlights the importance of undergoing fertility preservation (FP) prior to starting any hormone treatment.

Study design, size, duration: Data for adolescents wanting to FP was prospectively maintained. A total of 122 patients were referred for FP, of which 23 declined (19%).

Participants/materials, setting, methods: Young people were counselled for FP and serum testosterone, FSH, and LH were recorded prior to providing semen samples. Semen samples were classified by concentration; normal >15mil/ml, oligospermia <15mil/ml, poor <1sperm/slide, azoospermia. Insufficient samples, or unwillingness to masturbate, meant SSR was offered in a stepwise manner using electroejaculation (EE), TESE and mTESE.

Main results and the role of chance: Most patients (n = 78, 64% - median age 16.7) were able to produce semen by masturbation, cryopreserving an average of 6.6 straws. Sperm concentration was normal in 44% of samples produced, 36% were oligozoospermic, 9% were poor sperm concentration, and 12% were azoospermic. Mean blood results showed: testosterone 12.32nmol/l, FSH 3.83 IU/L, LH 4.39 IU/L.

21 patients required SSR - median age of 14.9. EE was successful in 4 patients, 9 underwent TESE, and 8 underwent mTESE. 4 mTESE patients were azoospermic. Success rate was 77%, with an average of 5.5 vials stored. Semen parameters in this cohort were poor - however possibly adequate for ICSI. Hormone levels were similar to the masturbation cohort: mean testosterone 11.7nmol/l, FSH 3.4 IU/L, LH 3.5 IU/L.

3 patients used GnRHa pre-SSR. Following a washout period, 1 patient remained azoospermic despite a testosterone of 21 nmol/l. The other 2 patients had sperm found with abnormal morphology and motility.

This is the largest UK cohort of transgender girls referred for FP. The results showed that semen parameters were abnormal in 67% of the masturbation samples produced, and within the SSR group, all patient's testosterone levels were >8nmol/l, with an average of 5.5 vials saved.

Limitations, reasons for caution: The quantity of long-term data on whether adolescents end up using their gametes post FP is a factor to

consider with this study, as more evidence is needed in order to display the actual long-term usage of gamete cryopreservation.

Wider implications of the findings: Masturbation is often possible in this group, but alternatively, SSR provides patients with a safe opportunity to potentially have their own biological children in the future.

Trial registration number: not applicable

Abstract citation ID: dead093.331

O-277 Safety of pregnancy after early breast cancer in young women with hormone receptor-positive disease: a systematic review and meta-analysis

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Study question: Is it safe to have a pregnancy in women with prior history of hormone receptor-positive early breast cancer?

Summary answer: Pregnancy following breast cancer treatments in young women with history of hormone receptor-positive disease is safe with no detrimental effect on patients' prognosis.

What is known already: Breast cancer is the most common malignancy diagnosed in women of reproductive age. Both physicians and patients continue to have concerns about a potential detrimental effect of pregnancy after breast cancer, particularly in the setting of hormone receptor-positive disease. In recent years, several studies have demonstrated the safety of pregnancy after anticancer treatments in breast cancer survivors.

Study design, size, duration: A systematic literature search of Medline, Embase and Cochrane library with no language or date restriction up to January 1st, 2023, was performed following the PRISMA guidelines. We included retrospective or prospective case-control and cohort studies as well as prospective clinical trials comparing survival outcomes of premenopausal female patients with reported pregnancy or not after diagnosis and treatment for hormone receptor-positive breast cancer.

Participants/materials, setting, methods: Included patients were childbearing potential age women with a prior history of hormone receptor-positive early breast cancer. Outcomes of interest were disease-free survival and overall survival. Hazard ratios (HR) with 95% confidence intervals (CI) were extracted. Higgins I^2 index was used to evaluate the degree of inconsistency in the results of the included studies. Pooled HRs were considered statistically significant with a P value of < 0.05 (two-sided).

Main results and the role of chance: Eight studies were eligible to be included in the final analysis. A total of 3,805 patients with hormone receptor-positive breast cancer were included in these studies, of whom 1,285 had a pregnancy after treatments. Median follow-up of the included studies ranged from 3.81 years to 15.8 years.

In three studies (n = 987 patients) reporting on disease-free survival outcomes, no difference was observed between patients with or without a subsequent pregnancy (HR 0.96, 95% CI 0.75 – 1.24, p = 0.781). Six studies (n = 3,504 patients) reported outcomes in terms of overall survival: patients with a pregnancy after breast cancer had better overall survival compared with those without a pregnancy (HR 0.46, 95% CI 0.27 – 0.77, $p < 0.05$).

At the subgroup analysis on timing of pregnancy, no detrimental effect of pregnancy after breast cancer in terms of disease-free survival was observed for patients achieving a late pregnancy (defined as 2 or 5 years after diagnosis) as compared to patients without a subsequent pregnancy (HR 1.08, 95% CI 0.80 – 1.46, p = 0.611). Increased disease-free survival was observed in patients with an early pregnancy (HR 0.63, 95% CI 0.47 – 0.85, $p < 0.05$).

Limitations, reasons for caution: This meta-analysis is based on abstracted data and most of the studies are retrospective cohort studies. Median follow-up in a large proportion of the studies was shorter than 10 years. Adjuvant hormone therapy before and after pregnancy was not available in many studies included.

Wider implications of the findings: Our results strengthen the evidence that having a pregnancy in women with prior history of hormone receptor-positive breast cancer is safe.

Trial registration number: not applicable

Abstract citation ID: dead093.332

O-278 Medical and ethical perspectives of performing ovarian stimulation and oocyte cryopreservation for fertility preservation (FP) in adolescents: 5 years' experience from a tertiary centre

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Study question: What are the medical and ethical challenges of performing ovarian stimulation and oocyte cryopreservation in adolescents and what are the barriers to provision?

Summary answer: Oocyte cryopreservation for benign and malignant indications in adolescents is associated with unique medical and ethical challenges.

What is known already: An improvement in childhood cancer survival, increased transgender care and benign conditions such as haemoglobinopathies requiring stem cell transplant have led to higher representation of adolescent birth-registered females in FP clinics.

FP in adolescents has increased complexities, both physical (pubertal stage, diagnosis) and psychological (accepting diagnosis, processing information, decision-making). Concerns relate to reliability of AMH in adolescents, anticipated response to ovarian stimulation and oocyte quality. Counselling and consenting young people and their families about fertility decisions, often alongside receiving a life-changing diagnosis, is an ethically complex area. Assessing capacity to consent is not straightforward. We report our clinical experience.

Study design, size, duration: This was a retrospective, observational cohort study of 26 adolescent birth-registered females aged 13 to 18 years who underwent ovarian stimulation and oocyte cryopreservation in a specialist unit for fertility preservation between 2018 and 2022. The primary outcome was oocyte yield, secondary outcomes included complications and drop-out rate.

Participants/materials, setting, methods: We included post-pubertal adolescent birth-registered females aged 13 to 18 years who were referred for fertility preservation for high risk of gonadotoxicity from treatment and who commenced ovarian stimulation for oocyte cryopreservation. Data was retrieved from a prospectively managed database. We noted demographic data, ovarian reserve, method of monitoring, response to ovarian stimulation and oocyte cryopreservation and route of egg collection. Documentation on counselling conversations and psychological maturity was retrieved from clinical records.

Main results and the role of chance: There were a total of 29 ovarian stimulation cycles performed in 26 adolescent birth-registered females aged between 13 and 18 years. Indications for fertility preservation included malignancy 62% (16/26), immunological disorders 19% (5/26), gender reassignment treatment 8% (2/26), recurrent ovarian cyst surgery 8% (2/26) and benign haematological disease 4% (1/26). The youngest was aged 13 years and 10 months at the time of egg collection. Minimum time from menarche to ovarian stimulation was 11 months. 23% (6/26) adolescents had previously received chemotherapy. AMH was 2.8-37 pmol/L and AFC 2-36. AFC correlated well with AMH in the majority. Random-start was performed in 54% (14/26) and dual stimulation in 12% (3/26). Number of cryopreserved oocytes was 3-45 and number of MII oocytes cryopreserved 3-35. Ultrasound monitoring was performed transabdominally in 88% (23/26) and transvaginally in 12% (3/26). Egg collection was performed transvaginally in all cases in this cohort, comprising adolescents from varying ethnicities. All cycles proceeded to completion. There were no complications in any cycle, including the 42% (11/26) performed for haematological malignancy. All adolescents were counselled in association with a family member to obtain informed consent. All were assessed as able to comprehend discussions.

Limitations, reasons for caution: There is limited data on performing oocyte cryopreservation in adolescents. Further studies expanding on our findings are needed to support clinicians to perform oocyte cryopreservation in this population. Concerns remain regarding increased aneuploidy rates in this age group compared to women in their 20s. Livebirth outcome data is needed.

Wider implications of the findings: This is the largest case series to date. Post-pubertal adolescents as young as age 13 can undergo ovarian stimulation and oocyte cryopreservation. Transvaginal egg collection is an accepted procedure when counselled appropriately. Clinician experience, correct setting and funding enable a permissive environment for oocyte cryopreservation in this population.

Trial registration number: N/A

POSTER DISCUSSION SESSION
SESSION 83: REPRODUCTIVE (EPI)GENETICS
Wednesday 28 June 2023 Hall D2 10:00 - 11:45

Abstract citation ID: dead093.333
P-696 Identification of novel candidate genes associated with meitotic aneuploidy in human embryos by whole-exome sequencing (WES)

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Study question: Can certain genetic variants in women cause a genetic predisposition to aneuploidy?

Summary answer: Our results suggest that the maternal variants identified in genes regulating meiotic processes could be useful genetic biomarkers for predicting a predisposition to embryonic aneuploidies.

What is known already: Errors in chromosome segregation during meiosis as chromosome synapsis, crossing-over and spindle building, occur frequently in human oocytes and cause aneuploidy in embryos. These errors increase dramatically in the oocytes of older women. However, the rate of producing aneuploidy oocytes varies among IVF patients for a given age. Recent publications have identified genetic variants that are crucial for producing healthy oocytes in female IVF patients. The association between maternal genetic variants and embryonic aneuploidy risk suggests the potential of using genomic data to predict embryonic aneuploidy risk. Our aim was to identify novel variants and candidate genes for embryonic aneuploidy.

Study design, size, duration: A prospective observational cohort study was done including 127 trophoectoderm biopsies from 29 couples who performed whole-exome sequencing (WES) and PGT-A between November 2019 and March 2022. Women were 35 years old or younger and normal karyotype for men and women. Patients were divided in two groups according to the embryo aneuploidy rate expected by their maternal age: $\leq$ 50% of aneuploid blastocysts as control group(n = 14), and $\geq$ 50% of aneuploid blastocysts as study group(n = 15).

Participants/materials, setting, methods: WES was performed using Trusight One Expanded Sequencing Panel (Illumina®). The following criteria were used for filtering and annotation of candidate variants: (1) minor allele frequency (MAF) <0.05 in the gnomAD and 1000 genomes, (2) variants in genes previously associated with chromosome segregation, chromatin cohesion, meiosis and cell division processes, (3) exonic/splicing boundaries variants, (4) variants having potentially strong/moderate functional effects on the protein evaluated using three in silico prediction algorithms (SIFT, PolyPhen-2 and MutationTaster).

Main results and the role of chance: Overall, the mean female and male age was 26.21 + 4.11y and 40.69 + 9.07y respectively. No differences were shown between groups for patient characteristics. Regarding PGT-A cycle no differences were seen between the number of oocytes and MII retrieved. However, the mean number of biopsied embryos per patient (5.36 + 2.50 vs 3.47 + 1.36; $p < 0.05$) as well as the percentage of embryos biopsied on day 5 (85.33 vs 32.69; $p < 0.05$) were higher in the control group. Finally, as expected, the percentage of aneuploidies was higher in the study group (18.64 vs 71.89; $p < 0.05$). 45 variants were identified in genes potentially associated to the mechanism related to the phenotype. After the variant filtering six probably pathogenic variants were identified. The variants found in the genes were: c.1397T>C (*TLE6*), c.169G>A (*IKBKG*), c.1227A>C (*BUB1B*), c.277G>A (*TP73*), c.190C>T (*PLXNA3*) and c.744C>G (*AURKC*). All variants were heterozygous and all of them were of maternal origin. The females carrying the candidate variants belonged to the study group, and two variants in different genes were identified in one patient. No potentially pathogenic variants were detected in patients from the control group. Therefore, the average detection rate of genetic variants with potential impact in embryo aneuploidy was 33.33% (5/15).

Limitations, reasons for caution: A notable strength of our study is the inclusion of both (male and female) genomic data. On the other hand, our study has certain limitations including the inherent challenges of any observational study, also, the pathogenicity of the identified variants should be validated in further functional studies.

Wider implications of the findings: We described new candidate genes that have never been associated to meiotic embryo aneuploidy and are involved in important biological processes (cell division and chromosome segregation). WES may be considered as an efficient tool to identify patients with higher risk of embryo aneuploidy allowing an individualized genetic counselling in advance.

Trial registration number: Not applicable

Abstract citation ID: dead093.334
P-715 NGS-based metagenome analysis of endometrial microbiome in women with implantation failure after in vitro fertilization: results of a prospective cohort study

S. Hadjidekova[1], D. Parvanov[2], T. Tihomirova[3], S. Chapanova[3], R. Staneva[1], B. Rukova[1], M. Pancheva[4], M. Serafimova[4], R. Ganeva[2], M. Ruseva[2], M. Handzhiyska[2], N. Vidolova[2], D. Metodiev[5], G. Stamenov[3]

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Study question: We aimed to compare the endometrial microbiome between pregnant and non-pregnant women after frozen embryo transfer (FET) with euploid embryos.

Summary answer: Specific bacteria taxa had significantly higher relative abundance in the endometrium of patients with implantation failure after FET with euploid embryos.

What is known already: Several studies revealed a significant association between the endometrial microbiome composition, including the abundance of Lactobacilli and pathogen species and the occurrence of implantation failure or miscarriage. However, there is still scarce information about the influence of relative abundance of certain bacteria taxa on the pregnancy outcome in patients with idiopathic infertility and FET.

Study design, size, duration: In this prospective observational cohort study endometrial biopsies were collected from 30 women aged between 34 and 45 during the mid-luteal phase (LH + 7) in a natural cycle. FET was performed with euploid embryos (after preimplantation genetic testing for aneuploidy (PGT-A)), up to 6 months after the biopsy. The exclusion criteria for the patients were history of recent inflammatory disease, chronic endometritis, recent antibiotic treatment, moderate or severe endometriosis, adenomyosis, uterine hyperplasia, and endometrial polyps.

Participants/materials, setting, methods: Endometrial microbiota composition was analyzed using 16S rRNA (v4-v5 region) next generation sequencing (NGS). The sequencing data was assigned to bacterial taxonomy and the background-contaminated bacteria were excluded from the analysis. The Student t-test or Mann-Whitney U test for the relative abundance of each bacteria taxa were applied according to normal or not normal distribution of data. A Bonferroni correction was applied to the significant levels obtained.

Main results and the role of chance: The performed analysis of 30 patients with different clinical outcomes, including no pregnancy (n = 16), clinical pregnancy confirmed by ultrasound (n = 14) revealed differences in the endometrial microbiome composition.

Baseline characteristics including age, BMI and duration of infertility were comparable between the studied groups of patients.

In total, 271 distinct bacterial species and 668 bacterial genera were identified. The number of unique species, found in non-pregnant women was 62 (22.88%), while in pregnant patients it was 39 (14.39%). Among them, bacteria with high frequency of occurrence such as *Cutibacterium granulosum, Isoptericola, Acetomicrobium, Marivivens*, *Syntrophomonas and Bacteroides* were found only in patients with unsuccessful implantation while *Bosea* was present only in women with successful implantation.

Based on the analysis of bacteria relative abundance, *Lactobacillus* was the most prevalent genus in both groups. The abundance of this genus was not significantly different between the studied groups. In contrast, *Serratia marcescens, Delftia, Massilia, Glutamicibacter, Lactococcus and Staphyloccocus* were significantly enriched in the non-pregnant group ($p < 0.05$).

Limitations, reasons for caution: Our study is a single-center study with a relatively small sample size.

Wider implications of the findings: The microbiome showed specific composition in pregnant compared to non-pregnant women. It could be hypothesized that an appropriate treatment for optimization of endometrial microbiome content in women with diagnosed microbiome dysbiosis could be beneficial for improvement of pregnancy rates.

Trial registration number: The current research was funded by National Science Fund, Ministry of Education, Bulgaria, Contract № KP-06-N53/14/16.11.2021.

Abstract citation ID: dead093.335
P-720 Biallelic variants in IQCN cause sperm flagella assembly defects and male infertility

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Study question: What is the effect of defects in the manchette protein IQ motif-containing N (*IQCN*) on sperm flagellar assembly?

Summary answer: Deficiency in IQCN is responsible for sperm flagella assembly defects and abnormal computer-assisted sperm analysis (CASA) parameters, which result in male infertility.

What is known already: Manchette is a transient structure that is involved in the shaping of the spermatid nucleus and protein transport of flagella. Most recently, our group reported that the manchette protein IQCN is essential for fertilization. Variants in *IQCN* led to total fertilization failure (TFF) and defective acrosome structure phenotypes. However, the related function of IQCN in sperm flagella assembly is still unknown.

Study design, size, duration: Fifty infertile males were recruited from the Reproductive and Genetic Hospital of China International Trust Investment Corporation (CITIC)-Xiangya from January 2014 to October 2022.

Participants/materials, setting, methods: Genomic DNA was extracted from the peripheral blood samples of the affected individuals for whole-exome sequencing. The ultrastructure of the flagella was detected by transmission electron microscopy (TEM). CASA was used to test the parameters of sperm motility. An *Iqcn* knockout (*Iqcn*$^{-/-}$) mouse model was generated by CRISPER-Cas9 technology. Immunoprecipitation (IP) followed by liquid chromatography–mass spectrometry was used to select the differentially expressed IQCN-binding proteins. Immunofluorescence was used to validate the localization of IQCN-binding proteins.

Main results and the role of chance: Biallelic variants in *IQCN* (c.3913A>T and c.3040A>G; c.2453_2454del) were identified in our infertile cohort. The sperm from the affected individuals showed irregular "9 + 2" structure of sperm flagella, which resulted in abnormal CASA parameters. Similar phenotypes were observed in *Iqcn*$^{-/-}$ male mice. The CASA parameters in the sperm of *Iqcn*$^{-/-}$ male mice were significantly lower than those in *Iqcn*$^{+/+}$ male mice. The partial of peripheral doublet microtubules (DMTs) and outer dense fibers (ODFs) were absent, or the chaotic arrangement of DMTs were observed in the principal piece and end piece of sperm flagella. The hyperactivation ability of sperm from *Iqcn*$^{-/-}$ male mice was decreased than sperm from *Iqcn*$^{+/+}$ male mice. The CDC42 and IFT protein families are hub proteins that interact with IQCN and regulate flagella assembly during spermiogenesis. Deficiency in IQCN causes the loss of binding with CDC42 and IFT74, which leads to the absence or abnormal localization of CDC42 and IFT74 in mouse and human spermatozoa.

Limitations, reasons for caution: More cases are needed to demonstrate the relationship between the *IQCN* variations and the phenotypes.

Wider implications of the findings: Our findings expand the genetic and phenotypic spectrum of *IQCN* variants in causing male infertility, thus providing a genetic marker for sperm motility deficiency and male infertility.

Trial registration number: N.A.

Abstract citation ID: dead093.336
P-742 Universal genome-wide haplotyping-based preimplantation genetic testing: a systematic seven-year experience at a single center

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Study question: To record the outcome and clinical benefit of universal genome-wide haplotyping-based preimplantation genetic testing (PGT).

Summary answer: Universal PGT allows for standardization of protocols and reduces turnaround time. Apart from accurate genetic diagnosis, it also provides chromosome information, improving embryo selection strategies.

What is known already: Developments in genome-wide technologies leveraged the implementation of universal PGT that combines haplotyping and copy number typing across the whole genome. Universal PGT provides diagnosis for any familial monogenic disorder (PGT-M) or structural rearrangement (PGT-SR), and allows for concurrent aneuploidy screening (PGT-A) in PGT-M/SR cycles, all using the same generic protocol and the same biopsy. For this reason, various universal PGT approaches have been developed in the last 2-3 years and are increasingly making their way to the clinic worldwide.

Study design, size, duration: Universal PGT as a first-tier test was implemented at UZ Leuven mid-2014, using in-house developed algorithm (siCHILD/haplarithmisis). This is a retrospective analysis of universal PGT data, collected between 1 January 2015 and 31 December 2022. Data on PGT indication, PGT cycles and biopsy results was extracted. Implantation rate (IR; with IU/EU fetal sac) per embryo transfer was calculated from frozen embryo transfer (FET) cycles for patients with known result by the end of 2021.

Participants/materials, setting, methods: By the end of 2022, 718 couples have successfully finalized preclinical PGT workup. At the time of writing, 573 couples (80%) have further proceeded with PGT cycles. In total, 1264 PGT cycles were performed with day-3 (D3) cleavage-stage (n = 3344) or trophectoderm (TE) biopsies (n = 1524). For 403 patients with known outcome from 954 FET cycles, IR per embryo transfer was calculated per biopsy type and embryo ranking, considering genome-wide chromosome information.

Main results and the role of chance: Couples were referred to PGT due to various autosomal dominant (AD, 65%), autosomal recessive (AR, 14%) and X-linked disorders (XL, 10%). Various PGT-M indications encompassed >200 genes in total, but almost 20% of couples enrolled due to inherited BRCA1/BRCA2 mutations. PGT-SR was indication in 7% of cases, mainly for familial microduplications/deletions (64%). For all PGT cycles, approximately 15% and 18% of all performed D3 and TE biopsies, respectively, were scored as abnormal largely due to meiotic errors in the embryo. All unaffected embryos were further ranked as R1 (euploid) or R2 (mosaic), and the number of R2 embryos was expectedly higher after D3 biopsies, compared to TE biopsies (250/1063, 23.6% vs 37/510, 7.3%, $p<0.001$). In accordance with inheritance mode, the number of unaffected embryos suitable for transfer was significantly lower in AD PGT cycles, compared to AR (32.3% (1116/3455) vs 44.0% (262/596) ; $p<0.0001$)). For 403 patients with FET cycles, IR was higher in R1 vs R2 embryos after D3 biopsy (231/590, 39.2% vs 30/159, 18.9 %, $p <0.0001$), whereas no difference was observed between R1 and R2 embryos after TE biopsy, although low number of R2 embryos after TE biopsy was transferred (85/142, 59.9% vs 7/12, 58.3%, *p=ns).*

Limitations, reasons for caution: The analyzed data is derived from a single center and the clinical outcome may not reflect the experience of other IVF/PGT centers due to confounding factors, such as PGT indication, used PGT technology and IVF lab practice.

Wider implications of the findings: The current dataset provides a comprehensive overview on general trends and outcome of universal PGT practice. Apart from accurate genetic diagnosis, universal PGT provides information on chromosomes, which improves embryo selection for transfer. This in turn impacts clinical counselling, interpretation and reporting of the results, and embryo transfer policies.

Trial registration number: Not applicable

Abstract citation ID: dead093.337

P-727 New methods reveal the true incidence of DNA contamination in PGT-A samples for the first time and avoid errors that could result in serious misdiagnoses

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Study question: What is the occurrence of contamination in embryo biopsy samples and could this lead to incorrect interpretation of preimplantation genetic testing for aneuploidy (PGT-A) results?

Summary answer: On average, contamination affects 0.4% of biopsy samples, but can be significantly more common in some clinics. Misdiagnosis can occur when contamination is not detected.

What is known already: Until recently, most commercially available platforms for PGT-A have utilised whole genome amplification followed by sequencing of a random selection of DNA fragments scattered across the genome using next generation sequencing (NGS). However, the simple quantitative measurements of DNA fragments derived from each chromosome, provided by such methods, cannot reveal when a biopsy sample is contaminated with non-embryonic DNA. Negative controls are seldom used during PGT-A and are inadequate as they do not evaluate contamination in the actual tube containing the biopsy specimen. The extent to which failure to detect DNA contamination is a problem for PGT-A is unknown.

Study design, size, duration: This was a retrospective study involving analysis of 49,287 trophectoderm biopsy samples that underwent PGT-A over a three-year period. Embryos found to have a contaminated biopsy specimen typically underwent a second biopsy. In such cases, results from the two samples were compared to ascertain whether the contaminated sample would have been misdiagnosed if the analysis had been restricted to examining only the relative chromosome copy number, as is the case for most NGS-based PGT-A methods.

Participants/materials, setting, methods: All trophectoderm biopsies underwent targeted DNA amplification and next generation sequencing using a highly validated PGT-A method that evaluates the relative chromosomal copy number, similar to traditional PGT-A methods, but combines this with analysis of variations in DNA sequence (single nucleotide polymorphisms - SNPs). The genotype of each SNP, and the relative quantity of DNA fragments containing each of the different alleles, allows detection of otherwise invisible states, such as triploidy, haploidy, and contamination.

Main results and the role of chance: From the 49,287 TE biopsies analysed, contamination with non-embryonic DNA was detected in 218 (0.44%). There was variation in the rates of contamination between the 25 clinics that provided samples, ranging between 0% and 1.5%. Additionally, one clinic had a contamination rate of 7.7%, but the number of biopsies derived from that site was considered too small for reliable evaluation (n = 26). 156 of the embryos with a contaminated biopsy specimen underwent secondary biopsy (71.5%), allowing the relative chromosome copy number result from the contaminated specimen to be compared to that obtained from an uncontaminated sample. The results were split into three categories: 1) no change in interpretation between the first (contaminated) and second biopsy specimens; 2) false positive – the contaminated sample was euploid but would have been wrongly interpreted as triploid and would have been erroneously discarded, potentially impacting the patients chances of achieving a pregnancy; 3) false negative – the contaminated sample was fully aneuploid but would have been incorrectly classified mosaic or euploid and could have been eligible for transfer, potentially leading to implantation failure or abnormal pregnancy. 19% of contaminated samples gave a false negative result, while 24% gave a false positive, appearing to be triploid.

Limitations, reasons for caution: It is not possible to determine the origin of contaminants with certainty without having DNA from the contamination source for comparison. Additionally, we were unable to conclude whether contamination is more likely to occur in IVF or ICSI cycles as only 3% of samples were fertilized using IVF.

Wider implications of the findings: Contamination detection during PGT-A is important to prevent misdiagnosis of embryos. Misclassification due to undetected contamination can lead to discard of potentially viable embryos. It can also lead to the transfer aneuploid embryos, wrongly classified as mosaic, which could lead to increased rates of implantation failure, miscarriage and aneuploid pregnancy.

Trial registration number: Not applicable

Abstract citation ID: dead093.338
P-743 Semen samples quality and blastocysts diversity after PGT-A

C. Albanese[1], D. Perruzza[1], S. Azzena[1], A. Crippa[1], S. Graziosi[1], G. Bilotta[1], M.C. Magli[1], L. Gianaroli[1]

[1]*S.I.S.Me.R., Reproductive medicine unit, Bologna, Italy*

Study question: Does semen samples quality contribute to blastocyst diversity by correlating with the presence of DNA in the blastocoelic fluid (BF)?

Summary answer: Semen samples quality is associated with the detection of DNA in BFs from both euploid and aneuploid blastocysts

What is known already: DNA was detected by whole genome amplification (WGA) in BFs from expanded blastocysts dependently on the blastocyst chromosome condition (assessed by PGT-A on trophectoderm (TE) cells), being more frequent in aneuploid blastocysts. TE-euploid blastocysts with positive BF-WGA also seem to have less chances to implant compared to those with failed BF-WGA. The extrusion of abnormal cells from the embryo proper was proposed as a mechanism triggered by the mosaic embryo to eliminate abnormal cells. Severe male factor samples are at risk of causing embryo mosaicism. Possible association between semen quality and DNA in BFs has not yet been investigated.

Study design, size, duration: This retrospective cohort study included 115 patients (maternal age 37.9 ± 4.1), which underwent PGT-A in the last three years. The aim of the study was to evaluate whether the detection of DNA in BFs was correlated with semen samples indices. Both TE-euploid and TE-aneuploid blastocysts were considered for the analysis and stratified according to the semen samples quality as normozoospermic (N), moderate oligoasthenoteratozoospermic (m-OAT) and severe OAT (s-OAT) following the 2021 WHO criteria.

Participants/materials, setting, methods: BF and trophectoderm (TE) biopsies were collected from high grade expanded blastocysts before vitrification, and submitted to WGA. Amplification after WGA was evaluated by loading an aliquot of the amplified product onto a 1.5% agarose gel. 24-chromosome analysis was performed on TE biopsies. To assess the impact of semen samples quality on the detection of DNA in BFs, a multiple logistic regression analysis was performed correcting for maternal age as a confounding factor.

Main results and the role of chance: The BF from 590 blastocysts (265 TE-euploid and 325 TE-aneuploid) were submitted to WGA. In TE-euploid blastocysts, 125 derived from N semen samples, 52 from m-OAT, and 88 from s-OAT. The incidence of positive BF-WGA was proportional to the severity of the male factor condition and occurred in 48.0% of blastocysts originated from N samples, in 55.8% of blastocysts derived from m-OAT, and in 60.2% of blastocysts from s-OAT. In TE-aneuploid blastocysts, 178 originated from N semen samples, 80 from m-OAT, and 67 from s-OAT. The incidence of positive BF-WGA showed an opposite trend compared with the TE-euploid group being detected in 64.0% of blastocysts originated from N samples, in 66.3% of blastocysts derived from m-OAT, and in 52.2% of blastocysts obtained from s-OAT. The relationship between semen quality and positive BF-DNA amplification was confirmed by a multiple logistic regression model setting the BF-WGA as the outcome variable. In the TE-euploid blastocyst cohort, s-OAT showed an odd ratio (OR) of 1.78 (95% CI: 1.02-3.14; p=0.045), whereas in the TE-aneuploid blastocyst group, s-OAT displayed an OR of 0.55 (95% CI: 0.31-0.98; p=0.043).

Limitations, reasons for caution: This is a retrospective cohort study including a limited number of cases where the different categories of sperm samples were not equally represented. Although the results of BF-WGA were evaluated per single blastocyst, they cannot be considered independent variables.

Wider implications of the findings: In s-OAT samples, abnormal cell elimination is especially active in TE-euploid blastocysts, whereas in TE-aneuploid blastocysts the efficiency of this process is reduced suggesting the difficulty of eliminating abnormal cells in an aneuploid background. BF-DNA amplification is a minimally invasive tool contributing additional knowledge to elucidate blastocyst diversity.

Trial registration number: None

INVITED SESSION
SESSION 84: PRACTISING EVIDENCE-BASED EMBRYOLOGY

Wednesday 28 June 2023 Hall A 12:00 - 13:00

Abstract citation ID: dead093.339
O-279 Proven and unproven techniques currently used in the IVF lab

S. Mastenbroek[1]

[1]*Amsterdam UMC, Amsterdam, The Netherlands*

Abstract citation ID: dead093.340
O-280 Reading between the lines: Evaluating the clinical benefit of laboratory interventions from published studies

J. Wilkinson[1]

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Embryologists rely on both randomised and non-randomised studies evaluating the effectiveness and safety of interventions. However, many of these studies contain subtle but critical methodological limitations, which are not identified by peer reviewers. Embryologists must therefore be critical consumers of the published literature. In this talk, I will highlight some common, serious methodological flaws occurring in studies of embryological interventions, and will give intuitive explanations of both the problems and the impact they may have on study results. This includes methods of analysing randomised controlled trials which nullify the benefits conferred by randomisation, and flawed methods of identifying study groups in observational studies which prevent any meaningful evaluation of an intervention's benefit. I will discuss underappreciated methodological challenges in studies evaluating artificial intelligence algorithms for embryo selection, which limit their usefulness, and will outline superior approaches to evaluate these interventions.

INVITED SESSION
SESSION 85: FRONTIERS IN ENDOMETRIAL RESEARCH

Wednesday 28 June 2023 Hall D3 12:00 - 13:00

Abstract citation ID: dead093.341
O-281 Single cell transciptomics of the human endometrium: What can we learn

R. Vento-Tormo[1]

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Abstract citation ID: dead093.342
O-282 Role of extra-cellular vesicles in reproduction

R. Machtinger[1]

[1]*Sheba Medical Center and Tel-Aviv Univeristy- Israel, Obstetrics and Gynecology, Herzlia, Israel*

Extracellular vesicles (EVs) are bilayer-membrane-bound vesicles isolated from biofluids and tissues. Recently, these organelles have been recognized as a novel mode for inter and intra-cellular communication. The three critical

subtypes of EVs include exosomes, microvesicles, and apoptotic bodies. They can be discriminated according to their size, cargo, biogenesis, release pathways, and functions.

These organelles serve as vehicles for transferring bioactive molecules as lipids, proteins, cytokines, and regulatory molecules as RNAs, locally and remotely. A wide range of cell types releases EVs, and their cargo differs under normal and pathological conditions. These facts make them possible biomarkers for diagnosing and treating different medical conditions.

Isolation of EVs from various female and male reproductive tissues such as prostate, epididymis, seminal fluid, fallopian tube, cumulus, and mural granulosa cells, oocytes, embryos at various developmental stages, endometrium, and decidua has been described.

Oogenesis, sperm maturation, fertilization, embryo development, and implantation are composite processes highly reliant on the interaction between tissues and cells. Existing data show that miRNAs and proteins in epididymal fluid associated with testicular maturation are transferred to the sperm by EVs

Ovarian follicular growth is a synchronized process comprising bidirectional communication between the oocyte, cumulus, and mural granulosa cells. Some studies have identified EVs from several species' follicular fluid (FF), including bovine, equine, and human. To date, data linking EVs with oogenesis are primarily descriptive. EV micro-RNA (EV-miRNA) isolated from FF target pathways as mitogen-activated protein kinase (MAPK), wingless signaling pathway (WNT), epidermal growth factor receptor (ErbB), and transforming growth factor beta (TGFß). These pathways are related to folliculogenesis, meiotic resumption, and ovulation.

EV-miRNAs isolated from follicles that contained mature oocytes were associated with fertilization potential and embryo quality. In addition, EVs derived from microvillous-rich oolemma may neutralize acrosome reaction and prevent polyspermy.

Animal and human studies have confirmed that embryos at various developmental stages secrete EVs into the extracellular medium, and embryos internalize EVs. The number, miRNA profile, and size of EVs secreted vary across the cycle and according to embryos' developmental potential.

Endometrial EVs release miRNAs essential for the blastocyst's adhesion. In ovine, EVs were identified from the uterine lumen and uterine flushing during implantation. Uterine lumen flush EVs were internalized by the trophectoderm cells in the conceptus, reducing trophectoderm cell proliferation.

The study of EVs in reproduction has increased exponentially in the last few years. Evidence support that these organelles contribute to oogenesis, fertilization, embryo development, and implantation. Further research in EVs will help us expand noninvasive methods and identify new biomarkers in the reproductive field.

INVITED SESSION

SESSION 86: WEIGHT-LOSS INTERVENTION PROGRAMS IN SUBFERTILE WOMEN: TOO MUCH FOR TOO LITTLE ?

Wednesday 28 June 2023 Hall D1 12:00 - 13:00

Abstract citation ID: dead093.343
O-283 Impact of metabolic syndrome and effect of treatment on male and female reproductive capacity

R. Legro[1]

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Obesity is associated with sub fertility and multiple adverse pregnancy outcomes including miscarriage and later pregnancy complications such as pre-eclampsia and gestational diabetes. Weight loss preconception is recommended for women with obesity and infertility. The etiology of infertility may affect the response to weight loss with WHO type 2 patients more likely to ovulate spontaneously or with oral medications after modest weight loss. The evidence for weight loss improving fertility outcomes is weak for women with unexplained infertility. There may also be unrecognized harms to weight loss including increased risks of miscarriage and weight rebounding excessively during pregnancy. Unique hurdles to weight loss interventions including the types, duration and best comparators will be discussed. Blanket recommendations that weight loss improves fertility and pregnancy outcomes should be avoided. Other factors such as age and ovarian reserve should be factored into any preconception weight loss strategies

Abstract citation ID: dead093.344
O-284 Female obesity and pregnancy outcome following IVF

G. Griesinger[1]

[1]*University of Luebeck and University Hospital of Schleswig-Holstein, Campus Lueb, Luebeck, Germany*

INVITED SESSION

SESSION 87: WHAT WAS AND WHAT IS NORMAL IN MALE FERTILITY EVALUATION

Wednesday 28 June 2023 Hall D4 12:00 - 13:00

Abstract citation ID: dead093.345
O-285 The 6th edition of the WHO manual for the examination and processing of human semen: How should it affect our practice?

L. Björndahl[1]

[1]*Björndahl- Lars, Andrology Laboratory- ANOVA- Karolinska University Hospital, Stockholm, Sweden*

The WHO laboratory manual (World Health Organization 2021) has widened the scope of semen examination from primarily a prognostic tool for Medically Assisted Reproduction (MAR) by indicating the importance of evaluating men with possible disorders in male reproductive functions. Practical instructions for basic examination have been clarified and focused to measures of sperm production and motility as judged from sperm number, motility (vitality if poor motility) and sperm morphology. In addition to the recommendations by the WHO, an international standard based on the same laboratory science as the WHO manual has been published (International Organization for Standardization 2021) to facilitate for laboratories to provide reliable results of semen examination.

Each ejaculate must be handled with great care before analysis begins. Time and temperature between sample collection and start of assessments is crucial. To reduce assessment variability, it is essential to ascertain that the examined aliquots are representative for the entire ejaculate and that the number of observations is sufficient to reduce the risk for significant influence of random factors. The former is handled both by aliquot volume (at least 50 µL for sperm concentration) and replicate assessment and comparison that the two assessments don't differ too much. Regarding number of observations, 400 sperm counted in sperm concentration and in sperm motility assessments is required to reduce this source of errors to ± 10%. For assessment of sperm morphology and vitality a minimum number of observations is 200 spermatozoa.

To achieve acceptable performance, thorough in-house training is necessary. There are protocols how to set up and run such schemes (Mortimer 1994, 1994). Basic medical laboratory standards also require that the laboratory regularly performs internal quality control to monitor that inter- and intra-personal variability is under control. Each laboratory should also participate in an external scheme for quality assessment (EQA) (International Organization for Standardization 2012).

For the proper interpretation of semen examination results reference limits are important. However, the reference limits suggested by the WHO (World Health Organization 2021) come from a very mixed group of men and should therefore not be mistaken for true limits between fertility and infertility. The new WHO manual therefore argues for development of decision limits (when is it reasonable to act?) that are much more important than limits from a mixed population.

It has been argued that semen examination must be better standardized (Björndahl et al. 2016), but the compliance has been almost non-existent (Vasconcelos et al. 2022) leading to even stronger appeals for stronger improvement of basic laboratory investigations of male reproductive functions (Björndahl et al. 2022)

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World Health Organization. WHO laboratory manual for the examination and processing of human semen. 6th edn, 2021. World Health Organization, Geneva.

Abstract citation ID: dead093.346

O-286 Male genital tract ultrasound normative values and utility in fertility evaluation: An update

POSTER DISCUSSION SESSION

SESSION 88: ETHICS AND LAW

Wednesday 28 June 2023 Hall D2 12:00 - 13:00

Abstract citation ID: dead093.347

P-387 Exploring the Potential of Artificial Intelligence in Providing Infertility Consultations and Answers: can it replace the infertility specialist? A hybrid study.

G. Buzzaccarini[1], G. Grecchi[1], G.A. Papa[1], G. Carnemolla[1], N. Salmeri[1], R. Villanacci[1], P. Giardina[1], S. D'Alessandro[1], M. Molgora[1], D. Marzanati[1], L. Pagliardini[1], V.S. Vanni[1], A. Alteri[1], M. Candiani[1], E. Papaleo[1]

[1]IRCCS San Raffaele Scientific Institute, Obstetric and Gynecology Clinic, Milano, Italy

Study question: What are the differences between the accuracy of answers given by an AI-based infertility consultation system compared to those of an infertility specialist?

Summary answer: OpenAI, an AI platform, does not have the potential to provide proper consultation to women searching for answers regarding their infertility.

What is known already: Artificial intelligence (AI) has been recently introduced in the field of human reproduction, both in controlled ovarian stimulation algorithms and laboratory decisions. Additionally, AI-enabled technology has been used to facilitate online communication between patients and healthcare professionals. However, the recent widespread use of AI-based chatbots is causing serious concerns about their use in providing medical advice. Furthermore, the capacity of AI-based chatbots to answer intricate questions about infertility and its treatment has yet to be explored. Despite AI being declared to not be able to provide accurate medical advice, a study assessing this has not yet been conducted.

Study design, size, duration: The aim of this prospective observational study was to assess the ability of OpenAI, an artificial intelligence (AI) platform, to provide appropriate infertility answers to women asking online questions. OpenAI generated answers and answers provided by infertility specialists (control) were both judged by external, blinded infertility specialists in terms of scientific appropriateness (Correctness), detailed feature (Accuracy), ability to properly answer the infertility question (Precision) and providing human kindness (Empathy).

Participants/materials, setting, methods: 20 online infertility questions were retrieved from the web. For each question, the openAI playground was questioned "Answer this question. You are the infertility specialist". The AI platform was available on the internet website https://beta.openai.com. The evaluators were four different infertility specialists with more than 20 years' experience and they were blinded to the purpose of the study and the presence of AI answers. Four criteria were used (1-5): Correctness, Accuracy, Precision and Empathy.

Main results and the role of chance: The mean total score for the human answer without the empathy criteria was 11.18/15 (14.53/20 with empathy). The AI answer provided total score of 8.95/15 and 12.50/20 respectively. At the Mann-Whitney test, both the total scores showed a statistically significant difference ($p < 0.001$). With a tolerance of -/+ 0.5 total score without empathy, the AI answers were evaluated similar for 4/20 answers, compared to the human answers and better than the human infertility specialist answer in 2/20. Of outmost importance, none of the evaluators suspected the presence of an AI engine tool. Intriguingly, evaluating the absolute impact, the scores obtained from AI were higher than we expected and showed great results. This result strongly suggests that, although not able to overcome an infertility consultation, AI has shown great abilities in mimicking an infertility specialist. AI usage in providing infertility answer or consultation should be not considered due to lack of scientific appropriateness, answering details, and question-points precision. However, the results obtained from AI showed borderline performance with the human's even at the evaluation of a team of blinded infertility specialists. Indeed, the AI tool should be appropriately addressed given its worldwide spread.

Limitations, reasons for caution: Further research is needed to understand the limitations and assess the validity of this engine in providing appropriate medical advice. Caution and ethical concerns should be taken into consideration for the coming future.

Wider implications of the findings: OpenAI also contributed to develop this study, by giving answers to our questions on the potential study design. Moreover, and intriguingly, this abstract is the first hybrid-written (human and AI) of the history of ESHRE abstract presentation.

Trial registration number: Not Applicable

Abstract citation ID: dead093.348

P-390 Uterus transplantation, is it ethical to authorize living donor programs when no other alternative to this type of donation is possible?

I. Carton[1], L. Dion[1], B. Hofmann[2], V. Lavoué[1]

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[2]University of Oslo, Senter for medical ethics, Oslo, Norway

Study question: Can we guarantee a freely taken decision for a living donor when she represents the only possibility for a loved one to have a uterus-transplantation?

Summary answer: The autonomy and freedom of the donor may be reduced by the awareness that they are the only possibility for the relative to achieve parenthood.

What is known already: The subject is unknown unlike to the many studies that compare surrogacy to uterus transplantation. The closest study is Lisa Guntram's 2021 paper "May I have your uterus? The contribution of considering complexities preceding live uterus transplantation" which looks at the difficulty of simply having to ask this question. She highlights that the answer, including a refusal, can have both short-and long-term consequences.

However, it does not specifically address the constraint that this represents, or rather adds to the already complex problem of not offering any alternative to the living donor. As a reminder, this is a complex and risky surgery

Study design, size, duration: This is a qualitative study based on semi-structured interviews. The study protocol was submitted to an independent ethics committee for approval. The study was carried out during the year 2022. Communication was made through patient associations and participants volunteered to take part in the study.

Participants/materials, setting, methods: We interviewed 9 health professionals involved in uterus transplantation programmes in France and Sweden and 9 women with Rokitansky syndrome. The health professionals were gynaecologists or psychologists. The interviews were recorded, manually transcribed and analyzed using a thematic content analysis method. The analysis of the interviews was carried out in double reading.

Main results and the role of chance: Many centres that offer the possibility for women with Rokitansky syndrome to be transplanted do not offer a deceased donor uterus transplant programme or surrogacy.

According to some of the professionals interviewed, mothers, the main potential donors identified, already feel guilty about their daughters' disease (Rokitansky syndrome). They cannot therefore consider, despite the risks involved, refusing to donate their uterus. But how can an agreement based on guilt be considered? Furthermore if there is no other solution if they refuse to allow their daughter to become both a legal and biological mother.

In the case of uterus transplantation, is the institution that has legalized such a procedure putting them in this difficult position bears some responsibility?

A total ban, however, would remove the hopes of these women.

According to some professionals and women, the constraint is reduced if women know that in the event of the absence of an identified donor in their entourage, the possibility of resorting to a deceased donor is possible.

Thus, freedom is possible at all levels: asking or not for a uterus transplant and having the possibility to accept or not to donate

Limitations, reasons for caution: This study is based on 18 interviews, which is a small number. Furthermore, all the interviews are in Europe, and only in 2 countries, which may make extrapolation to a larger scale difficult. It has the merit of opening the discussion

Wider implications of the findings: Even if it seems difficult to legislate on a global scale, it is necessary to reflect on the prerequisites before endorsing a transplant programme, including the notion of an alternative to be offered to donor/recipient couples by encouraging the development of a deceased-donor programme, altruistic donors, or by legalizing surrogacy.

Trial registration number: not applicable

Abstract citation ID: dead093.349

P-385 Are you completely aware of your citations? A cross-sectional study on improper citations of retracted articles in medically assisted reproduction

S. Minetto[1], D. Pisaturo[2], G.C. Cermisoni[1], E. Rabellotti[1], L. Pagliardini[1], M. Candiani[1], E. Papaleo[1], A. Alteri[1]

[1]IRCCS San Raffaele Scientific Institute- Milan- Italy, Obstetrics and Gynaecology Unit, Milan, Italy
[2]CIONIC- Inc, CIONIC- Inc, San Francisco, U.S.A

Study question: Are authors aware that they have cited a retracted paper in their manuscripts in medically assisted reproduction (MAR)?

Summary answer: Most corresponding authors were unaware of their own inappropriate retracted citations, mainly due to improper notification of retraction status.

What is known already: Retraction is a severe penalty in scientific research for various reasons, ranging from honest mistakes made in good faith to deliberate misbehaviour. Scientific publications with compromised integrity should be retracted and, once retracted, only be cited in the context of that retraction. However, it has long been recognized that large numbers of citations of retracted papers occur, usually without mentioning the retraction. Concern about the study of inappropriate citation of retracted articles has been gaining momentum in recent years. However, this topic has yet to be considered in medically assisted reproduction.

Study design, size, duration: From December 23, 2022 to January 29, 2023, a cross-sectional study based on an online survey was conducted to acquire information on the irregular citation pattern from corresponding authors who cited a retracted article. The survey was set up in Google Forms and included seven questions. The survey was distributed via e-mail to corresponding authors who cited a retracted paper in their study using the Mailchimp platform. Three reminders were sent about ten days apart.

Participants/materials, setting, methods: A dataset of retracted articles in MAR published up to July 2022 was collected from PubMed and Retraction Watch, according to our systematic review (registered with PROSPERO, CRD42020185769). For each retracted article, a complete list of cited articles published was retrieved from PubMed and Google Scholar. Search, and screening were performed independently by two authors, and any disagreement about the eligibility of a study was resolved through discussion with a third author.

Main results and the role of chance: Forty-three MAR retracted articles were selected, of which 36 were included. Specifically, manuscripts that cited a retracted article in the year of publication of the retraction notice or the following year were excluded from this analysis ($n = 7$) to reduce instances of citation by authors potentially unaware of the retraction of the cited article. The survey was sent to 267 corresponding authors and 39 filled out the survey (participation rate 14.6%). Most respondents (79.5%) were unaware of the retraction status of the cited articles, mainly due to inadequate notification of retraction status in the research database (33.3%), inadequate notification of retraction status in the journal (24.2%), or use of stored copies of the retracted manuscript (15.2%). Regarding bibliography building, 48.7% of respondents declared that they use both online databases and previously printed copies to search scientific manuscripts. Notably, the majority of authors (61.5%) declared that references were entered and checked by only one author before submission and that, during the review process, no one received concerns from editors and/or reviewers about the retracted references. Finally, according to 53.8% of participants, no check of retraction notices is performed, while only 12.8% check both the journal website and scientific databases.

Limitations, reasons for caution: This online survey on citation inappropriateness provided some insight into the justifications correlated with it. Despite the approach used to identify retracted articles in the context of MAR and citations, some may have been missed. In addition, incorrect or disused e-mail addresses constituted a limitation for this study.

Wider implications of the findings: Correcting publications containing references that are subsequently retracted is significant for systematic reviews, meta-analyses, and guidelines. Citations of retracted articles perpetuate

erroneous scientific data, even though assessing the accuracy of citations requires considerable effort. Proper notification of retraction status and cross-checking of citations can help prevent errors.

Trial registration number: N/A

Abstract citation ID: dead093.350
P-388 Is access to egg freezing equitable and fair? A comparison between policies in Belgium and France

H. Mertes[1], M. De Proost[2]

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[2]*Ghent University, Department of Philosophy and Moral Science, Ghent, Belgium*

Study question: What underlying norms and values are the inclusion and exclusion criteria for egg freezing in Belgium and France based on? Are they equitable and fair?

Summary answer: Exclusion criteria are desirable from a distributive justice viewpoint. While age limits are justifiable, criteria such as oncological vs non-oncological and gender are not equitable.

What is known already: In 2018, the Belgian federal institute for health insurance (RIZIV or ENAMI) decided on health insurance coverage for the retrieval, freezing and storing of gametes and gonadal tissues for fertility preservation. This coverage is however not available to all candidates for egg freezing, but strongly directed towards oncology patients. France took a different approach. Since the French Law on Bioethics was passed in August 2021, egg freezing is available to all women between their 29th and 37th birthday. Egg harvesting is covered by public health insurance, but not the storage costs.

Study design, size, duration: This is a normative analysis in which the different inclusion and exclusion criteria from the Belgian and French system are critically analysed in light of existing ethical scholarship on access to egg freezing from the past decade. Special attention is paid to arguments relating to justice as fairness.

Participants/materials, setting, methods: Literature research; normative analysis

The method that is used to bring empirical data (as found in literature research) and normative ethics together is the 'Wide Reflective Equilibrium', the most commonly used method in bioethics.

Main results and the role of chance: While the French system, allowing access to a great range of people, appears to be the most fair, the opportunity costs involved plead for the implementation of inclusion and exclusion criteria to make sure that resources are allocated in a more equitable manner. These criteria should be based on effectiveness and social justice. Looking at the currently implemented criteria, the distinction between medical and non-medical or between oncological and non-oncological conditions is difficult to justify given the large grey area where these categories overlap. For example, in Belgium several categories of people at risk of losing their fertility are excluded: transmen receiving gender-confirming therapy, people needing a stem cell transplant for other than hematopoietic reasons (e.g. sickle cell anaemia) or women nearing the end of their reproductive lifespan (also if due to previous cancer treatment). Arguments in favour of age limits are effectiveness and the avoidance of false hope for people of advanced reproductive age. Arguments against are founded on concerns of ageism and social injustice. Of the potential arguments arguing for gender limits – specifically the exclusion of transmen – none appear convincing, while access for transmen is problematic both in Belgium and France.

Limitations, reasons for caution: This analysis is a case study of two countries, not a comprehensive or representative analysis of European policies.

Wider implications of the findings: Countries that are considering implementing systems for coverage of egg freezing by public funding, can learn from these case studies to better finetune their inclusion and exclusion criteria.

Trial registration number: N/A

SELECTED ORAL COMMUNICATIONS
SESSION 89: CLINICAL APPLICATION OF PGT

Wednesday 28 June 2023 Hall A 14:00 - 15:15

Abstract citation ID: dead093.351
O-287 Preimplantation genetic testing for monogenic disease (PGT-M) where results are only based upon analysis of linked polymorphisms/haplotypes risks serious diagnostic errors

A. Haj Ali[1], M. Ampiah[1], H. Abdous[1], D. Babariya[1], A. Raberi[1], D. Wells[1]

[1]*Juno Genetics, Laboratory, Oxford, United Kingdom*

Study question: PGT-M often involves diagnoses based upon the analysis of polymorphisms linked to the mutant gene. Are such methods sufficiently reliable to be used alone?

Summary answer: Several problems can lead to misdiagnosis when linkage analysis is used in isolation. Therefore, PGT-M should also include direct mutation testing (DMT) whenever possible.

What is known already: Many PGT-M strategies involve analysis of DNA sequence polymorphisms in close proximity to the mutant gene, which have specific alleles that are inherited along with the disease. Unlike diagnostics that focus on detection of specific mutations, which can be unique to individual families, PGT-M protocols using linked polymorphisms can usually be reused for multiple families. Indeed, strategies such as karyomapping, which assess thousands of polymorphisms across the genome, provide a single method applicable to numerous diseases. Such methods are attractive since the work-up required for individual cases is minimal, reducing costs and patient waiting times. However, are such methods safe?

Study design, size, duration: Over a period of three years, we carried out 261 PGT-M cases covering 312 different mutations in 116 genes. Each couple requesting PGT-M provided blood samples from which DNA was extracted. The patients underwent IVF and embryos that reached the blastocyst stage were subjected to trophectoderm biopsy. All PGT-M cases involved the use of DMT to interrogate the mutation site(s). The DMT result was supplemented by analysis of multiple informative linked polymorphisms, as described below.

Participants/materials, setting, methods: Embryo biopsy samples were subjected to multiple displacement amplification (MDA). Mutation site(s) were amplified from MDA products using PCR and mutations were revealed using minisequencing, Sanger sequencing, or DNA fragment size analysis. Parental and embryo samples were also analysed using karyomapping, involving the genotyping ~300,000 polymorphisms scattered across the genome with a microarray. Where possible, samples from additional family members were also tested, allowing determination of which alleles of linked polymorphisms accompanied mutant gene copies.

Main results and the role of chance: Multiple PGT-M cases were identified where DMT prevented potentially serious errors. In five cases, diagnostic reports provided to the PGT laboratory were incorrect. These reports are vital for defining the genetic status of an individual, allowing specific alleles of linked polymorphisms to be correctly associated with mutant or normal gene copies. In three cases, DMT carried out on patient samples during the initial work-up revealed that, contrary to the report, the patient did not carry a mutation. Therefore, PGT-M was not indicated, saving patients from the stress and expense of an unnecessary PGT-M cycle and avoiding discard of healthy embryos. In the other two cases, errors in the reports would have inverted all results based upon linkage analysis, leading to transfer of affected embryos and discard of unaffected embryos, potentially causing a serious misdiagnosis. Thirteen more cases had recombination events extremely close to the mutation site, preventing determination of the status of the embryos based on analysis of nearby polymorphisms. Two further cases displayed consanguinity (undisclosed by the patients), leading to large areas of homozygosity in the

genome, precluding use of linked polymorphisms for diagnosis. In all these cases DMT unequivocally confirmed the true status of patients and their embryos.

Limitations, reasons for caution: In ~8% of PGT-M cases at least one embryo could not be accurately diagnosed without DMT, while in ~1% of cases our routine use of DMT averted a serious misdiagnosis. Nonetheless, even when DMT and linkage analysis are combined, it must be acknowledged that misdiagnoses remain possible (although extremely rare).

Wider implications of the findings: PGT-M is a valuable reproductive strategy for patients at high-risk of transmitting a single gene disorder. Linkage analysis is a valid strategy for PGT-M, but misdiagnoses are possible when testing relies entirely on such methods. These errors can be virtually eliminated by including direct testing of the mutation site.

Trial registration number: Not applicable

Abstract citation ID: dead093.352
O-288 Implantation, ultrastructure and metabolic profiling of embryos from PGT-M and PGT-A Cycles

A. Chatzimeletiou[1], A. Sioga[2], N. Petrogiannis[3], I. Panagiotidis[4], P. Konstantina[2], G. Theodoridis[5], N. Raikos[5], K. Nikolaides[6], E. Kolibianakis[1], B. Tarlatzis[1], G. Grimbizis[1]

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Study question: Can metabolic profiling predict embryos at risk of single gene defects and chromosomal abnormalities and how are these reflected in their ultrustructure?

Summary answer: Different metabolic profiles are observed between embryos with chromosomal abnormalities and monogenic disorders and normal embryos, linked to altered mitochondrial and other organelles' structure/function

What is known already: Down's syndrome embryos and Monosomy 21 embryos have previously been shown to have differential expression of metabolites compared to normal embryos, but limited studies, have investigated in detail the metabolic profiling of embryos with other abnormalities or those with monogenic disorders in comparison to chromosomally normal embryos or their reflection in their ultrustructure.

Study design, size, duration: 520 embryos were biopsied on day5 for PGT-A (n = 178-47 cycles) or on day3 for PGT-A (n = 178-33 cycles) and PGT-M (n = 164-24 cycles). Following transfer of normal embryos, spare embryos, rejected for transfer following day5 or day3 biopsy were processed for TEM (n = 60 day3 biopsied, n = 60 day5 biopsied/vitrified), 60 unbiopsied/vitrified embryos from egg donation cycles used as control. Culture media were collected from the embryos and analysed by hydrophilic interaction liquid chromatography tandem mass spectrometry (HILIC-MS/MS).

Participants/materials, setting, methods: PGT-A and PGT-M were performed in 2 IVF Units. Metabolic profiling was conducted in a Forensic Toxicology Laboratory by HILIC-MS/MS (100 metabolites). Ultrastructure analysis by TEM was carried out in an Academic Hospital and Histology/Embryology Laboratory following embryo fixation in 3% glutaraldehyde, 1% osmium tetroxide, washes in PBS and staining with 1% aqueous uranyl acetate.

Main results and the role of chance: The implantation rates (+ve hCG/ET) for the PGT-M cycles was 68.2% and the for the PGT-A (blastocyst and cleavage biopsy cycles) 64.5% and 65% respectively. Characteristic patient specific metabolic profiles after screening for >100 primary metabolites were observed which differed between normal embryos that had resulted in a viable pregnancy and aneuploid and chaotic embryos although it was more difficult to find clear patterns in embryos with monogenic disorders. Logistic regression analysis revealed a number of metabolites with high predictive value which in combination with embryo score could serve in the future as non-invasive markers for the detection of chromosomal abnormalities before embryo transfer. TEM analysis revealed differences in the quality of cells and organelle activity which were reflected in the embryo metabolic profiles. Abnormal but well developed hatching blastocysts had mainly cells with good mitochondrial morphology/ activity, nice Golgi apparatus and well developed rough and smooth endoplasmic reticulum but depending on the aneuploidy or gene mutation involved, inner cell mass cells with limited organelles and occasionally lipofuscin droplets in the trophectoderm were evident. Chaotic poor quality embryos showed a lower number of mitochondria, often with no cisternae, increased number of vacuoles, and frequently problems in junctions between cells.

Limitations, reasons for caution: Although metabolic profiles were compared between normal and abnormal embryos, all the normal embryos were transferred to the uterus or remain vitrified for clinical purposes. Therefore the ultrastructure analysis is based only on biopsied abnormal embryos and as control unbiopsied embryos from egg-donation cycles with high chance of being normal.

Wider implications of the findings: This study shows high implantation rates after PGT—M and PGT-A and identified distinct differences in the metabolic profiles of normal and abnormal embryos providing unique metabolites which in the future could serve as non-invasive biomarkers for the detection of abnormalities before embryo transfer.

Trial registration number: Not applicable

Abstract citation ID: dead093.353
O-289 Morphology should be prioritised over presumed mosaicism status in PGT-A cycles: data from a non-selection study of 2,621 embryo transfers

S. Caroselli[1], D. Cimadomo[2], M. Poli[1], L. Picchetta[1], M. Figliuzzi[1], A. Vaiarelli[2], E. Alviggi[3], B. Iussing[4], N. Barnocchi[5], S. Canosa[6], F.M. Ubaldi[2], L. Rienzi[2,7], A. Capalbo[1]

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Study question: Is morphological grade or presumed low-mosaicism status the most impactful parameter on live birth rate (LBR) when selecting the embryo to transfer in PGT-A cycles?

Summary answer: Among euploid/presumed low-mosaic embryos, morphology provides a better selection parameter for improving live birth in PGT-A cycles.

What is known already: NGS-based technologies enable the detection of intermediate chromosomal copy number (CN) values, which are commonly interpreted as embryonic chromosomal mosaicism. So far, studies showed that low morphological grade blastocysts are associated with poorer prognoses. Prospective blinded trials also showed that embryos displaying putative mosaic profiles produce comparable clinical outcomes to uniformly euploid ones. Nonetheless, scientific societies suggest different approaches on embryo prioritisation: PGDIS and COGEN advise a selection based on presence of mosaicism, while ESHRE suggests parallel evaluation of mosaicism and morphology. However, the combined effect of morphology and mosaicism on live birth rate (LBR) hasn't yet been thoroughly investigated.

Study design, size, duration: This retrospective multisite cohort analysis is based on a euploid/low-mosaic non-selection study, where the presumed mosaicism was not disclosed to clinics and did not affect the embryo selection procedure. It includes 2,339 consecutive IVF/PGT-A treatments (January2018-December2021) that led to 3,999 transferable blastocysts (euploid or presumed mosaic <50%) and resulted in 2,621 frozen single embryo

transfers (SETs). Morphological (i.e., good/poor quality) and chromosomal (i.e., euploid/mosaic) features were assessed. Clinical outcomes were followed up for each subgroup.

Participants/materials, setting, methods: Morphological grading was assessed using Gardner's criteria, <BB for poor-quality and >BB for good-quality embryos. PGT-A analysis and CN values calculations were performed on IonTorrentS5 and Ion-Reporter software (ThermoFisher). Based on raw NGS data, CN variations <30% and 30%-50% were internally categorised euploidy and mosaicism, respectively. Nevertheless, embryos belonging to either category were reported to clinics as euploid. The association between morphology, chromosomal status, and LBR was evaluated using Fisher's exact test and multivariate analysis.

Main results and the role of chance: In this study population, low-mosaicism had an incidence of 15.8% (N = 630/3,999; 95%CI:14.7-16.9). Overall, LBR and miscarriage rate did not differ between euploid and presumed mosaic SETs: 45.0% vs. 44.8% (P=NS), and 11.2% vs. 13.2% (P=NS), respectively. Also, euploid and presumed mosaic embryos showed similar incidence of poor morphology (7.4% vs. 9.7%; P=NS). In the good-quality category, LBR and miscarriage rate were 46.2% vs. 44.8% (P=NS), and 11.3% vs. 13.1% (P=NS) for euploid and presumed mosaic embryos, respectively. In the poor-quality category, LBR was 21.0% vs. 23.8% (P=NS), respectively; whilst miscarriage rates could not be compared due to insufficient number of cases across the whole morphological category (N = 4/33). Accordingly, the focused scenario that employed poor-quality uniformly euploid embryos instead of good-quality mosaic embryos showed the highest impact on LBR (21.0% vs. 44.8%; $P < 10^{-5}$). Finally, the proportion of cases where both selection strategies (i.e., mosaicism vs. morphology) could be applied accounted for 6.1% (N = 44/717; 95%CI:4.6-8.1) of all cycles where >1 transferable embryo was available (N = 717/2,339).

Limitations, reasons for caution: The results were obtained through the analysis of raw NGS data independent from any proprietary diagnostic algorithm or chromosome-specific consideration commonly used by PGT-A laboratories. These findings concern putative whole-chromosome mosaic aneuploidies only and cannot be extended to segmental mosaic configuration. Follow-up studies in prenatal diagnosis are needed.

Wider implications of the findings: This study suggests that, when selecting embryos for transfer, morphological grading should be considered as a higher criterion than low-mosaic conformation (i.e., mosaicism <50% of copy-number). Reporting mosaicism based on intermediate copy-number categorization ranges <50% appears to provide no clinical utility in the current technological landscape.

Trial registration number: not applicable

Abstract citation ID: dead093.354

O-290 Clinical utility of putative mosaicism detected using concurrent copy-number and genotyping PGT method: outcomes from multisite, prospective, non-selection study including 9828 single embryo transfer cycles

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Study question: What is the clinical utility and associated outcomes of mosaic whole chromosome or segmental aneuploidies detected using concurrent copy-number and genotyping analysis in PGT-A cycles?

Summary answer: Although high-level whole-chromosome mosaicism is linked to reduced sustained implantation, it has limited clinical significance in PGT-A cycles when co-evaluated with other clinical/ embryological factors

What is known already: NGS-based PGT-A can detect intermediate chromosomal copy number (CN), commonly interpreted as mosaic chromosomal aneuploidies in embryos. A prospective non-selection approach is the most effective way to assess the clinical utility of reporting putative mosaicism findings in PGT-A, wherein the presence of mosaicism is not disclosed and does not influence embryo selection. Conflicting results have been reported previously, possibly due to technological limitations in mosaicism assessment or retrospective analysis methods. This study reports the results of the largest multisite prospective non-selection clinical study examining the predictive value of whole-chromosome and segmental mosaicism, assessed through combined CN and genotyping data analysis.

Study design, size, duration: A multisite study involving seven IVF clinics was conducted from Feb 2020 to Oct 2022, including 6951 patients and 9828 single embryo transfers. The study involved a prospective non-selection approach, where embryos suspected of having whole chromosomal or segmental mosaicism were reported as negative for non-mosaic aneuploidies. Embryos were chosen for transfer based solely on standard morphological features. The primary outcome was sustained implantation rate (SIR) defined as pregnancy continuing beyond 8 weeks of gestation.

Participants/materials, setting, methods: In this study, the trophectoderm biopsies were analyzed using a custom, targeted NGS assay that examined approximately 5000 loci across the genome, providing genotyping information to support aneuploidy classification. Mosaicism was identified by any copy number deviation from the expected two copies (LogR plots) and confirmed by corresponding SNP B-allele frequency (BAF) patterns. Confounding factors, such as clinical and embryological variables, were controlled for in the multivariate analysis.

Main results and the role of chance: The average female age in this cohort was 34.9 years (SD = 4.1), with aneuploidy rate of 30% in embryos and SIR of 61.2%. Of the embryos transferred, 6.5% (636/9828) were whole chromosomal mosaic (WCM) only; 9.6% (947/9828) were segmental mosaic (SM) only and 1% (83/9829) were a combination of WCM and SM. The rate of putative mosaicism ranged from 15%-89%. The SIR of embryos in the control group (non-mosaic), SM and WCM were 62% (5190/8328; 95%CI), 58% (549/947;95%CI) and 50.3% (320/636; 95%CI P < 0.01) respectively. A logistic model found that the level of WCM was associated to SIR, along with other embryological and clinical factors. In particular, WCM with a CN difference >50% as well as poor embryo morphology were associated with lower SIR (OR = 0.5; 95% CI:0.32-0.76), but low-level (<50%) mosaicism was not significant (NS). Notably, female age (OR = 0.98; 95% CI:0.97-0.99 per year), BMI (0.98; 95% CI:0.98-0.99) and previous ET failures (OR = 0.58; 95% CI:0.5-0.68) were strongly associated with SIR. A predictive model, taking into account all relevant variables, yielded a significant stratification of SIR, from 43% to 68%. Given the low incidence of WCM in our clinical setting, the multivariate SIR prediction (AUC) was 0.580 without WCM and 0.585 with mosaicism included.

Limitations, reasons for caution: This study did not have prenatal and post-natal data available at the time of the abstract's writing, hence conclusions about these outcomes wasn't possible. Despite the large sample size, chromosome specific analysis was not feasible. Furthermore, this study's data is platform-specific and cannot be translated to other PGT-A assays.

Wider implications of the findings: In this non-selection study, WCM of > 50% variation was associated with lower SIR. However, high-level WCM has a minimal overall impact on SIR when co-evaluating with other clinical/ embryological parameters. Decisions on reporting criteria for these findings must weigh the risk of discarding potentially viable embryos with substantial reproductive potential.

Trial registration number: Not applicable

Abstract citation ID: dead093.355

O-291 Clinical Application of Noninvasive PGT for Patients with Recurrent Pregnancy Loss or Repeated Implantation Failure

N. Li[1], J. Zhao[2], H. Xi[2], Y. Yao[3], S. Lu[1]

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Study question: Has noninvasive PGT been allied with the patients experiencing recurrent pregnancy loss (RIF) and recurrent implantation failure (RPL)? What are the clinical beneficiaries?

Summary answer: Embryo selection through noninvasive PGT can reduce the miscarriage rate in patients experiencing RPL and improve the clinical pregnancy rate in patients experiencing RIF.

What is known already: Chromosomal abnormalities exist in early human embryos, especially in patients with RPL and RIF. Therefore, embryos are usually evaluated through pre-implantation genetic testing for aneuploidy (PGT-A). However, a biopsy is an important concern for undetermined health risks. Meanwhile, the researchers observed genomic DNA contents in the embryo culture medium. Since then, multiple studies have been published using culture medium for analyzing chromosomal ploidy. In 2016, Xu et al. first reported a noninvasive chromosome screening (NICS) assay using a spent blastocyst culture medium. Nevertheless, the clinical application of NICS has not been evaluated in patients experiencing RPL or RIF.

Study design, size, duration: We designed a retrospective cohort study including 303 subjects from July 2018 to May 2021, according to the records of the Reproductive Centre at the Second Affiliated Hospital of Wenzhou Medical University.

Patients experiencing RPL or RIF who received the NICS for aneuploidy were included in the NICS group, while those who underwent conventional morphology embryo transfer during the same period were included in the non-NICS group.

Participants/materials, setting, methods: We included women with a history of RPL (≥2 pregnancies) or RIF (≥3 implantations), exclusion criteria were antiphospholipid syndrome (APS), diabetes, hypothyroidism, or other severe complications.

Routine IVF/ICSI was performed based on sperm quality. The embryos were placed in droplets. Approximately 30 mL of blastocyst medium from each embryo was transferred into cell lysis buffer. Whole-genome amplification was performed using culture media, followed by library preparation using ChromInst (Yikon Genomics; EK100100724 NICS Inst Library Preparation Kit).

Main results and the role of chance: For the patients experiencing RPL, the miscarriage rate per FET was significantly lower in the NICS group than in the non-NICS group (17.9% vs. 42.6%), whereas the ongoing pregnancy rate (40.7% vs. 25.0%) and live birth rate (38.9% vs. 20.6%) were significantly higher in the NICS group compared to the non-NICS group. Nevertheless, no differences were identified in pregnancy rates per patient between the NICS and non-NICS groups (49.6% vs. 44.9%).

For the patients experiencing RIF, the pregnancy rates per FET were significantly higher in the NICS group than in the non- NICS group (46.9% vs. 28.7%), whereas the live birth rate and ongoing pregnancy rate per FET and per patient were no significant difference in the NICS group than in the non-NICS group. Nevertheless, no differences were identified in the miscarriage rate per clinical pregnancy between the NICS and non-NICS groups (23.3% vs. 25.9%).

Limitations, reasons for caution: As a retrospective study, patients in the NICS groups had different clinical prognoses than those in the non-NICS groups, introducing bias in the study results.

Wider implications of the findings: Non-invasive PGT can be used not only for aneuploidy detection but also for a comprehensive evaluation of morphology and DNA concentration, mosaic ratio, resolution, etc. The accumulation of clinical outcomes can also be combined with the clinical data of patients as an index to predict the clinical outcomes of embryos.

Trial registration number: NA

SELECTED ORAL COMMUNICATIONS

SESSION 90: NEW CONCEPTS: OVULATION AND OVULATION TRIGGER

Wednesday 28 June 2023 Hall D3 14:00 - 15:15

Abstract citation ID: dead093.356

O-292 Does ovulation trigger enhance live birth in a natural cycle for frozen embryo transfer: A randomized controlled trial

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[5]CHU and University of Montpellier, Department of Reproductive Biology, Montpellier, France

Study question: Does ovulation trigger enhance pregnancy rate in a natural cycle (NC) for frozen embryo transfer (FET)?

Summary answer: Pregnancy rates after a FET are similar in a natural cycle with detection of a spontaneous LH surge or with a triggered ovulation.

What is known already: Several studies have explored whether one approach might appear to be more beneficial in terms of pregnancy or live birth rates, but with inconsistent results. Thus, the best protocol to identify the time of ovulation in NC and to plan the FET is still undefined.

Study design, size, duration: This was a bicentric, two-arm, randomized, controlled and open-label trial, including 108 patients for 18 months. In the spontaneous-NC group, the LH surge was defined as a rise of the serum LH level at least three times above the basal LH serum value with a serum progesterone level three days after the LH surge above 3 ng/mL.

Participants/materials, setting, methods: Patients included were aged 18- to 39-year-old, had regular cycles and were planned for an autologous frozen day-5 blastocyst transfer in a NC. They did not have endometriosis stage AFSr 3 nor 4 nor adenomyosis. Fifty-four patients per group were included. In the modified-NC group, a spontaneous LH surge was observed in 16 women before the hCG-trigger could be administrated: they were re-allocated to the spontaneous-NC for the per-protocol (PP) analysis.

Main results and the role of chance: Patients characteristics were comparable in both groups. Pregnancy rates were similar in the spontaneous-NC and the modified-NC group in the intention-to-treat (ITT) (41.3 versus 37.8% respectively, $p = 0.85$) and in the PP analysis (39.3% versus 40% respectively, $p = 0.9$). Ongoing pregnancy, miscarriage and live birth rates were also similar. Significantly more visits were needed in the spontaneous-NC (3.4 ± 0.81 versus 2.59 ± 1.0, $p < 0.0001$ in the ITT analysis and 3.18 ± 0.93 versus 2.63 ± 1.03, $p = 0.0064$ in the PP analysis).

Limitations, reasons for caution: Due to group redistribution (spontaneous LH surge before hCG trigger), the number of subjects in the modified-NC group is low for the PP analysis. This may reduce the power of the trial which may fail to detect a small difference.

Wider implications of the findings: The results reinforce previous data that showed similar efficacy of the s-NC and the m-NC. This allows the patient to choose from several options depending on her preference.

Trial registration number: NCT03428165

Abstract citation ID: dead093.357

O-293 An Artificial Intelligence Based Approach for Selecting the Optimal Day for Triggering in Antagonist Cycles

M. Youngster[1], S. Reuvenny[2], A. Luz[3], R. Hourvitz[4], M. Baum[5], M. Ettie[5], A. Hourvitz[1]

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Study question: Can a machine learning (ML) algorithm suggest an optimal trigger day for maximizing the number of mature (MII) oocytes retrieved during an antagonist protocol cycle?

Summary answer: Using ML algorithm for trigger day selection may increase the mean number of MII oocytes and usable embryos by 3.6 and 1.1 per cycle respectively.

What is known already: ML has been increasingly used in the field of reproductive medicine, mainly in lab related technologies such as embryo selection. Only a few studies, aimed at improving clinical decision making, were published. A recent study used ML to optimize trigger day selection based on a retrospective analysis, showing an increase of 2.3 MII oocytes and 1.0 usable embryo.

Study design, size, duration: A retrospective cohort study including data of 9,622 antagonist protocol cycles performed between August 2017 to November 2022. The evaluation of the ML algorithm was conducted using a test dataset including the following quality groups: 1. "Freeze all- oocytes" cycles – a unique population of patients mostly for social fertility preservation. 2. "ICSI only" cycles – for maturation rate evaluation 3. "Fertilize all" cycles including IVF and ICSI, for evaluation of the number of embryos.

Participants/materials, setting, methods: The ML algorithm suggested optimal trigger days for maximizing the number of MII oocytes retrieved by considering the MII prediction, prediction errors and outlier detection results. The model suggested one, two, or three days as trigger options, depending on the difference in potential outcomes. It recommended the days that have a 10% higher prediction of MII oocytes with a confidence level of over 50% compared to less optimal options if they exist.

Main results and the role of chance: To evaluate the performance of the management algorithm, it was applied to cycles in the test sets. For each cycle, the algorithm provided a suggestion for trigger days, and this was compared to the actual trigger day chosen by the physician. When the day chosen by the physician was one of the algorithm's suggestions, the result was labeled as "correct", otherwise it was labeled as "incorrect".

Comparing the "correct" and "incorrect" groups, using the trigger management algorithm resulted in a higher number of total and MII oocytes retrieved, 2PN and usable embryos. Specifically, when using the algorithm in the "Freeze All" test set, an average increase of 4.8 oocytes and 3.4 MII oocytes retrieved in the "correct" group (consisting 36.2% of the quality group subset) compared to the "incorrect group" (63.8%). In the "ICSI-only" test set, the algorithm resulted in an average increase of 4.5 oocytes, 3.8 MII oocytes, 2.4 2PN and 1.1 usable embryos in the "correct" group (26.8%) compared to the "incorrect group" (73.2%). Lastly, in the "Fertilize all" test set, the algorithm resulted in an average increase of 3.6 oocytes, 2.1 2PN, and 0.9 usable embryos in the "correct" group (25.1%) compared to the "incorrect group" (74.9%).

Limitations, reasons for caution: The trigger management algorithms' decision-making is based solely on the predicted number of MII oocytes to be retrieved. Moreover, it was developed and applied exclusively for antagonist cycles.

Wider implications of the findings: The trigger management algorithm may improve oocyte yield and IVF outcomes in antagonist cycles. Moreover, provision of two or three trigger options allows for more flexibility when choosing the trigger day and enables taking into account other factors without negatively affecting the outcome.

Trial registration number: NA

Abstract citation ID: dead093.358

O-294 Dual trigger versus hCG-only trigger in ICSI patients: an analysis of 8500 cycles

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Study question: Among patients undergoing ovarian stimulation for ICSI, does dual trigger with hCG and GnRH agonist offer any benefit over mono trigger with hCG alone?

Summary answer: Dual trigger is associated with a higher number of mature oocytes without any impact on the pregnancy rate.

What is known already: In ART cycles, final oocyte maturation and resumption of meiosis are generally triggered by the administration of hCG as a surrogate for the natural LH surge. Several studies have investigated the role of a combination of a bolus of GnRH agonists and hCG to mimic the natural peak of endogenous LH and FSH, without clear answers regarding clinical benefit. A meta-analysis showed highernumbers of good-quality embryos and increased ongoing pregnancy rate after dual trigger and a recent systematic review demonstrated significantly higher live birth rate (LBR) per cycle after administration of dual trigger compared to hCG-only trigger.

Study design, size, duration: The study was a retrospective, single-centre cohort study. In this study, 8,525 ICSI cycles were included between January 2017 and April 2022 at a tertiary referral University Hospital. We included all patients undergoing ICSI in a GnRH antagonist ovarian stimulation cycle. The mono trigger consisted of recombinant or highly purified urinary hCG. In the dual trigger group, a bolus of GnRH agonist was combined with the hCG trigger.

Participants/materials, setting, methods: Our cohort of 8,525 cycles was divided into two groups: Group A, the mono trigger group, (7,022 cycles), and Group B, the dual trigger group (1503 cycles). Patients who underwent IVF, pre-implantation genetic testing, oocyte donation, and fertility preservation were excluded. Patients with uterine anomalies and endocrine disorders were also excluded, as well as patients who received a triptorelin-only trigger because of hyperresponse.

Main results and the role of chance: There was no difference in the mean age between the dual vs mono trigger cohorts (35.93 $\pm$ 4.90 vs 35.52 $\pm$ 4.85). The most common indication for ART was male factor infertility in both groups (21.36% in group B vs 27.11% in group A). Stimulation was shorter in the dual trigger cohort (10.41 vs 12.08 $p = 0.04$). The total number of cumulus oocyte complexes (8.26 $\pm$ 5.32 vs 7.45 $\pm$ 4.68, $p < 0.001$), mature oocytes (6.39 $\pm$ 4.15 vs 5.99 $\pm$ 3.80, $p = 0.006$) and fertilized oocytes (4.79 $\pm$ 3.59 vs 4.33 $\pm$ 3.27, $p = <0.001$) was higher in the dual trigger group compared to those in the mono trigger group. Day 5 embryo transfer was more prevalent in the dual trigger group (46.07% vs 33,84%, $p < 0.001$). Embryo utilization rate was higher in group A (61.37% $\pm$ 31.98) than in group B (53.47% $\pm$ 30.91 $p = <0.001$). Ongoing regnancy rate was similar in both groups (27.29% in group A vs 27.08% in group B). Pearson chi2, Mann Whitney and logistic regression tests were used for the analysis.

Limitations, reasons for caution: In spite of the large sample, this study is retrospective and holds the possibility of unmeasured confounders.

Wider implications of the findings: Although a dual trigger may increase the number of mature oocytes, this does not translate into a higher pregnancy nor live birth rate. Reduced oocyte competence of the "surplus" oocytes may underlie this observation. Further research should identify specific subgroups that may benefit from dual trigger across meaningful outcome parameters.

Trial registration number: not applicable

Abstract citation ID: dead093.359

O-295 Dual trigger does not improve reproductive outcomes in advanced maternal age women

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Study question: Could dual trigger increase oocyte yield and maturation rate in women of advanced maternal age (≥ 40) undergoing IVF?

Summary answer: Dual trigger does not result in higher oocyte yield or maturation rates compared to hCG monotrigger

What is known already: Advanced maternal age is related to poor ovarian response (POR) and remains a major therapeutic challenge in routine IVF practice, because of the association with low live birth rates and high cancellation rates. hCG is used at the end of controlled ovarian hyperstimulation as a surrogate LH surge to induce final oocyte maturation. Recently, the co-administration of GnRH agonist and hCG for final oocyte maturation (dual trigger) has been suggested to improve IVF outcome (by improving oocyte quantity and quality) in normal responders, while evidence in poor responders remains controversial.

Study design, size, duration: This is a retrospective cohort study including patients attending a private IVF clinic from 1st January 2018 until 1st June 2022.

Participants/materials, setting, methods: All women who underwent IVF/ICSI in antagonist protocol in our center were included. Patients may have undergone triggering of the final oocyte maturation either with 250mcg of rhCG or 0.2 mg of GnRH agonist. Mature oocytes were inseminated using ICSI.

Main results and the role of chance: In total, 2242 patients were included, 454 (20.2%) in the rhCG group and 1788 (79.8%) in the dual trigger group. There was no significant difference in female age [41.3 (1.12) vs 41.3 (1.19), p value 0.94]. Total stimulation units and duration were also comparable between groups. The number of oocytes and MII oocytes did not differ significantly between rhCG and dual trigger group [5.8 (4.1) vs 6.3 (4.7) and 4.7 (3.3) vs 4.9 (3.4), p = 0.15 and 0.49, respectively]. Maturation rates were similar 81.7% (22.4) and 79.8% (23.5), p = 0.15, as well as fertilization rates (defined as the number of oocytes fertilized divided by the total number of cumulus-oocyte complexes recruited) [57% (29.7) vs 58.8% (29.4), p value=0.2]. Embryo utilization rates (defined as the total number of embryos transferred and cryopreserved divided by the number of oocytes fertilized) were comparable between the two arms: 69.7% (34.2) vs 70% (33.9), p value =0.22.

Multivariate Poisson regression analysis adjusting for relevant confounders (AMH, total stimulation units) showed that the type of triggering strategy (dual trigger vs rhCG) was not associated with either the number of MII oocytes (coefficient 0.2, p value=0.24) or maturation rates (coefficient -1.8, p value 0.12).

Limitations, reasons for caution: The main limitation is the retrospective design of our study, with an inherent risk of bias.

Wider implications of the findings: To the best of our knowledge, this is the largest study evaluating dual trigger strategy in advanced maternal age women. Our data demonstrate that dual trigger cannot improve the outcome of low prognosis women and should not be used as a panacea for all IVF patients.

Trial registration number: NA

Abstract citation ID: dead093.360

O-296 Progesterone level is an alternative marker to detect ovulation time in natural cycle frozen-thawed embryo transfers

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Study question: Do serum progesterone or LH levels better predict the optimal timing for embryo transfer in true natural cycle frozen-thawed embryo transfers (NC-FET)?

Summary answer: Performing embryo transfer according to serum progesterone levels (PL) had similar ongoing pregnancy rates compared to embryo transfer according to serum LH levels.

What is known already: With efficient and safe embryo vitrification techniques, there is an increasing trend in frozen-thawed embryo cycles (FET) over fresh cycles. FET protocols are programmed cycle, NC-FET and modified NC-FET. NC-FET is increasing in popularity, since it is associated with favourable obstetric and perinatal outcomes. In NC-FETs, ovulation time is the critical parameter for synchronization of embryo and endometrium. In the literature, LH surge is the most commonly used test to define ovulation time.

Study design, size, duration: This prospective cohort study included 183 NC-FET cycles in an IVF clinic between March 2022 and November 2022.

Main outcome measure is ongoing pregnancy.

Participants/materials, setting, methods: 183 subfertile women aged between 18-40, having regular menstruation with a cycle length between 24-38 days are included. Protocol for NC-FET included serial hormone measurements and ultrasound monitoring when the dominant follicle reached the diameter of 15-16 mm. Serum LH level ≥15 IU/l defined as 'ovulation -1' in Group 1 and blastocyst embryo transfer was performed after 6 days. Serum PL > 1 ug/ml was defined as the 'ovulation day' in Group 2.

Main results and the role of chance: There were no significant differences in baseline characteristics including female age, female body mass index, infertility duration, infertility diagnosis and number of failed IVF cycles between two groups. Also, cycle characteristics regarding endometrial thickness, number of embryos transferred, embryo quality and PLs at transfer day were similar. The overall clinical pregnancy was 59.6% and ongoing pregnancy was 50.8%. The implantation rate was 61.7%. The clinical pregnancy and ongoing pregnancy of Group 1 and Group 2 were 49.5%, 50.5% and 47.3%, 52.7%, respectively (p > 0.05). PLs in order to detect ovulation time were calculated according to a mathematical modelling (PL(Ov)=a1 eb1 Ov)(Ov:ovulation day; a1,b1:regression coefficients; e:euler number≅2.718) described in our previous study. Embryo transfer timing was planned after 5,4 or 3 days based on PLs calculated by modelling.

Limitations, reasons for caution: Since this is the first study planning embryo transfer in NC-FET based on progesterone levels, sample size should be increased and also serial change of progesterone levels were unknown in women >40 years of age.

Wider implications of the findings: Progesterone has a particular increasing pattern that enables ovulation day prediction accurately and may be used instead of serum LH levels in timing of embryo transfer.

Trial registration number: NCT05690360

SELECTED ORAL COMMUNICATIONS

SESSION 91: ART OUTCOME AND MALE CONTRIBUTION

Wednesday 28 June 2023 Hall D1 14:00 - 15:15

Abstract citation ID: dead093.361

O-297 Contribution of Frozen Embryo Transfer Cycles to ICSI Clinical Outcomes in Male Factor Infertility

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Study question: What is the benefit of adding frozen embryo transfer (FET) cycles to ICSI clinical outcomes in male factor infertility?

Summary answer: FET carried out on spare embryos that reached blastocyst stage remarkably contributed to additional clinical pregnancies in ICSI cycles for male factor infertility.

What is known already: Current trends in reproductive medicine lean toward full-preimplantation development and eventually to PGT-A to select a single euploid embryo for transfer. The utilization of this approach, while beneficial in most couples, is not ideal for male factor infertility due to the tendency of being characterized by impaired embryo development. So, we wonder if the utilization in FET cycles of leftover embryos that reached to blastocyst stage or those that eventually reached day 5 for aneuploidy testing contributed to the clinical outcomes.

Study design, size, duration: In the past 7 years, we included 22,289 couples who underwent ICSI while the large majority (84.8%) received a fresh embryo transfer, of which 70.9% were transferred at day 3. Leftover embryos, together with those euploid after PGT-A at blastocyst stage, were replaced in subsequent FET cycles. The clinical outcomes including clinical pregnancy rate (CPR) and deliveries were compared between the fresh embryo transfer and those after FET in total and after PGT-A.

Participants/materials, setting, methods: Couples with male factor underwent ICSI in standard fashion using exclusively ejaculated sample. For fresh embryo transfer cycles, embryos were transferred either at day 3 or at day 5. For FET cycles, embryos were cultured up to exclusively blastocyst stage and cryopreserved by vitrification. For aneuploidy, NGS was carried out for PGT-A. FET was carried out in natural or programmed cycles.

Main results and the role of chance: In the cohort underwent fresh embryo transfer, 18,896 couples underwent 37,751 ICSI cycles, where 322,916 oocytes were injected and 243,768 (75.5%) fertilized. Additionally, 3,393 patients received 4,712 FET in total with a fertilization of 69.8% (46,163/66,171) that did not differ from the fresh transfer group. The number of average embryos transferred in fresh cycles was 2.4 ± 2 while FET was carried out exclusively on single embryo. Fresh transfer yielded 37.3% (14,087/37,751) CPR, while overall FET cycles achieved a higher CPR at 52.2% (2,462/4,712, $P<0.0001$). Similarly, the delivery rate in fresh cycles was 31.8% (12004/37751) and became 45.5% (2145/4712) in the FET ($P<0.0001$).

To identify the advantage of selecting a single euploidy embryo, we compared FET on leftover unscreened blastocyst to those that were planned for PGT-A. Spare embryo transfers involved 1774 patients in 2282 cycles with a fertilization rate of 67.7% (18,905/27,937). For the PGT-A cycles, 1619 couples in 2430 cycles achieved a comparable fertilization rate of 71.3% (27,258/38,234), In this comparison, the FET on spare unscreened embryos achieved a CPR at 47.0% (1,072/2,282) while in the PGT-A group reached CPR at 57.2% (1390/2430, $P<0.0001$). Similarly, the delivery rate was 37.6% (857/2,282) in the FET and PGT-A was 53.0% (1,288/2,430) ($P<0.0001$).

Limitations, reasons for caution: The comparison is retrospective and is carried out on male factor infertility where day 3 embryo transfer were performed almost exclusively on fresh cycle to overcome poor embryo development. Nonetheless, those spare embryos that reached blastocyst stage and those that electively underwent aneuploidy testing significantly contributed to enhance clinical outcomes.

Wider implications of the findings: ICSI can overcome most of male infertility; however, the risk of impaired embryo development proposes a transfer at cleavage stage. The advanced embryo culture condition together with time-lapse allowed us to monitor embryos up to the blastocyst stage that once transferred, improving clinical outcomes, especially for embryos with confirmed euploidy.

Trial registration number: N/A

Abstract citation ID: dead093.362

O-298 Poor semen parameters alter embryo morphokinetic patterns during preimplantation development but do not compromise clinical outcomes

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Study question: Does semen quality affect preimplantation development and clinical outcomes in ICSI cycles using donor oocytes?

Summary answer: Poor semen parameters led to altered morphokinetic parameters during preimplantation development but did not compromise blastocyst quality nor clinical outcomes in oocyte donation cycles.

What is known already: The quality of semen used for in vitro fertilization (IVF) may compromise the kinetics and morphology of developing embryos, ultimately impairing implantation and reducing live birth rates. Such effects are particularly pronounced when testicular or epididymal spermatozoa are used for IVF treatment. However, current studies assessing the impact of poor semen parameters on embryo development and clinical outcomes are often confounded by the use of oocytes from infertile patients. Performing such evaluations in oocyte donation cycles will provide more definitive insights into the role of semen during preimplantation development.

Study design, size, duration: This retrospective study included 616 embryos from 99 oocyte donation ICSI cycles, performed between February 2018 and July 2019. We compared embryo morphokinetics and clinical outcomes (cumulative pregnancy and live birth rates) across three groups with variable semen parameters. These included donor semen (control group, DD, $n=294$), partner sperm obtained by testicular sperm extraction (TESE group, $n=72$) and partner semen with altered parameters (including poor concentration, motility and morphology, ALT group, $n=250$).

Participants/materials, setting, methods: We assessed several morphokinetic parameters, including second polar body extrusion (tPB2), pronuclei appearance (PN1a, PN2a), 2-3-4-5-8-cell stages (t2 to t8), start of blastulation (tSB) and blastocyst formation (tB). Kaplan-Meier survival curves were built for each parameter per study group. Survival curves were analysed by Cox regression. Qualitative parameters (even size PN and blastomeres, percent fragmentation, abnormal cleavages, blastocyst grade) were assessed using a Chi-Squared test, while Fisher's exact test was used to evaluate clinical outcomes.

Main results and the role of chance: Interestingly, TESE embryos reached several developmental stages faster than the DD group: tPB2 (3.03 vs. 3.08, $p<0.001$), PN1a (5.55 vs. 6.19, $p<0.001$), PN2a (6.78 vs. 7.36, $p<0.001$), tPNf (21.30 vs. 22.20, $p=0.001$), t3 (34.02 vs. 35.79, $p=0.016$), t4 (35.90 vs. 36.92, $p=0.015$), t5 (46.70 vs. 48.07, $p=0.036$), t8 (52.48 vs. 55.28, $p=0.049$), tSB (85.53 vs. 94.62, $p<0.001$) and tB (104.09 vs. 106.60, $p=0.002$). ALT embryos were faster in early stages: PN1a (6.63 vs. 6.19, $p=0.005$), PN2a (7.84 vs. 7.36, $p<0.001$), t5 (48.12 vs. 48.07, $p=0.028$), t8 (55.57 vs. 55.28, $p=0.032$), but slower in the late stages: tSB (93.71 vs. 94.62, $p=0.001$), tB (105.60 vs. 106.60, $p=0.002$). Compared to DD, ALT and TESE embryos showed a lower rate of even pronuclei (ALT $p=4.6\times10^{-06}$; TESE $p=8\times10^{-05}$) and even blastomeres at the 2-cell (ALT $p<0.001$; TESE $p=0.006$) and 4-cell stage (ALT $p=0.004$; TESE $p=0.005$). Moreover, embryos derived from testicular sperm showed significantly higher fragmentation rates at the 8-cell stage ($p=0.005$), while no significant differences in the frequency of irregular divisions nor in the number of top-quality blastocysts were detected in ALT and TESE. Importantly, cumulative live birth rates per cycle were similar across all study groups (DD 31/53, ALT 31/51, TESE 8/11; $p>0.05$).

Limitations, reasons for caution: The main limitation resides in the retrospective nature of this study and the limited number of embryos in the TESE group. We also note variability in the testicular biopsy (obstructive and non-obstructive azoospermia) and non-normozoospermic diagnoses (oligozoospermia, teratozoospermia or asthenozoospermia), which may confound the analysis.

Wider implications of the findings: Although poor semen parameters significantly altered embryo morphokinetics, they did not compromise embryo quality, pregnancy or cumulative live birth rates, resulting in similar clinical outcomes as with double donation. Overall, our findings support the use of autologous sperm with donor oocytes, even in severe cases of male factor infertility.

Trial registration number: not applicable

Abstract citation ID: dead093.363
O-299 Sperm DNA fragmentation by TUNEL influences live birth in egg recipients after euploid embryo transfer

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Study question: Does sperm DNA fragmentation (SDF) by TUNEL affect reproductive success measured as live birth rate (LBR) in egg recipients after euploid embryo transfer?

Summary answer: SDF increases miscarriage rates in egg recipients with PGT-A. Therefore, live birth rates depends on sperm DNA damage in egg recipients after euploid embryo transfer.

What is known already: Sperm DNA integrity is important for optimal fertilisation, implantation and pregnancy. Although controversial, several studies have shown that sperm DNA fragmentation correlates with adverse pregnancy outcomes in infertile men. Ooplasmic sperm DNA fragmentation repair mechanisms may occur in female gametes, mainly in young women. However, research on the impact of DNA fragmentation on LBR are limited. Also, chromosomally normal embryos analysed by PGT-A offers opportunity to isolate female factor and study male factor. The aim of this study is to assess whether high level of semen DNA damage has an impact on reproductive outcomes in terms of live birth rate.

Study design, size, duration: This is a retrospective study of 467 PGT-A cycles in egg recipients. The study population consists of infertile couples for euploid embryo transfer (February 2017-November 2022). Semen samples were obtained to measure DNA damage from 447 men. PGT-A was performed in 1831 blastocysts. Trophectoderm biopsies (d5/d6 blastocysts) were analysed by NGS (Veriseq Illumina). Embryos were frozen and 568 blastocysts transferred in the subsequent cycle. Conventional hormone replacement was used for endometrial preparation in recipient patients.

Participants/materials, setting, methods: Cohort study with 447 infertile couples attending to private fertility clinic undergoing ART with their autologous semen, donated oocytes and PGT-A. SDF was measured by Terminal-deoxyribonucleotidyl-ARTSferase-mediated dUTP Nodule End Labelling (TUNEL) assay using the FITC-labelled-dUTP in situ cell death detection kit (Roche). The cohort was divided into two groups according the DNA fragmentation index (DFI): high SDF group (DFI>20%) and normal SDF group (DFI≤20%). To evaluate reproductive outcomes Chi-square and Linear regression tests were used.

Main results and the role of chance: Of the 447 couples included (average paternal age 41.29 + 7.32 years), 66.5% of male partners had normal semen parameters. We reported high sperm DNA fragmentation in 8% of patients. Overall, the embryo aneuploidy rate was 31.7%. For the further statistical analysis confounding factors such as, female and male age, embryo quality, day of embryo biopsy were included. PGT-A analysis showed an aneuploidy rate of 32.1% in the high DNA fragmentation group and 30.3% in the normal DNA fragmentation group ($p = 0.453$). Regarding clinical data, the overall implantation rate was 34.9%. There was no significant difference between elevate DNA fragmentation and normal DNA fragmentation with respect to biochemical miscarriage rate (4.3% vs 7.6%, $p = 0.799$). Although not significant differences between groups, a lower implantation rate near to reach significance was found in the high SDF group (20.0% vs 34.4%, $p = 0.050$). Pregnancy and clinical pregnancy rates were significantly lower in the high DNA fragmentation group (26.1% vs 44.9%, $p = 0.042$; 21.7% vs 31.3%, $p = 0.044$;), respectively. Also, miscarriage rate was significantly higher in the high SDF group (40% vs 12.5%, $p = 0.014$). Therefore, the live birth rate was significantly lower in the high SDF group (13.0% vs 32.6%, $p = 0.024$).

Limitations, reasons for caution: Larger studies including a higher number of samples are needed to confirm the correlation observed between LBR in eggs recipients with semen DNA fragmentation. Those patients with a DFI higher than control values should be treated with antioxidants. The pregnancy outcomes should be analyzed after treatment of DNA fragmentation.

Wider implications of the findings: Our findings suggest that autologous semen DNA fragmentation influence in live birth rate in egg recipients. Our data show that live birth rates decreased in patients with a high DFI after PGT-A. This study confirms published studies in the literature showing that ART outcomes are affected by sperm DNA fragmentation.

Trial registration number: Not Applicable

Abstract citation ID: dead093.364
O-300 Human sperm selection using cumulus oophorus complexes compared with conventional sperm preparation method on sperm quality and ICSI outcomes: A pilot study

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Study question: We compared a sperm selection protocol using cumulus oophorus complexes with a swim up preparation, evaluating the effects on sperm parameters and ICSI outcomes

Summary answer: Spermatozoa selected using cumulus oophorus complexes showed higher rates of normal morphology, good motility compared with basal semen and the traditional pellet swim up protocol.

What is known already: It is known that the head of a mature spermatozoa has a hyaluronan receptor that allows them to bind with hyaluronan acid. Cumulus oophorus complexes (COCs) extracellular matrix is made mainly by hyaluronic acid (HA). Various studies have confirmed the importance of COCs in fertilization, demonstrating that spermatozoa that are able penetrate through a layer of COCs are more likely to have a normal morphology and be more effective in producing acrosome reactions than spermatozoa that are not able to do. Moreover, these spermatozoa have been shown to have higher chromatin integrity and zona-binding capacity, resulting in increased fertilization competence.

Study design, size, duration: A prospective study was conducted between July and December 2022. A total of 50 participants were recruited for the study, randomly selected between patients attending ICSI. The patients were classified as group A when the sperms were selected using pellet swim up and COC selection and as group B when it was used pellet swim up treatment alone. Patients older than 39 years, with endometriosis or repeated IVF failure or severe male factor were excluded.

Participants/materials, setting, methods: 3 drops of 30 mlt aligned and connected each other were prepared, covered with mineral oil. In group A, in the middle drop, was transferred COC fragments, while the central drop was empty in group B. In the left drop were added 20 mlt of sperm suspension after swim up, and from the right drops were recovered the sperms after passing the middle drop with or without COC, that were used for ICSI treatment.

Main results and the role of chance: The aim of the study was to verify the effectiveness of COCs, collected after ovarian stimulation, in selecting the best sperms to be used in ICSI procedure. No statistical difference was found between group A and B in the median age of female patients, FSH/LH units administred, E_2 and endometrium thickness at triggering using hCG, in the sperm concentration, motility and normal morphology in the baseline seminal

samples. We found a statistical difference in the traditional seminal parameters comparing the two different treatments. In particular, we found an higher rates of sperms with rapid and progressive motility and normal morphology in group A between basal semen sample and after sperm selection using COC filter (86.7 vs 31.3%; 8.2 vs 4.4 %). We found a lower sperm concentration in group A vs group B after sperm treatment (2.3 vs 4.7 M/ml), and an higher rates of rapid progressive motility and normal morphology in group A vs group B (86.7 vs 38.6 %; 8.2 vs 4.6%). We found an higher but not statistically significative pregnancy and implantation rates in group A compared with group B probably because of the limited number of patients analyzed (25 vs 19.2 %; 25 vs 20.6%)

Limitations, reasons for caution: The study was conducted on a small participant cohort. We analyzed the traditional sperm parameters, but it is necessary to evaluate the chromatine integrity of the sperms after COC selection to confirm that the higher sperm competence in fertilization and embryo development could be based on a better genetic pattern

Wider implications of the findings: Data seem to demonstrate that the use of COC allows to select higher competent sperms if compared with traditional pellet swim up treatment. The clinical and biological ICSI outcomes seem to be improved by COC treatment but it is necessary to increase the sample size in future experimental trial

Trial registration number: not applicable

Abstract citation ID: dead093.365

O-301 Can advanced sperm selection techniques improve blastocyst euploidy rate of abnormal sperm DNA fragmentation cases to the level of normal ones; prospective randomized controlled trial

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Study question: To compare PGT-A outcomes of Physiological ICSI (PICSI) and magnetic activated cell sorting (MACS) sperm selection techniques to a control of normal sperm DNA fragmentation.

Summary answer: PGT-A outcomes did not differ between using normal sperm DNA fragmentation semen to the semen processed by PICSI or MACS.

What is known already: Sperm DNA fragmentation can negatively affects pre-implantation embryo development in all stages after ICSI. Advanced sperm selection techniques like PICSI and MACS are thought to be efficient to improve fertilization, cleavage, and blastocyst formation rates in cases with abnormal SDF. It has become evident that a blastocyst's aneuploidy is not only contributed by the oocyte,but can also be caused by the sperm.The relation between sperm selection techniques such as PICSI or MACS and blastocyst euploidy is not investigated yet.

Study design, size, duration: A prospective randomized controlled trial of 351 ICSI cycles in a private fertility center, from October 2019 to December 2022. Cases were included in the study if: female had ≥5 MII oocytes, male had abnormal sperm DNA fragmentation (SDF) index (>20% by TUNEL assay), all blastocysts (≥4BB) had trophoectoderm biopsy.

Participants/materials, setting, methods: Included cases were randomized on the day of ICSI to PICSI or MACS group, along with a control arm of cases with normal SDF needed for comparison. PGT-A was done using Illumina platform (Miseq), and the results were reviewed by artificial intelligence (Cooper Surgical). Cases were categorized according to the female age into 2 subgroups(<35, and > 35 years). Statistical analysis of all pre-implantation embryo development parameters and PGT-A results were done using SPSS.

Main results and the role of chance: We found no significant differences between the female age, male age, count, motility, SDF, no. mature oocytes between the studied groups.

Limitations, reasons for caution: The results included all pre-implantation embryo parameters but, subsequent post-implantation events like implantation, pregnancy and miscarriage rates will add more data about the competency of the resulted embryos.

Wider implications of the findings: The usage of advanced sperm selection techniques like PICSI or MACS has showed comparable results to that of normal SDF cases, through eliminating the adverse effects of SDF on ICSI and PGT-A outcomes.

Trial registration number: NCT05494216

O-301 Table I

Study results

	Control		Total S.S (PICSI + MACS)		PICSI		MACS	
Female age group (years)	**<35**	**>35**	**<35**	**>35**	**<35**	**>35**	**<35**	**>35**
No. of cases	114	66	87	82	46	40	41	42
Fertilization rate (%)	72.07	76.4	74.4	77.7	69.9	79.6	78.03	75.9
High quality Day3 Cleavage rate (%)	62.9	61.1	64.5	51.3	64.9	53.9	60.6	49
Blastocyst development rate (%)	63.3	60.1	70	57.2	70.6	53.2	69.8	60
Top quality blast. rate (%)	24.2	15.9	30	17.4	26.4	18	28.8	16.8
Good quality blast. rate (%)	18.08	18.18	20.9	25.5	17.6	29.2	22.5	21.9
Fair quality blast. rate (%)	11.5	11.7	21.9*	21.4	23.4	14.5	21.1	28*
Euploidy rate (%)	60.1	35.2	51.6	31.5	51.7	30.1	51.5	32.9
Aneuploidy rate (%)	23.9	54.4	29.3	56.9	30.2	61	28.4	53.1
Low mosaic rate (%)	4.5	2.09	4.1	4.2	2.8	4.7	5.5	3.9
High mosaic rate (%)	7.6	5.23	11.6	7.3	12.9	7.05	10.1	7.5

Data are mean. n = no. of cases included in the analysis. * is the significant difference ($p < 0.05$).

SELECTED ORAL COMMUNICATIONS

SESSION 92: IMPAIRED FEMALE FERTILITY: GENETIC CAUSES AND CONSEQUENCES

Wednesday 28 June 2023 Hall D4 14:00 - 15:15

O-302 Triploid conceptions are predominantly caused by female meiosis II errors and their risk increases with advancing maternal age

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Study question: What are the incidence and origin of ploidy abnormalities in embryos derived from 2 pronuclei (2PN) zygotes, and is their risk linked to maternal age?

Summary answer: While embryo haploidy is usually due to male meiosis errors, triploidy is mainly caused by female meiosis II errors, whose incidence increases with maternal age.

What is known already: Normal fertilization is denoted by the appearance of 2PN 16-18 hours after insemination. Deviation from 2PN is considered evidence of abnormal fertilization and ploidy anomalies. Nonetheless, even 2PN embryos can be diagnosed with ploidy abnormalities during preimplantation genetic testing (PGT). The incidence of 3PN has already been linked to advanced maternal age. However, no conclusive and well-powered studies have yet investigated the incidence, origin, and maternal age correlation of genetically-diagnosed ploidy abnormalities. A targeted NGS-based approach that simultaneously analyzes copy number and genotyping data provides a comprehensive ploidy status assessment and helps address numerous basic and clinical research questions.

Study design, size, duration: Retrospective study including 96,660 trophectoderm biopsies analyzed between 2020 and 2022 using a targetedNGS-based PGT platform. A total of 1,063 embryos carrying haploidy or triploidy were used to investigate maternal age correlation, and parental/meiotic origin of the anomaly. Of these, 57 embryos were from concomitant PGT-M (PGT for monogenic disorders) cycles where also parental DNA was available. These trios (embryo-parents) were used to independently estimate parental/meiotic contribution to the ploidy abnormality and genome-wide recombination events.

Participants/materials, setting, methods: Targeted-NGS's accuracy in genotyping and copy number (CN) detection were previously validated on triploid cell lines and multifocal blastocyst biopsies with known ploidy status derived from abnormally fertilized oocytes. Parental and meiotic origins were estimated using two independent approaches. First, they were inferred using sex chromosomes CN from 1,063 embryos with altered ploidy status. Secondly, they were directly calculated using genotyping data from 57 trios (embryo and parents) from PGT-M cycles.

Main results and the role of chance: The prevalence of ploidy abnormalities in 2PN-derived embryos was 1.1% (n = 1,063/96,660), with triploids accounting for 83.0% (n = 882/1,063) and haploids for 17.0% (n = 181/1,063). Remarkably, the incidence of ploidy anomalies is positively correlated with maternal age (OR = 1.046 per year; $p < 0.001$). Triploidy showed strong correlation with age (OR = 1.059 per year; $p < 0.001$), while haploidy did not (OR = 0.96 per year; $p = 0.1$). Based on sex chromosomes CN analysis, the extra haploid set of triploid embryos was almost completely of maternal origin (94.6%; 95%CI:93.0-96.1), with male errors accounting for only 5.4% (95%CI:4.0-7.1). On the other hand, haploid embryos were the result of paternal errors in 97.8% of cases (95%CI:94.4-99.4), with the missing haploid set being of maternal origin in the remaining 2.2% (95%CI:0.6-5.6). In terms of triploidy's meiotic origin, two-thirds of the errors occurred during MII (95%CI:63.4-69.8), while one-third occurred during MI (95%CI:30.2-36.5). An independent method using genotyping data of 57 trios confirmed the predominance of paternal error in haploidy (n = 12/14) and the exclusively maternal origin of all embryonic triploidies (n = 43/43). The extra haploid set resulted from an error during MI in 27.9% (n = 12/43) and during MII in 72.1% (n = 31/43) of cases. Interestingly, 16.3% of triploids (n = 7/43) showed no genome-wide recombination events.

Limitations, reasons for caution: Despite the uniquely large sample size, the inference based on sex chromosomes CN suffers the limitations of the modeling assumptions (independence between parental and meiotic errors), which require further validation. Due to the low prevalence of paternal errors, clinical correlation with male factor could not be investigated with sufficient power.

Wider implications of the findings: Thanks to the exceedingly high sample size, this is the first study to reveal an increased risk of triploid conception with advancing female age (76% higher at age 40 than at age 30), providing meaningful insights for patients counseling. Importantly, the meiotic origin of ploidy anomalies in embryos were unveiled.

Trial registration number: not applicable

Abstract citation ID: dead093.367

O-303 Contribution of targeted sequencing for genetic diagnosis of premature ovarian insufficiency in clinical practice

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Study question: What is the diagnostic yield of custom designed gene panel for patients with premature ovarian insufficiency (POI)?

Summary answer: The diagnostic yield of our POI gene panel (POIGP) is 7.3%

What is known already: POI is a specific female syndrome with a high clinical and genetic heterogeneity. It is characterized by a premature exhaustion of the ovarian function and infertility and affects approximately 1% of women. POI can be related to genetic factors which include chromosomal abnormalities, FMR1 premutation and rare variants in numerous genes. The advent of high throughput sequencing methods has led to the identification of an increasing number of variants implicated in the development of POI over the last decades. However, POI etiologies still remain undetermined in the majority of cases.

Study design, size, duration: An observational analytic cohort study of 150 patients presenting idiopathic POI (normal karyotype, absence of FMR1 premutation, absence of adrenal and/or ovarian antibodies) recruited prospectively at three Belgian academic and university hospitals between 2016 and 2021.

Participants/materials, setting, methods: Patients were included if they experienced POI, as defined by ESHRE guidelines on POI (2016). POI genes included in the panel were selected from PubMed using different key words mainly premature ovarian insufficiency, gonadal dysgenesis, hypergonadotropic hypogonadism, ovarian failure and genetics. The panel included 156 genes, variants were filtered based on allele frequency (≤1%) in latest available population databases and classified according to ACMG/AMP (American College of Medical Genetics/Association for Molecular Pathology) guidelines 2015.

Main results and the role of chance: Our analysis revealed a potential causative variant for 11 patients in the following genes: MEIOB, BMP4, CFTR, FANCA, FSHR, FANCG, MLH1, MRPS22 and STARD9. This means that the diagnostic yield of our POI gene panel (POIGP) is 7.3%.

Patients were mainly Caucasian (63%), North African (17%) and sub-Saharan African (13%). They presented primary amenorrhea in 14.7% of cases. Consanguinity and/or a family history of POI or early menopause in 28% of cases. Mean patient's age (years) at POI diagnosis was 28.9 ± 8.5 (mean ± SD). The overall mean coverage was 229X, and more than 95% of the target exome was represented with more than 30-fold coverage.

Limitations, reasons for caution: The present study was limited to monogenic etiologies of POI, potential oligogenic causes have not been searched. Functional studies and/or family segregation were not performed for the identified variants.

Wider implications of the findings: Our findings show the importance of targeted next generation sequencing in clinical practice and highlight the limit of our current genetic knowledge in the field of POI. A regular update of genes included in POIGP will improve its diagnostic yield.

Trial registration number: P2016/196/CCB B406201628264

Abstract citation ID: dead093.368

O-304 Whole-exome sequencing in women with reproductive failure is a potentially useful diagnostic test in clinical practice

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Study question: Could whole-exome sequencing (WES) be useful in clinical practice for primary infertile female involving oocyte or embryo defects and unexplained recurrent miscarriage (RM) patients?

Summary answer: Identifying key genes involved in oocyte and embryo development helps explain approximately 17% cases of reproductive failure. WES results will help optimize clinical therapeutic treatment.

What is known already: Dozens of genes have been reported to be the genetic causes of human infertility. Variants in these genes have specific effects on certain processes of human early reproduction and result in a spectrum of phenotypes, such as oocyte maturation arrest, fertilization defects, cleavage failure, early embryonic lethality, and recurrent miscarriage.

Study design, size, duration: This retrospective cohort study was conducted from July 2020 to August 2022. A total of 129 affected females were enrolled. Most of them were primary infertile women who suffered repeated failure of *in vitro* fertilization (≥2 cycles) due to abnormal development of oocytes or embryos (PI group, n = 125). Our cohort also comprised a small number of unexplained RM subjects without offspring (RM group, n = 4). All patients and their spouses had normal karyotype.

Participants/materials, setting, methods: The peripheral blood samples from all the participants and all of their available family members were obtained for DNA extraction. Genomic DNA samples from the probands were subjected to WES to identify candidate variants. Subsequently, the variants were validated by Sanger sequencing or qPCR. Familial co-segregation analyses were then carried out within the family members. The relationship between phenotype and genotype by clinical tests and the clinical records of the patients was studied.

Main results and the role of chance: Firstly, variants that are potentially relevant to the phenotypes of female infertility were identified in 17.05% of cases (n = 22), including *TUBB8* in 14 cases, *PATL2* in 2 cases, *ZP2*, *ZP3*, *PANX1*, *TLE6*, *NLRP2* and *NLRP7* in 1 case for each. A breakdown by phenotype revealed that we identified variants in 16.00% (n = 20) of PI group and 50% (n = 2) of RM group. Secondly, 16 *TUBB8* variants were identified in 14 cases, of which 8 were novel, 3 were previously reported, and 5 were novel amino acid change occurring at the same position as another previously reported change. We found 3 *TUBB8* variants (p.C211R, p.T232I, p.A342T) in 3 embryonic arrest families and 1 *TUBB8* mutation (p.A102V) in a RM family, which were maternally inherited. Thirdly, 75 patients in PI group without phenotypic variant underwent ≥1 cycles of ovulation and/or frozen embryo transfer after WES, and 49.33% had achieved clinical pregnancy (n = 37).

Limitations, reasons for caution: The genomic DNA of parents were not available in all these 22 families, so we cannot determine whether the variant is *de novo* or inherited in some cases. Additionally, further functional studies should be performed to prove that these novel mutations affect protein function.

Wider implications of the findings: The present study expands the kinds of variants and phenotypic spectrum of gene mutations with regard to female reproductive failure. Genetic tests for causative genes involved in oocyte and embryo defects were recommended for primary infertile patients who suffered from these conditions and for unexplained RM patients.

Trial registration number: not applicable

Abstract citation ID: dead093.369

O-305 Next-generation sequencing of a cohort of 100 patients with diminished ovarian reserve reveals an etiology in 27% of cases and may predict the fertility prognosis

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Study question: Identify the genetic cause of a cohort of patients with unexplained Diminished ovarian reserve (DOR) by next-generation sequencing and compare with pregnancy outcome.

Summary answer: A high-yield positive genetic diagnosis was obtained: 27% of cases. Defects of genes involved in DNA repair/meiosis appeared to have an unfavorable prognosis.

What is known already: Ten percent of women undergoing Medically Assisted Procreation-MAP have a DOR defined by an AMH level <1.2 and an antral follicle count (AFC) <5. However, most causes of DOR are unknown. There is no known criteria of success in MAP. Primary ovarian insufficiency (POI) corresponds to a complete cessation of ovarian function in 1-4% of women under 40 years. We have very recently shown in a large cohort of POI that a custom-made target next-generation sequencing (NGS) POI panel allowed a genetic diagnosis in 30% of unexplained POI and leads to personalized medicine (Heddar et al., EBioMedicine. 2022 doi: 10.1016/j.ebiom.2022.104246).

Study design, size, duration: Prospective genetic study of a cohort of 100 patients with undergoing MAP using a custom-made NGS-POI panel comprising 88 validated POI-causing genes. These patients were for 77% European, 20% North African and 3% Asian. The classification of the variants detected were based on the American College of Medical Genetic criteria 2015. Only

pathogenic and likely pathogenic variants were used for diagnosis. Candidate gene studies were performed in negative patients.

Participants/materials, setting, methods: One hundred patients aged 16-35 with DOR, normal karyotype and FMR1 gene, were studied with the NGS POI panel. The segregation of the variants in the available families was performed by Sanger sequencing. Cytogenetic studies of chromosomal fragility was performed if necessary. The outcome of the pregnancy (spontaneous or induced) was recorded. A correlation between the result of the genetic study and the outcome of the pregnancy has been performed.

Main results and the role of chance: One family included two sisters, one with POI and the other with DOR, highlighting the proximity of the two syndromes. A high positive genetic yield was found: 27% of cases. Genes were involved 1) in follicular growth and gonadal development (*SOX8, AR, NOBOX, BMPR1A, BMPR1B, GNAS*) (35%) 2) DNA repair (*BNC1, ERCC6, BRCA1, ATM*) (31%) 3) metabolism and mitochondrial functions (*POLG, STAR*) (12%), 4) cellular aging (24%), in particular the *LMNA* gene.

In a patient with isolated DOR, we identified for the first time, bi-allelic truncating variants of *BRCA1*, a major breast/ovarian cancer susceptibility gene. Cytogenetic studies revealed increased chromosomal breaks with radial figures typical of Fanconi Anemia (FA). However there was no sign of FA or cancer in the patient and family. This observation is reminiscent of the family with isolated POI and biallelic mutations of *BRCA2* (Heddar et al, J Med Genet 2021; DOI: 10.1136/jmedgenet-2019-106672).

Among the 27 patients with an established genetic diagnosis, six were able to obtain a pregnancy. However, no pregnancies were achieved in the DNA repair gene suggesting an unfavorable prognosis for this gene family. On the other hand, patients with mutations of other gene families seem to have a better prognosis of fertility.

Limitations, reasons for caution: The size of the cohort should be implemented to confirm these results. The issues of pregnancy are not available for the whole cohort.

Wider implications of the findings: This is the first genetic study of a cohort of DOR and the first implication of *BRCA1* in isolated DOR. It shows i) the need for genetic studies of DOR ii) the genetic link between POI and DOR iii) NGS study could give information on the fertility prognosis of DOR.

Trial registration number: not applicable

Abstract citation ID: dead093.370
O-306 Genome-wide association meta-analysis supports the association between MUC1 and ectopic pregnancy

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Study question: Can we identify genetic variants associated with ectopic pregnancy by undertaking the first genome-wide association study leveraging two large-scale biobank initiatives?

Summary answer: We identified two novel genome-wide significant associations with ectopic pregnancy, highlighting MUC1 as the most plausible affected gene.

What is known already: Ectopic pregnancy is an important cause of maternal morbidity and mortality worldwide. Despite being a common early pregnancy complication, the genetic predisposition to this condition remains understudied and no large scale genetic studies have been performed so far.

Study design, size, duration: A GWAS meta-analysis including 7,070 women with ectopic pregnancy and 248,810 controls from Estonian Biobank and the FinnGen study. Several post-GWAS analysis were conducted to characterise the genetic signals, as well as to analyse the genetic and phenotypic relationships with the condition.

Participants/materials, setting, methods: We identified ectopic pregnancy cases from national registers by ICD codes (ICD-10 O00), and all remaining women were considered controls.

Main results and the role of chance: We identified two genome-wide significant loci on chromosomes 1 (rs4971091, p = 5.32x10-9) and 10 (rs11598956, p = 2.41x10-8). Follow-up analyses propose MUC1, an epithelial glycoprotein with an important role in barrier function, as the most likely candidate for the association on chromosome 1. We also characterise the phenotypic and genetic correlations with other phenotypes, identifying a genetic correlation with smoking and diseases of the (genito)urinary and gastrointestinal system, and phenotypic correlations with various reproductive health diagnoses, reflecting the previously known epidemiological associations.

Limitations, reasons for caution: The main limitation is that the findings apply to European-based ancestry populations and we only captured maternal genomes.

Wider implications of the findings: This study encourages the use of large scale genetic datasets to unravel genetic factors linked to ectopic pregnancy, which is difficult to study in experimental settings. Increased sample size might bring additional genetic factors associating with ectopic pregnancy and inform its heritability.

Trial registration number: not applicable

POSTER VIEWING
ANDROLOGY

Abstract citation ID: dead093.371
P-001 ClpX is required in maintaining mitochondrial functions during meiosis and spermatogenesis

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Study question: What is the role of ClpX in spermatogonia differentiation and spermatogenesis?

Summary answer: ClpX is required to maintain normal mitochondrial functions in spermatocytes, deficiency of ClpX resulted in meiosisarrest and diruption of spermatogenesis in mice.

What is known already: ClpX is mitochondria-specific quality control protease, it maintains proteostasis via degrading misfolded or damagedproteins in mitochondrial matrix. ClpX is a AAA+ protease that uses the energy of ATP binding and hydrolysis to degradeunfolded or misfolded proteins. ClpX is reported working as a complex with ClpP, the complex consists of a hexamer ofClpX and a tetradecamer of ClpP. ClpX recognizes the protein substrates via binding with their unstructured peptidesequences, termed as degradation tags or recognition signals. The fragments of the cleaved polypeptides can then exitthe chamber and be further degraded.

Study design, size, duration: Study design: we generated a germ cell specific ClpX knockout mice line and evaluated size, weight, inner structure oftestis tissue. We also applied biochemical techniques and high-throughput sequencing techniques to investigate themechanism of ClpX in regulating spermatogenesis.

Size: more than 50 mice were used in this study, including the control mice.

Duration: 2 years

Participants/materials, setting, methods: Participants/materials: we generated a ClpX conditional knockout (cKO) mice line by CRISPR-gene editing and Cre-LoxP system, which specifically knockout ClpX in the germ cells of male mice.

Setting: the siblings of ClpX WT mice and ClpX cKO mice were compared in various of experiments.

Mehods: we performed morphology comparement to check the weight and size of tetes in the control and ClpX cKOmice, and applied immunofluorescence, western blot, histological study, high-throughput sequencing andpharmocological treatment.

Main results and the role of chance: We found ClpX deficiency reduced mitochondrial functions and quantity in spermatocytes, affected energy supply duringmeiosis and attenuated zygotene-pachytene transformation of the male

germ cells. The affected spermatocytesexhibited disorder of chromosome synapsis and cross-over events. Their telomeres failed to attach to the nuclearenvelop, which was associated with failure of α-tubulin formation in the ClpX deficient spermatocytes. The dysregulatedspermatocytes finally underwent apoptosis resulting in decreased testicular size and vacuolar structures within theseminiferous tubules. Both transcriptome analysis and m6A-methylation sequencing analysis highlighted dysregulationof metabolic pathways including the mTOR signaling in the isolated spermatocytes. We confirmed over-activation of themTORC1 pathway with increased expression of phosphorylated S6 and 4EBP after deletion of ClpX in spermatocytes.Long-term inhibition of the mTORC1 signaling via rapamycin treatment in vivo partially rescue spermatogenesis in theseminiferous tubules with much less apoptotic germ cells and formation of late stage of meiotic germ cells, such asround spermatids and elongated spermatozoon. The data reveal the novel roles of ClpX in regulating meiosis andspermatogenesis.

Limitations, reasons for caution: This study is based on animal experiments, thus, these results only indicated the important roles of ClpX in micespermatogenesis rather than human beings. Clinical data has not yet found a Clpx gene mutation in reproductivedisease.

Wider implications of the findings: Clinical report has already found the mutation in ClpP can induce autosomal recessive Perrault syndrome, while themutation of ClpX has not been reported yet from clinical data. Our resutls demonstrated severe effect onspermatogenesis after knockingout of ClpX, this might give some insights to the clinic.

Trial registration number: not applicable

Abstract citation ID: dead093.372
P-002 SARS-CoV-2 tropism spectrum for human testicular cells

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Study question: In human testicular cells, how are SARS-CoV-2 receptors and their priming proteases for the viral spike (S) protein distributed spatially?

Summary answer: Human testicular tissue expressed both the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) receptors and proteases investigated, key factors for cellular susceptibility to SARS-CoV-2 entry/infection.

What is known already: Male reproductive function is altered on numerous levels by SARS-CoV-2 and coronavirus disease. Angiotensin-converting enzyme 2 (ACE2) is the main SARS-CoV-2 host membrane receptor but requires transmembrane protease serine 2 (TMPRSS2) for S protein priming (activation by proteolysis). Other proteases, like cathepsin L (CTSL), might nevertheless contribute to SARS-CoV-2 entrance. This mostly happens in cells with low or nonexistent ACE2 expression, which calls for alternative receptors like cluster of differentiation 147 (CD147). Although co-expression of receptors and proteases is essential for successful SARS-CoV-2 infection, it is still unknown how viral receptors and accompanying proteases are distributed geographically in testicular cells.

Study design, size, duration: To overcome this restriction, we focused on using immunohistochemistry to map the spatial distribution of the SARS-CoV-2 receptors ACE2 and CD147, as well as their priming proteases for the viral spike (S) protein, TMPRSS2 and CTSL, which are necessary for viral fusion with host cells, in human testicular tissue.

Participants/materials, setting, methods: Laboratory experiments were executed under the Joint Ethics Committee of the Hospital and University (approval number 2020-094 (077-DEFI-078-CE)). Human testicular tissues were obtained from cases presenting Sertoli cell syndrome, maturation arrest, and hypospermatogenesis at the Hospital Pathology department, with five patients per pathology. Formalin-fixed paraffin-embedded tissue sections 4 µm were incubated with anti-antibodies ACE2 (orb638860, 1:100 and 1:150, Byorbit), TMPRSS2 (ab109131, 1:1000, Abcam), CD147 (MA5-29060, 1:2500, Invitrogen) and CTSL (MA5-32602, 1:100, Invitrogen).

Main results and the role of chance: The present study did not intend to study the virus entry proteins in different testicular pathologies. As spermatogenesis is a complex process involving many intricately linked cells, we selected these syndromes specifically to identify individual cells more clearly, Sertoli cells in Sertoli cell-only syndrome cases, spermatocytes in maturation arrest cases and spermatids in hypospermatogenesis cases. The hypospermatogenesis group consisted of cases whose azoospermia was due to duct obstruction, showing conserved spermatogenesis. Interstitial cells, including endothelium, Leydig and myoid peritubular cells, and the seminiferous epithelium (Sertoli cells, spermatogonia, spermatocytes, and spermatids), were discovered to contain ACE2 and TMPRSS2, demonstrating co-expression of both receptor and protease in all testicular cells. All cell types, with the exception of endothelium and peritubular cells, showed the presence of CD147, whereas CTSL was only found in Leydig, peritubular, and Sertoli cells. As a result, only Leydig and Sertoli cells co-expressed CD147 and CTSL. The findings of proteomic databases indicating testicular cells have SARS-CoV-2 receptors and proteases are supported by our discoveries, demonstrating that testicular cells may be directly infected and injured, halting spermatogenesis and acting as viral vectors for the SARS-CoV-2.

Limitations, reasons for caution: Our results support viral tropism for human testicular cells and raise the possibility of testicular manifestations, although this does not necessarily mean that the virus infects testicular cells directly and further studies are needed to discern SARS-CoV-2 infection and transmission targeting alternative receptors and to identify the underlying mechanisms.

Wider implications of the findings: Male reproductive system susceptibility to viral infections has long been demonstrated. To prevent infection by allowing the virus to attach to germ cells, impair sperm production, and increase the risk of meiotic errors and viruses spreading through sperm, our study emphasizes the importance of safe practices in the wider community.

Trial registration number: not applicable

Abstract citation ID: dead093.373
P-003 Obstructive azoospermia; MESA should be employed as the method for sperm retrieval, not TESE

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Study question: TESE is widely used for obstructive azoospermia (OA) as a surgical method for sperm retrieval, but is it benefiting the patient?

Summary answer: Since MESA-ICSI has a very good fertilization rate, clinical pregnancy rate and delivery rate, MESA should be employed for the OA subjects, not TESE.

What is known already: TESE and micro-TESE are technically simple, and widely used as a sperm retrieval surgery because they do not require microsurgical skills. Some review articles have shown no significant differences in the rates of cleavage, good-quality embryos, implantation, clinical pregnancy

between ICSI with epididymal sperm or testicular sperm. Clinical usefulness of MESA is still controversial according to previous reports.

Study design, size, duration: We studied 110 patients diagnosed with OA and treated with MESA at the Asada Ladies Clinic between April 2004 and December 2021.

Participants/materials, setting, methods: The MESA was performed using a micropipette with a micropuncture technique under operative microscope. When no sperm were present or motility was not observed, additional punctures to the epididymal tubule were performed. Aspirated samples were transferred into modified human tubal fluid and sent to the in vitro fertilization (IVF) laboratory for cryopreservation.

Main results and the role of chance: Motile sperm were recovered in all cases (110 cases). Of these, ICSI using frozen thawed sperm was performed in 101 cases. The rate of normal fertilization rate was 76%. Of the 399 embryo transfer (ET) cycles, 168 had a clinical pregnancy (41% per ET). Of the 101 patients who underwent ART, 94 (93% per case) had clinical pregnancies resulting in 90 (89.1%) deliveries. It should be emphasized that since MESA does not involve incision of the testes, there are fewer postoperative peritoneal irritation symptoms and no concerns about postoperative testicular atrophy or low testosterone levels. Some review articles showed no significant differences in the rates of cleavage, good-quality embryo, implantation, clinical pregnancy between ICSI with epididymal sperm or testicular sperm. Although clinical usefulness of MESA is still controversial, if the ART results of MESA-ICSI and TESE-ICSI are the same, MESA should be performed as it is less invasive on the patient and reduces the burden on the embryologist who have to process the TESE tissue.

Limitations, reasons for caution: Some authors have reported that since MESA specimens contain DNA fragmentated sperm when compared with specimen of TESE, subsequent ICSI results in poorer fertilization and pregnancy rates. We did not evaluate sperm DNA fragmentation.

Wider implications of the findings: A large quantity of uncontaminated sperm can be retrieved using MESA which is less invasive for the patient, and there is no need for special processing before cryopreservation. Additionally, it can reduce the laboratory workload.

Trial registration number: not applicable

Abstract citation ID: dead093.374
P-004 Sperm concentration of less than 1 million/ml in Y microdeletion patients may affect ICSI clinical outcomes: a propensity score matching analysis

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Study question: To investigate which semen parameter in AZFc microdeletion patients affect the intracytoplasmic sperm injection (ICSI) clinical outcomes.

Summary answer: AZF-microdeletion caused oligospermia affected the ICSI clinical outcome when the sperm concentration is less than $1x10^{6/ml}$.

What is known already: To reduce the impact of female factors on clinical outcomes, this study employed a propensity score matching analysis to retrospectively analyze the laboratory and clinical pregnancy results of patients with AZFc deletion who underwent ICSI.

Study design, size, duration: A propensity score matching analysis in a retrospective ICSI cohort of 99 AZFc microdeletion patients and 198 oligospermia patients without AZFc microdeletion.

Participants/materials, setting, methods:

Patients/Animals: oligospermia patients with or without AZFc microdeletion undergoing ICSI.

Intervention: ICSI for couples attempting conception.

Main Outcome Measures: Outcomes included normal fertilization rate, high-quality embryo rate on Day 3, clinical pregnancy rate, abortion rate, and live birth rate.

Main results and the role of chance: Patients with AZFc microdeletion had a lower normal fertilization rate (69.55%) and Day-3 high-quality embryo rate (50.73%) than the control group (77.17% and 58.44% respectively, $p = 0.000$). There was no significant difference between the two groups in the clinical pregnancy rate (67.21% vs. 68.16%, $p > 0.05$), miscarriage rate (13.41% vs. 12.54%, $p > 0.05$), and live birth rate (58.20% vs. 59.18%, $p > 0.05$). Subgroup analysis showed that the fertilization rate, high-quality embryo rate, clinical pregnancy rate, and live birth rate were comparable between the AZFc-microdeletion group and the control groups when the sperm concentration was no less than $10^{6/ml}$. Interestingly, when the sperm concentration was below $1x10^{6/ml}$, the fertilization rate (65.60% vs. 73.79%, $p = 0.001$) and high-quality embryo rate (47.74% vs. 58.48%, $p = 0.001$) in the AZFc-microdeletion group were significantly lower than those in the control group. Furthermore, the clinical pregnancy rate and live birth rate were lower, while the miscarriage rate was higher in the AZFc-microdeletion group; however, the statistical difference is not significant.

Limitations, reasons for caution: the retrospective study was limited, and the cases of obtaining testicular sperm by microsurgery were not included, so the results may be biased, and prospective studies with larger sample size need to be designed for further confirmation.

Wider implications of the findings: We recommend screening for AZF in Chinese male infertile population when sperm concentration is below $5X10^{6/ml}$.The male physician should advise the patient to undergo genetic counseling before pregnancy assistance, and choose whether to choose PGT assisted pregnancy according to the patient's wishes.

Trial registration number: not applicable

Abstract citation ID: dead093.375
P-005 Association between aging and semen parameters: a retrospective study on over 2,500 men

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Study question: Is there an association between advancing male age and worsening seminal parameters (If so, is it possible to define an age-threshold for semen quality decline)?

Summary answer: Only sperm morphology and motility-related parameters exhibited a significant negative association with age, particularly from the age of 40 years.

What is known already: While the importance of advanced maternal age on reduced fertility and higher reproductive risks is well documented, scarce and controversial are the information regarding the role of aging on male fertility potential. Indeed, growing concerns arise from the trend in the advancing average parenthood age in Western countries.

Study design, size, duration: A retrospective study was conducted in 11,307 consecutive semen samples obtained from men who attended the Andrology Unit of the University Hospital of L'Aquila, during the period from January 1992 to January 2022 for evaluation of their fertility potential.

Participants/materials, setting, methods: Semen samples were collected by masturbation into sterile containers after a sexual abstinence of 2-7 days. Total sperm count (TSC) was calculated by multiplying sperm concentration and the volume of the ejaculate, motile sperm concentration (MSC) was calculated by multiplying sperm concentration and progressive motility (%), and the total motile sperm count (TMSC) was calculated by multiplying MSC and volume. Leukocytospermia was defined as $> 1x10^6$ peroxidase-positive cells/mL.

Main results and the role of chance: Three hundred and forty-nine patients with azoospermia or severe oligozoospermia ($<5x10^6$ spz/ml) were excluded. Moreover, 8,450 were excluded because they were only subjected to semen analysis without concomitant clinical evaluation. Therefore, 2,508 subjects have been included in the final analysis. Significant inverse correlations were observed between male age and semen volume (p=0.04), total (p<0.0001) and progressive sperm motility (p<0.0001), TMSC (p=0.01), total progressive motile count (p=0.008), and percentage of spermatozoa with normal morphology (p=0.001), but not with sperm concentration (p=0.8)

and TSC (p=0.2). After adjustment for abstinence time, smoking, history of genital tract infections, genital trauma, varicocele, cryptorchidism, orchiectomy, and the year the semen analysis, a significant negative association was found for total (p<0.0001) and progressive motility (p=0.0003), TSC (p=0.001), total progressive motile count (p=0.001), and normal morphology (p=0.035). The comparison of seminal parameters among age quartiles revealed that the age group above 40 years was associated with a significant reduction in total and progressive motility, total motile, and total progressive motile count, while the decline in the percentage of normal forms appeared to be earlier.

Limitations, reasons for caution: The present study did not analyze couple reproductive outcomes. Moreover, since sperm DNA fragmentation was available only for a limited number of subjects, it was not possible to assess its possible association with male aging.

Wider implications of the findings: In the light of the possible role of oxidative stress in affecting semen parameters in men aged over 40, the optimization of paternal lifestyle combined with targeted antioxidants treatments could be valuable preventive tools in the couple infertility work-up.

Trial registration number: not applicable

Abstract citation ID: dead093.376
P-006 In men with Raised Sperm DNA Fragmentation Index, would advance Sperm Preparation techniques alter Embryo Euploidy?

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Study question: In men with Raised Sperm DNA Fragmentation Index (DFI), Would advance sperm preparation techniques at Intra-Cytoplasmic Sperm Injection (ICSI) alter embryo euploidy status?

Summary answer: In men with raised Sperm DFI, use of advance sperm selection techniques are safe and do not appear to alter ploidy status of the embryos

What is known already: MACS (Magnetic Activated Cell Sorting), Microfluidic Sperm Sorting (MF), surgically retrieved Testicular Sperm (TESA) and Double Density Gradient sperm processing (DDG) have been shown to optimize sperm selection at ICSI for men with raised sperm DFI. Would any of these sperm selection methods alter embryo ploidy status is not proven

Study design, size, duration: This was a retrospective observational study conducted at a private fertility centre in-between years 2015 & 2022. Couples where women (<37yrs) had idiopathic Recurrent Implantation Failures (RIF) and men had raised sperm DFI (>25% DFI) and underwent Pre-Implantation Genetic Testing (PGT) were evaluated (n = 473). Based on sperm selection method the study population was divided into four groups. TESA (n = 84), MACS (n = 105), MF(n = 135), DDG (n = 148).A total of 1802 embryos were evaluated in this study.

Participants/materials, setting, methods: All women underwent Controlled Ovarian Stimulation (COS) and oocyte retrieval and male partners underwent sperm processing as per the clinics SOP. Appropriate counselling and written consents were obtained from all couples, where applicable. All oocytes subjected to ICSI, extended blastocyst culture and trophoectoderm (TE) biopsy. Post biopsy embryos were vitrified and TE tissue subjected to Next-Generation Sequencing (NGS) to assess the ploidy status. Ploidy status between various sperm selection methods was compared.

Main results and the role of chance: Euploidy rates between the study groups were as follows:

TESA (n = 311 embryos screened)- 48%
MACS (n = 445 embryos screened) – 51%
MF (n = 489 embryos screened) – 61%
DDG (n = 557 embryos screened)- 54%

Though numerically the ploidy status was highest in MF group for euploid embryos, there seems to be no statistical significance between the groups (p-value = 0.973). Different sperm selection techniques do not seem to alter embryo ploidy status.

Ejaculated sperm or Testicular sperms had similar euploidy status. Use of Testicular sperm, which is considered immature doesn't seem to increase embryo aneuploidy.

MACS, MF and DDG all seemed to have similar euploid embryos. This further warrants research to validate the role of MACS and MF as a routine intervention to optimize sperm selection and reproductive outcomes.

Limitations, reasons for caution: Retrospective design, un-equal group size and small sample size, a well-designed study is needed to further validate the findings of this study.

Wider implications of the findings: Role of advance sperm selection methods to optimize embryo implantation is yet to be proven. Data from this study showed no alarming increase in embryo aneuploidy in younger women and was comparable with published literature. Use of advance sperm selection techniques seems safe and doesn't alter embryo ploidy status.

Trial registration number: not applicable

Abstract citation ID: dead093.377
P-007 A Four Arm Prospective Randomized Control Trial to Investigate the Role of Advance Sperm Selection Techniques for Raised Sperm DNA Fragmentation Index

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Study question: Role of Testicular Sperm Aspiration (TESA), Magnetic Activated Cell Sorting (MACS), Microfluidics & Daily ejaculation, to reduce raised Sperm DNA Fragmentation Index (SDF) & evaluate impact on outcomes?

Summary answer: Amongst the advance sperm selection methods, Microfluidic sperm sorting seems to significantly reduce the SDF and offer the best reproductive outcomes

What is known already: Raised SDF is known to affect the reproductive outcomes of a couple. There are various sperm processing methods/interventions shown to reduce the sperm DNA fragmentation, but their effectiveness to optimize reproductive outcomes is still debatabl.

Study design, size, duration: RCT approved by the Institutional Ethical Committee. IEC No.:1312/Inst/TG/2019. Couples undergoing IVF with raised SDF were randomized into four arms by computer generated randomized numbers from October 2019 to May 2022 (n = 466). Of those 373 patients recruited to, TESA (n = 129), MACS (n = 92), Microfluidics (n = 116) Daily ejaculation (n = 36) . Males with SDF > 25% & Age 25-40 yrs were included. Women>37 yrs, BMI >30 and men with Smoking, Binge alcohol, varicocele excluded.

Couples with one failed IVF were offered SDF. SDF>25%was considered raised and were included in study.

Participants/materials, setting, methods: After Oocyte Pick Up (OPU), sample processed for ICSI and was sent for post intervention SDF. TESA done as per SOP of clinic. MACS & Microfluidics done as per instruction from manufacturer. Daily ejaculate group, patient ejaculated daily for 1week prior to OPU and at OPU, fresh ejaculate processed through density gradient. Men with high SDF, after randomization were subjected to OPU, ICSI, Blastocyst Culture, Freeze all policy and Frozen Embryo Transfer with 2blastocysts

Main results and the role of chance: Primary outcome-magnitude of reduction in SDF post intervention

Secondary outcomes – Blastocyst Rate (BR), Implantation rates (IR), Live birth (LBR)Mean of SDF on Raw Semen Sample Vs Post Intervention Sample:

TESA – 44% Vs 25.75%
Microfluidics - 34.4% Vs 9.8% (p < 0.05; Significant)
MACS – 32.2% Vs 27.95%
Daily Ejaculation 39.37% Vs 19.66%

Microfluidics seem to be beneficial intervention in significantly reducing the SDF. MACS seemed to have offered the least reduction of SDF.

BR: This was comparable between all groups with no statistical significance

Reproductive Outcomes for TESA, Microfluidics, MACS & Daily ejaculation:

IR – 39.3% Vs 57.27% Vs 46.4% Vs 61% (p = 0.1)

LBR – 60% VS 65% Vs 64% Vs 61% (p = 0.41)

Miscarriage Rates and Multiple Pregnancy rates were comparable between the groups and statistically not significant.

We observe that microfluidics was the best sperm processing method as it significantly reduced the DFI and had the best reproductive outcomes. It is noninvasive and can be done easily. Even though there was no statistical significance, Daily ejaculation yielded equivocal outcomes than the other 3 groups. Considering the ease, cost effectiveness and optimal reproductive outcomes Daily Ejaculation can also be offered to patients with raised SDF. Though TESA had acceptable outcomes, considering its invasiveness adequate counselling is warranted. Outcomes for MACS were guarded and be suggested with caution.

Limitations, reasons for caution: The sample size is small in the daily ejaculate arm as there were many dropouts.

The unequal size of the arms would affect the results. So, we are continuing the randomization and intend to look into the results with equal size of the arms in the future

Wider implications of the findings: Published literature has shown raised SDF to be detrimental to reproductive outcomes. There is an urgent need for an efficient intervention which is safe, economical, easy to use and offers the best reproductive outcomes. Daily ejaculation, a traditional concept which is cost effective needs due consideration over other techniques.

Trial registration number: CTRI. Trial No.: REF/2021/05/043439

Abstract citation ID: dead093.378

P-008 Acetate ameliorates doxorubicin-induced testicular toxicity by modulating Nrf2/NFkB pathway and apoptotic signaling

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Study question: Will acetate treatment ameliorate doxorubicin-induced testicular toxicity when used concomitantly?

Summary answer: Acetate ameliorated doxorubicin-induced testicular toxicity by modulating Nrf2/NFkB and apoptotic signaling.

What is known already: Doxorubicin is an antineoplastic agent that is effective in the management of several forms of cancers. However, a major limitation of this drug is its testicular toxicity. Studies have revealed that doxorubicin impairs male fertility by inducing testicular injury via downregulation of Nrf2 and upregulation of NFkB and apoptotic signaling. On the other hand, acetate upregulates Nrf2 and downregulates NFkB and apoptotic signaling. Yet, no study has reported the impact of acetate on doxorubicin-induced testicular toxicity.

Study design, size, duration: This is a prospective experimental study using animal model. Thirty two sexually mature litter-mate male Wistar rats of comparable weight were used for the study. The study lasted 8 weeks.

Participants/materials, setting, methods: Animals were acclimatized for two weeks, then randomized into four groups (n = 8). The control rats received 0.5mL of distilled water as vehicle *p.o* for 14 days, acetate-treated rats received 200 mg/kg/day of acetate *p.o* for 14 days, doxorubicin-treated rats received 7 mg/kg of doxorubicin *i.p* on day 8, while acetate + doxorubicin-treated rats received treatment as acetate-treated and doxorubicin-treated. The doses of drugs used were the Human Equivalent doses for rats.

Main results and the role of chance: Acetate ameliorated doxorubicin-induced rise in testicular activities of gamma glutamyl transferase, lactate dehydrogenase activity, and lactate levels. Also, acetate ameliorated doxorubicin-induced decline in the activities of testicular superoxide dismutase and catalase, and glutathione and Nrf2 concentrations. Acetate also alleviated doxorubicin-induced rise in testicular MPO activities, malondialdehyde, TNF-α, IL-1β, and NF-*k*B levels. More so, acetate ameliorated doxorubicin-induced reduction in spermatogenic indices and sperm quality, circulatory levels of FSH, LH, and testosterone, and testicular concentrations of 3β-HSD, 17β-HSD, and testosterone. These events were associated with dampening of doxorubicin-induced upregulation of Bax, caspase 3 and caspase 9 activities and doxorubicin-induced downregulation of BCl-2 by acetate.

Limitations, reasons for caution: This study was conducted in a rat model; therefore the results of the present study should be extrapolated to human with caution. Clinical trials are recommended to validate these findings.

Wider implications of the findings: The present study demonstrated the ameliorative effect of acetate treatment on doxorubicin-induced testicular toxicity. These findings provide substantive evidence of the possible protective role of acetate in doxorubicin-induced testicular toxicity.

Trial registration number: Not applicable

Abstract citation ID: dead093.379

P-009 Impact of organophosphate pesticide exposure on human semen quality and circulating testosterone: a systematic review and meta-analysis

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Study question: Does exposure to organophosphate pesticides (OP pesticides) alter human semen quality and circulating testosterone levels?

Summary answer: Exposure to OP pesticides reduced sperm count, concentration, total and progressive motility, and normal sperm morphology, possibly via a testosterone-independent mechanism.

What is known already: OP pesticides have been associated with a decline in semen quality, although there are still considerable arguments about the magnitude of the association. Also, human data on semen quality and male reproductive hormones in association with OP pesticide exposure is limited and inconsistent.

Study design, size, duration: This study provides a systematic review and meta-analysis of the impacts of OP pesticide on semen quality and male reproductive hormones.

Participants/materials, setting, methods: This study was conducted according to PRISMA guidelines. Following a pre-defined strategic protocol, 1, 001 studies were screened and 9 articles were identified as eligible for this study. Two of the studies were from China, and one each from Japan, Peru, France, Mexico, Malaysia, Venezuela, and Iran. Two of the studies did not specify the types of OP pesticide used, while the remaining 7 did.

Main results and the role of chance: A total of 766 male subjects (349 exposed to OP pesticide and 417 unexposed controls) were included in the meta-analysis. There was no significant difference in the ejaculate volume, seminal fluid volume, sperm multiple anomaly index, and leukocytes levels of the OP-exposed subjects compared to the control. In addition, OP pesticide exposure did not significantly affect serum concentrations of FSH, LH, and testosterone in subjects who were exposed to OP pesticide compared to their unexposed counterparts. However, we found a significant reduction in the sperm count, sperm concentration, progressive sperm motility, total sperm motility, and normal sperm morphology of OP pesticide-exposed subjects compared to the unexposed subjects. However, after subtype and sensitivity analyses, exposure to OP pesticide did not significantly reduce sperm count. In addition, after sensitivity analysis, OP pesticide exposure did not significantly alter progressive sperm motility.

Limitations, reasons for caution: The non-inclusion of non-English publications in this study and the scarcity of well-designed studies to be included

might have limited the pooled sample size and inadequately explored the impacts of OP. Also, the included studies are from a few countries, which may not necessarily be a good global representative.

Wider implications of the findings: The present comprehensive meta-analysis clearly demonstrates that exposure to OP pesticide causes reduced sperm count, concentration, total and progressive motility, and normal sperm morphology, possibly via a testosterone-independent mechanism. These findings strengthen existing evidence in the literature on the negative impacts OP pesticide exposure on semen quality.

Trial registration number: Not applicable

Abstract citation ID: dead093.380
P-010 The impact of COVID-19 Vaccination on Sperm Parameters in Indian Men: A Comparative Study

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Study question: To analyse the effect of COVID-19 vaccines on sperm parameters in Indian men seeking infertility treatment.

Summary answer: COVID-19 vaccination did not impact the sperm parameters of Indian men undergoing infertility treatment in this study.

What is known already: The COVID-19 pandemic triggered an expedited response from the scientific community to develop preventive vaccine programs against the deadly SARS-CoV-2 virus. Although COVID-19 is predominantly a respiratory system illness, initial reports suggested that it could have a short-term negative impact on the sperm parameters of the males affected by the disease too. Misinformation led to unfounded concerns about the potential consequences of the vaccines on male fertility, causing a great deal of initial vaccine hesitancy. To address these concerns, we compared the sperm parameters in men before and after COVID vaccination available in India at the time of this study.

Study design, size, duration: It was a multi-centre, prospective, observational study of 960 male participants conducted at our fertility clinics between June 2021 to December 2022.

The study participants were matched for age and had no known risk factors. We excluded 267 participants from this study since they did not meet the inclusion criteria, bringing our total study population down to 693.

Participants/materials, setting, methods: The study participants provided two semen samples for analysis before and after receiving any of the COVID-19 vaccines available in India at the time of this study. A trained embryologist analysed the pre-vaccination and post-vaccination sperm concentration and motility of these males, following the 6th Edition of WHO Manual for Semen Analysis. GraphPad Prism 9 was used for statistical analysis of the data & $p \leq 0.05$ was considered statistically significant.

Main results and the role of chance: The study enrolled 693 people, with 96.5% receiving at least one dose and 90.35% receiving two or more. The most common vaccines used among the group were Indian made-Covishield and Covaxin, received by **71.8**% & 24.3% of the participants, respectively.

In the group of participants who had tested positive for COVID (n = 259), the sperm concentration increased by 3.29 million/ml and the total motility increased by 3.24% post-vaccination. However, these changes were not statistically significant ($p = 0.140$ and $p = 0.099$, respectively).

In the group of participants who had not tested positive for COVID (n = 424), we observed a similar increase in sperm concentration and total motility post-vaccination. The sperm concentration increased by 1.52 million/ml and the total motility increased by 0.85%. However, these changes were also not statistically significant ($p = 0.580$ and $p = 0.404$, respectively). Subgroup analysis of different vaccines did not reveal any statistically significant differences either.

In conclusion, the study found that COVID-19 vaccination, including the type of vaccine used, did not significantly impact sperm parameters in men.

Limitations, reasons for caution: Supply chain disruptions in the COVID vaccination program meant we could not match the duration between the pre-vaccination and the post-vaccination semen analyses in this study. Additionally, the findings are specific to our study population and may not be generalizable to other populations or settings.

Wider implications of the findings: This study found that COVID-19 vaccination did not affect the chances of Indian men at fatherhood. Our work also supports the safety of Indian-made vaccines for men seeking infertility treatment, as it is one of the most extensive studies in this area.

Trial registration number: Not Applicable

Abstract citation ID: dead093.381
P-011 Slight decreases in cumulative live birth rates when using frozen-thawed semen samples for ICSI with donor oocytes do not mean we should stop freezing

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Study question: Does the use of autologous frozen semen samples for ICSI with donor oocytes make any difference in reproductive outcomes compared to using fresh samples?

Summary answer: Although it seems slightly detrimental in several intermediate outcomes, the advantages of using frozen samples in cycle logistics outweigh this potential decrease in results.

What is known already: Sperm freezing has become a part of day-to-day practice in infertility clinics due to its many logistic benefits. However, there is no clear consensus on the impact of freezing-thawing semen samples prior to ICSI on cycle outcomes. Moreover, most studies express results according to classical outcomes of reproductive success (i.e. pregnancy rate or live birth rate (LBR) per embryo transfer (ET)). Cumulative LBR (CLBR) are a more realistic approach to expressing the probability of achieving a livebirth when assessing the effect of a technique, since they compute the contribution of all used oocytes and embryos from the same cohort.

Study design, size, duration: Retrospective multicenter observational cohort study involving 45,120 ICSI procedures with donor oocytes (34,847 with fresh semen samples and 10,269 with frozen samples) from January 2008 to November 2021. The fresh sample group included real-world data from the clinical records of these patients and cycles from a cohort of 387,903 inseminated oocytes and 75,116 embryos transferred in 49,775 ET; whereas the frozen sample group considered 110,983 injected oocytes and 24,334 embryos replaced in 14,953 ET.

Participants/materials, setting, methods: Pregnancy rates and LBR per ET were computed and then adjusted to female and male patients' age and BMI, total progressive motile sperm, semen capacitation, transfer before or at blastocyst stage, and use of PGT, using generalized linear models to compute adjusted odds ratios (adjOR) and p-values comparing both groups. CLBR per ET, embryo replaced and used oocyte were plotted in Kaplan-Meier curves adjusted according to donors' and female patients' age in Cox regression models.

Main results and the role of chance: There were statistically significant differences between both groups in terms of biochemical, clinical and ongoing pregnancy rates per ET, and LBR per ET. After the multivariate analysis, these differences remained statistically significant with adjusted p-value <0.001. The adjOR were 1.091 (1.045-1.139), 1.133 (1.086-1.183), 1.115 (1.067-1.165) and 1.168 (1.114-1.223) respectively when considering the frozen sample group as the reference. CLBR was 57.73% (57.05-58.41) after three embryos were transferred and 73.31% (72.44-74.16) after five embryos replaced in the fresh sample group, compared to the 49.65% (48.40-50.88) and 66.58% (64.92-68.16) CLBR in the frozen sample group ($p < 0.001$). The adjusted hazard ratio for this comparison was 1.146 (1.100-1.195). The CLBR in the fresh sample group was 45.74% (45.10-46.37) and 69.25% (68.46-70.02) after 10 and 14 donor oocytes were used, while the rates for the frozen sample

group were 43.87% (42.66-45.05) and 68.83% (67.17-70.40) respectively (p = 0.0002). The adjusted hazard ratio was 1.067 (1.023-1.112), with an adjusted p-value of 0.002. In the fresh sample group, 5.6% of the used oocytes resulted in the couples' first newborn (267,462 oocytes and 15,153 newborns), whereas in the frozen sample group it was the 5.2% of oocytes (77,284 oocytes and 4,040 newborns), which is consistent with the published literature.

Limitations, reasons for caution: The retrospective nature of the study subjects data to biases and inaccuracies in their annotation in clinical sources from which they were exported, although the multivariate statistical analyses aimed to control those biases. Considering only donor oocytes also reduces some of the variability since it homogenizes factors concerning oocyte quality.

Wider implications of the findings: Although the frozen samples group had slightly decreased success rates, the CLBR per used oocyte remained between around two percent lower compared to using fresh samples. Thus, the large sample size and real-world data included show that the subtly lower rates do not outweigh the logistic benefits of sample freezing.

Trial registration number: Not applicable

Abstract citation ID: dead093.382

P-012 Time, risk & motion study of an integrated artificial intelligence robotic system for semen analysis

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Study question: Can integration of a robotic system for semen analysis reduce operational failure modes and eliminate risk in the IVF laboratory?

Summary answer: A robotic system for semen analysis reduces operational failure modes and eliminates severe risks including those associated with reduced patient experience and increased patient stress.

What is known already: Recently, the ever-evolving field of IVF has experienced a rise in innovations to boost laboratory performance. Considering the lack of robust evidence in some of them, decisions on implementation are often based on opinion and product cost. Currently, an approach to assess risk, time and effectiveness has not been fully described. IVF is not error-free (Sakkas et al., 2018), hence all new technology coming into the laboratory ought to reduce risk of errors happening.

Study design, size, duration: Failure mode effects analysis (FMEA) was carried out on the workflow integration into an IVF centre (>500 cycles per annum) to evaluate the possible procedural risks of manual semen analysis compared to a digitalised pathway. Possible sources of error were identified, and the Risk Priority Number (RPN), a product of likelihood, severity and detection of incidence was associated with each risk, as previously described by Rienzi et al., 2015.

Participants/materials, setting, methods: Using a systematic decision-making approach to quantify the value of incorporating the use of a robotic system (Mojo AISA) for semen analysis.

Main results and the role of chance: Five process phases were identified for conventional specimen analysis. There were eight associated process steps and 29 failure modes, among which 13 risks were given a moderate (RPN 15-50, i.e. data entry errors, incorrect count and sperm classification) and three were severe RPNs (RPN>50), resulting in reduction in efficacy of treatment. Protocol using the robotic system resulted in a reduction of process phases to four and failure modes to 16, with four moderate and none severe RPNs. Implementation of the robotic system improved workflow by optimising time in motion for the IVF specialist and further reducing active time by at least 30%.

Limitations, reasons for caution: FMEA is a proactive method to identify potential incidents and help to develop strategies to mitigate risks. Ultimately, this forms part of a scheme for responsible development. The likelihood of incidences and time analysis were estimated based on clinical experience. Risks inherent to sample handling remained present.

Wider implications of the findings: The robotic system has the potential to eliminate risks that exist when manually inputting data to electronic medical records, whilst offering support to IVF specialists.

Trial registration number: not applicable

Abstract citation ID: dead093.383

P-013 Evaluation of the efficacy of a short course of multidose menotropins in the treatment of male infertility in real clinical practice

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Study question: Is it possible to establish prognostic criteria for response to short course of multidose menotropin therapy in men with sperm pathology.

Summary answer: We found the increase of the effectiveness of menotropins with an increase in the duration of their use and an increase in dosage.

What is known already: Currently, menotropins are widely used to treat male infertility, both to stimulate spermatogenesis and to compensate for hormonal imbalance. But the actual practice of using menotropins may differ from the recommendations given by the manufacturer. Therefore, observational programs are particularly important, which allow assessing the effectiveness of a drug in routine practice. In real-world practice, hormonal treatment of the infertility is often carried out during the preparation of a couple for IVF and lasts no more than 4-6 weeks. At the same time, there is almost no scientific data in the literature of the effectiveness of such short treatment regimens.

Study design, size, duration: The study was designed as a multicentre, prospective, observational cohort study. The study included 1117 men with infertility enrolled between January 2020 and April 2021.

Participants/materials, setting, methods: The study included men with the absence of pregnancy during 12 months of regular unprotected sexual activity. The average duration of infertility was 20.6 months. As a treatment, men received multidose menotropin at a dosage of 75 IU, 112.5 IU or 150 IU every other day with hCG 2000 IU 2-3 times a week. Sperm parameters and hormones (total testosterone, SHBG, LH, FSH, Inhibin B, estradiol) were evaluated before treatment and 4 weeks after.

Main results and the role of chance: The age of the patients was 18 to 65 years (33.6 ± 7.0 years). The sperm concentration after 1 month of treatment increased by 23.8% from 17.6 ± 18.1 to 23.1 million/ml, the total sperm count increased by 32.1% from 40.2 ± 41.7 to 53.1 ± 49.1 million, progressive motility increased on average from 26.5 ± 16.2 to 32.0 ± 16.5%, the proportion of morphologically normal spermatozoa increased from 2.3 ± 2.5 to 3.0 ± 2.7 %. The level of total testosterone increased by 33.3% from 11.1 ± 4.6 to 14.8 ± 6.3 nmol/l. The levels of Inhibin B and estradiol did not change significantly. In a comparative analysis of the effect of dosage on the parameters of the ejaculate, the dose of 112.5 IU showed the greatest effect.

Limitations, reasons for caution: The main limitation of this study is the large heterogeneity of enrolled patients. There will be a very large variability in the age of men, as well as the severity of deviations from the norm in the analysis of ejaculate.

Wider implications of the findings: Thus, multidose menotropin, already when used for 4 weeks, can significantly improve the quality of the ejaculate (concentration, total count, progressive motility, morphology). In case of

insufficient effectiveness of a short course (1 month), it is advisable to continue treatment for at least 3 months or increase the dosage.

Trial registration number: not applicable

Abstract citation ID: dead093.384
P-017 Validation of origami microscope "Foldscope" as a preliminary screening tool for male infertility

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Study question: Is there a concordance between the new optical microscope based on origami (Foldscope) with a traditional microscope for semen analysis as a screening method?

Summary answer: There is a 91% direct concordance between the foldscope and a traditional microscope as screening for initial approximation to male infertility.

What is known already: Semen analysis is a key element on men fertility evaluation. In order to obtain a semen sample, patients have to assist to a fertility clinic where the sample is analyzed. However, due to either time or distance problems, many patients cannot attend to fertility clinics. On the other hand, Foldscope is an origami-based microscope that can provide over 2000x magnification, and it can be attached to a smartphone and take photos and videos, which can be sent to fertility specialists for analysis. This technology might provide important information about semen status, especially in areas with limited fertility care access.

Study design, size, duration: A retrospective observational study was carried out, we included semen samples obtained by masturbation from volunteers were analyzed for count, concentration, and motility from patients who were undergoing a fertility study between November 2021 and November 2022 in Clínica Alemana, Santiago, Chile.

Participants/materials, setting, methods: A total of 188 human semen samples were evaluated by a single embryologist under a standard microscope and later used the same sample to make a cell phone video with Foldscope. This information was randomized and the videos were subsequently analyzed by the same embryologist blindly. Finally, the matching of the results was carried out.

Main results and the role of chance: Moderate and significant positive correlation for concentration calculated as $>$ or $<$ 15 million/ml between Conventional Microscopy and Foldscope (correlation coefficient= 0.67, $p <$ 0.005) Sperm motility for progressives A+B recorded with Foldscope demonstrated a significant correlation with the traditional microscope results (correlation coefficient = 0.69, $p < 0.005$). Finally, the concordance for the sperm count between both methods was 91%, with a kappa index of 0.66 ($p < 0.0001$).

Limitations, reasons for caution: One of the most important limitations of semen analysis by foldscope is that it must be interpreted by an embryologist, which means that the images must be sent to a specialized center.

Wider implications of the findings: In countries with few economic resources and not many options to for reproductive medicine centers, the use of the foldscope microscope as a screening as an initial approximation to male fertility allows a quick and economical immediate referral to men with reproductive problems.

Trial registration number: not applicable

Abstract citation ID: dead093.385
P-018 Structural and biocompatibility analysis of gold nanoparticle integrated polymeric scaffolds for infertility diagnosis

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Study question: Can infertility diagnosis and sperm selection be improved by using bio- nanotechnologies?

Summary answer: Gold nanoparticle integrated biopolymeric scaffolds show good biocompatibility when interacting with human sperm cells, being suitable to be used as cell sorting and biosensing platforms.

What is known already: Microfluidic platforms are used in sperm selection for increasing the *in-vitro* fertilization success but they are limited by the exclusive analysis of sperm cell motility. The integration of microfluidic platforms with nanomaterials such as nanopatterned substrates can greatly enhance the selectivity and the sensitivity of the detection. The accuracy can be further improved by employing the intrinsic properties of gold nanoparticles (optical properties, high biocompatibility, surface chemistry favoring the conjugation of specific biomolecules), which makes them suitable for the successful development of precise and reliable detection assays, so far used to a limited extent in infertility research and clinical approaches.

Study design, size, duration: Concentration, motility and morphology of spermatozoa were analyzed in semen samples collected for spermogram. The normozoospermic and teratozoospermic samples were selected and prepared by density gradient centrifugation. The sediment obtained contained 100% motile spermatozoa, which were incubated on the thin films for 24h. After 24h, the probe from each incubation dish was collected and the survival rate was analyzed using the Makler chamber.

Participants/materials, setting, methods: Thin films of atactic polystyrene, chitosan and mixtures of each of the two polymers with gold nanoparticles were used to produce scaffolds of microchannels. The substrates were morphologically characterized by optical microscopy, scanning transmission electron microscopy and atomic force microscopy. Normozoospermic and teratozoospermic sperm samples were collected and incubated on the scaffolds. After 24h incubation, the viability was assessed by counting the motile spermatozoa under microscopic observation. Statistical analysis was performed using two-way ANOVA test.

Main results and the role of chance: Analysis of the sperm cell viability incubated on the polymeric scaffolds revealed good biocompatibility with higher survival rate in the case of chitosan-gold nanoparticle integrated samples. In multiple comparisons of normozoospermic samples, the survival rates of the sample incubated on the chitosan thin films to the control sample were 18% to 80% (-62%) ($p = 0.0143$), 16.66% to 45% (-28.34%) ($p = 0.0143$), 0% to 15.38% (-15.38%) ($p = 0.0239$), 4.76% to 55.35% (-50.59%) ($p = 0.0239$). In multiple comparisons of teratozoospermic samples, the survival rates of the sample incubated on the chitosan thin films to the control sample were 14.67% to 41% (-26.33%) ($p = 0.0143$), 0% to 44.82% (-33.82%) ($p = 0.0239$). For polystyrene, the viability was 0% which excludes its further use in infertility-related assays due to its high cytotoxicity. Overall, the colloidal gold nanoparticles integrated into the polymeric matrix improved the biocompatibility of the films. For example, citrate gold nanoparticles of spherical shape and sizes of about 20 nm increased the survival rate of normozoospermic samples, while PEGylation of nanoparticles showed further improvement of the biocompatibility. The influence of the particle type, composition and coating material onto the spermatozoa viability is currently under investigation for optimizing the effectiveness of the polymeric scaffolds.

Limitations, reasons for caution: NA

Wider implications of the findings: Due to the unique properties of gold nanoparticles, the nanoparticle-polymer scaffold systems have the potential to

become the foundation of a precise biosensor that can enhance the accuracy of the current approaches in infertility assessment.

Trial registration number: Not applicable

Abstract citation ID: dead093.386

P-019 Short ejaculatory abstinence time is associated with higher pregnancy and live birth rates and improved DNA fragmentation index (DFI): A Systematic Review

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Study question: Does short ejaculatory abstinence time (EA) improve DNA fragmentation and reproductive outcomes compared with long EA following assisted reproductive technology (ART)?

Summary answer: Shorter EA is associated with improved pregnancy and live birth rates following ART and lower DFI compared with longer EA at different cut-off points.

What is known already: When spermatozoa pass the epididymal tract, they are exposed to reactive oxygen species (ROS). ROS is known to cause DNA fragmentation. Testicular sperm has a lower DNA fragmentation when compared to ejaculated sperm, which indicates that DNA fragmentation is increased during the transition and storage in the epididymal duct. There are studies that suggest that a high DFI is associated with poor pregnancy and live birth rates following ART. WHO recommends 2-7 days of EA prior to semen collection; however, several recent studies have reported a correlation between shorter EA and improved reproductive outcomes and DFI.

Study design, size, duration: A systematic review was conducted according to the PRISMA guidelines and registered in PROSPERO (CRD42022379039). The electronic databases PubMed, Embase and Cochrane were searched for eligible studies by a research librarian in April 2022. Additional articles were manually retrieved after reviewing the reference lists from relevant publications.

Participants/materials, setting, methods: Two authors independently screened studies based on the eligibility criteria; studies including men referred to fertility treatment undergoing short and long EA reporting data on pregnancy rate, live birth rate and DFI. Study demographics, population characteristics and data on study outcomes including statistical analyses were extracted. Risk of bias in each study was evaluated according to The Scottish Intercollegiate Guidelines Network (SIGN). Any disagreements were resolved by a third author.

Main results and the role of chance: Out of 1237 studies initially identified, 24 of them met eligibility criteria and were included in the review with a total of 14.173 cases. The studies included in this review were conducted in Asia, North and South America and Europe. The cause of referral to fertility treatment includes both male, female, mixed and unexplained infertility. The EA varied from less than a one hour to as long as 15-20 days.

Nine of the 13 studies investigated the influence of varying EA on pregnancy rate, found a significantly higher pregnancy rate with short EA compared with long EA, five with EA as short as one day or less. Three studies evaluated live birth rates and found a significantly higher live birth rate when comparing short EA with long EA. Eleven of the 15 studies that reported DFI found significantly lower DFI with short EA compared with long EA. EA of one day or less were associated with the lowest rates of DFI in the studies. In general, pregnancy rate, live birth rate and DNA fragmentation are likely to be improved with short EA compared to long EA.

Limitations, reasons for caution: Many of the included studies used different EA intervals, leading to heterogenous data, and therefore, only allowed limited conclusion to be formulated regarding the ideal timeframe of EA. Four studies scored poorly in SIGN methodology checklist due to confounding and population size, suggesting their findings to be treated with caution.

Wider implications of the findings: Even though it is difficult to make a clear recommendation on the ideal timeframe for EA because of the heterogenous abstinence periods, this review suggest decreasing the EA limits of 2-7 days as recommended by WHO to improve pregnancy rate and decrease DNA fragmentation in semen, followed by ART.

Trial registration number: not applicable

Abstract citation ID: dead093.387

P-020 Association between male age and the frequency of sperm abnormalities

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Study question: Does advancing male age influence the frequency of occurrence of sperm abnormalities?

Summary answer: Advancing male age was associated with increased incidence of certain head and tail defects – elongated head, nuclear vacuoles, and coiled tail.

What is known already: Evaluation of male reproductive potential is based mainly on sperm motility and sperm morphology. Morphological sperm features serve as a reliable indicator in predicting the fertilizing capacity of sperm. Nowadays, there are various sperm selection techniques based on sperm morphology. However, advancing male age has been associated with decrease in semen quality. Therefore, the aim of this study was to examine the effect of aging on the frequency of occurrence of sperm abnormalities.

Study design, size, duration: This retrospective study includes 3980 men aged between 15 and 72 years, with a mean of 36 years. Semen samples were collected between October 2015 and December 2022 in a private in-vitro hospital. Men were divided into three groups according to their age: 1) from 15 to 25 years ($n = 144$), 2) from 25 to 45 years ($n = 3447$), and 3) over 45 years ($n = 393$).

Participants/materials, setting, methods: Sperm morphology was evaluated according to the Kruger's strict criteria. Totally, 23 types of abnormalities were determined. Head defects included small, large, amorphous, elongated, round, pear-shaped, double, detached head, acephalic cells, small or large acrosomal areas, and spermatozoa without acrosome. Midpiece defects included thick, thin, bent, asymmetric midpiece and cytoplasmic droplets. Tail defects included short, coiled, and double tail. Acrosomal vacuoles and nuclear vacuoles were also evaluated. Statistics: ANOVA, followed by LSD post-hoc test.

Main results and the role of chance: The results are presented as mean ± SD. Among the studied population the percentage of normozoospermic men was 44%, while the percentage of teratozoospermic semen samples was 56%. The total mean frequencies of occurrence of head, midpiece and tail abnormalities were 85.9 ± 7.9%, 34.1 ± 12.8% and 4.7 ± 5.1%, respectively. Only 3 (13%) of the analysed 23 types of sperm abnormalities were significantly more common with advanced male age – elongated head, nuclear vacuoles, and coiled tail.The frequency of occurrence of elongated head differed significantly between the age groups 1-2, and 1-3: 11.38 ± 10.1% vs. 13.79 ± 11.58%, $p = 0.015$, and 11.38 ± 10.1% vs. 14.4 ± 11. 8%, $p = 0.008$, respectively. No difference in the incidence of this defect was observed between groups 2 and 3 ($p > 0.05$).Furthermore, the frequency of nuclear vacuoles significantly increased with age as observed in the following age group comparisons 1-2, 1-3, and 2-3: 4.28 ± 4.93% vs. 5.4 ± 6.1%, $p = 0.04$, 4.28 ± 4.93% vs. 6.48 ± 7.89%, $p < 0.001$, and 5.4 ± 6.1% vs. 6.48 ± 7.89%, $p=0.001$, respectively.Finally, coiled tail was observed more frequently in age groups 2 and 3 compared to age group 1: 13.79 ± 11.58% vs. 11.38 ± 10.1%, $p < 0.001$, and 14.4 ± 11. 8% vs. 11.38 ± 10.1%, $p < 0.001$, respectively.

Limitations, reasons for caution: This is a single-institution retrospective study.

Wider implications of the findings: The findings of this study showed that aging is associated with an increased percentage of several sperm abnormalities: spermatozoa with elongated head, nuclear vacuoles, and coiled tail. Further studies are required to clarify the negative impact of aging on spermatogenesis leading to morphological defects.

Trial registration number: NA

Abstract citation ID: dead093.388
P-021 A novel SNP in HUWE1 promoter confers increased risk of NOA by affecting the RA/RARα pathway in Chinese individuals

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Study question: The present study aimed to investigate the role of HUWE1 in non-obstructive azoospermia (NOA) etiology and germ cell differentiation.

Summary answer: An SNP mutation in *HUWE1* promoter region downregulates HUWE1 expression in NOA patients, the genetic polymorphisms of *HUWE1* are related to the pathogenesis of NOA.

What is known already: HUWE1, a ubiquitin ligase, is crucial to the development of male reproductive system and embryonic development. However, the specific role of HUWE1 in regulating germ cell differentiation is unclear, and clinical evidence for the relationship between HUWE1 and the pathogenesis of male infertility is lacking.

Study design, size, duration: 190 NOA Chinese patients were recruited and analyzed single nucleotide polymorphisms (SNPs) in *HUWE1* gene.

Participants/materials, setting, methods: Regulation of *HUWE1* gene expression by *RARα* factor was evaluated by performing ChIP assay, EMSA, and siRNA-mediated *RARα* knockdown. We used C18-4 cells to determine whether HUWE1 participated in RA-mediated RARα signaling and performed luciferase assay, CCK-8 assay, immunofluorescence staining, RT-qPCR, and western blot analysis. The expression level of *HUWE1* and *RARα* was evaluated in clinical samples obtained from NOA or obstructive azoospermia (OA) patients using RT-qPCR.

Main results and the role of chance: We explored the mechanism by which polymorphism of the *HUWE1* promoter leads to spermatogenesis disorders, and focused on the identification of mutations in the SNP site of the *HUWE1* regulatory sequence among patients with NOA, which may influence HUWE1 expression. The results of in vitro experiments showed that RARα binds to the *HUWE1* promoter region and regulates its expression. However, the regulatory effect of *RARα* weakened following introduction of the mutation into the *HUWE1* regulatory sequence. The expression of spermatogonial differentiation-related gene *STRA8* was impaired, and γH2AX foci accumulated following inhibition of *HUWE1* gene expression using siRARα. Finally, to determine whether *HUWE1* expression was important in patients with NOA, its expression was verified using testicular puncture samples collected from patients with NOA and the results revealed that *HUWE1* expression was abnormal ($P < 0.01$). In addition, *RARα* expression was significantly downregulated ($P < 0.001$). Overall, our result support the hypothesis that *HUWE1* expression is induced by the RA/RARα signaling pathway and it inhibit γH2AX foci accumulation, thereby considerably promoting sperm differentiation and meiotic prophase.

Limitations, reasons for caution: The effect of SNPs in HUWE1 on male infertility is further studied in a wider population.

Wider implications of the findings: Our study of HUWE1 and RA/RARα signaling pathway provides new information on the regulatory mechanism of spermiogenesis, suggesting that SNPs in HUWE1 promoter are etiological factors in patients with NOA and providing a rationale for further drug development to target HUWE1 in these patients.

Trial registration number: the National Key Research and Development Program of China (2018YFC1003603)

Abstract citation ID: dead093.389
P-022 The ICSI Outcome of Frozen Thawed Testicular Sperm Utilization in Azoospermic Men with AZFc Microdeletion

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Study question: What is the efficacy of frozen thawed surgically retrieved spermatozoa in azoospermic men with AZFc microdeletion undergoing Introcytoplasmic sperm injection (ICSI)?

Summary answer: The comparison between frozen thaw to fresh testicular sperm did not show statistically significant difference in terms of fertilization, blastocyst development and pregnancy rates.

What is known already: In male infertility, Y chromosome microdeletion (YCM) may be the cause up to 10% of men with oligozoospermia or azoospermia. AZFc region deletion was mostly detected in YCM. In men with azoospermia and AZFc deletion spermatozoa can be retrieved via testicular sperm extraction (TESE). Today, couples suffering azoospermia due to AZFc deletion may have genetically own child by using testicular sperm with ICSI. Because of the rare incidence of AZFc deletion, the outcome of ICSI cycles in which frozen thawed spermatozoa utilized for assisted conception has not been widely known.

Study design, size, duration: The data of 611 men with non-obstructive azoospermia (NOA) who underwent microTESE surgery between 2014 and 2022 was retrospectively analyzed. Assisted reproduction treatment (ART) outcome of couples whose men with AZFc deletion was further analyzed according to the fresh or frozen thawed spermatozoa usage for ICSI.

Participants/materials, setting, methods: All of the patients were genetically screened with karyotype and Y chromosome microdeletion analysis and microTESE operation was performed for testicular sperm recovery. The patients who diagnosed AZFc deletion and had successful sperm recovery divided to two groups according to cryopreserved or fresh sperm utilization for ICSI. The spouse and men age, fertilization rate, blastocyst development, pregnancy and live birth rate was compared between the groups.

Main results and the role of chance: Twenty-seven (4.4%) men were diagnosed YMC deletion in total of 611 men with NOA who underwent microTESE. Spermatozoa was successfully retrieved in 14 men (51.8%) men who were diagnosed AZFc deletion. The mean age of women and men were 30.5 (3.5) and 32.8 (4.1) years, respectively. Oocyte retrieval and ICSI were accomplished in 18 cycles. In 7 (38.8%) cycles fresh testicular spermatozoa and in 11(61.2%) cycles frozen thawed testicular spermatozoa were used. In one cycle, embryo transfer was cancelled due to total fertilization failure. In total, fertilization rate as 2PN/M2 oocytes was 57% (119/206). In 5 cycles cleavage stage embryos were transferred whereas in remaining 13 cycles blastocysts were transferred. Blastocyst development rate as day 5 blastocyst/2PN was 45.2% (42/95). Comparison of fertilization and blastocyst development rates according to fresh TESE spermatozoa and frozen thawed TESE spermatozoa groups were 63.2% vs 54.4% and 50.0% vs 38.7% respectively ($p > 0.5$ for both). Clinical and live birth rates per embryo transfer cycle were 57.1% vs 40.0% and 42.8% vs 30.0% respectively ($p > 0.5$ for both).

Limitations, reasons for caution: Fresh and frozen thawed groups demonstrated no statistical difference in terms of ICSI outcome. However, relatively reduced fertilization and blastocyst development rates should be interpreted cautiously because beta type error. Further data is needed to clarify this difference.

Wider implications of the findings: The best of our knowledge our study is the largest in literature comparing frozen thawed and fresh sperm utilization for ICSI in men with AZFc deletion. This data may help for physicians and couples to give decision diagnostic microTESE surgery and cryopreservation of sperm further ICSI treatment.

Trial registration number: Not applicable

Abstract citation ID: dead093.390
P-023 Weight loss in obese fathers improves hepatic lipid metabolism in offspring

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Study question: Does paternal obesity affect offspring lipid metabolism and whether paternal weight loss can correct this effect?

Summary answer: The lipid metabolism of male offspring of obese fathers is abnormal, especially under the second hit, and paternal weight loss could rescue it.

What is known already: So far we have known that epigenetic modifications in spermatogenesis, are directly involved in the etiopathology of adult diseases transmitted by sperm. It was found that the offspring had more weight gain and impaired glucose tolerance and insulin resistance in adulthood, although they kept a normal diet through microinjecting ncRNA of obese mice spermatozoa into fertilized eggs from normal parents. The above evidence supported that epigenetic modifications can undertake part of the transmission from father to offspring.

Study design, size, duration: The 30 4-week-old C57 male mice were divided into three groups, normal diet as the control group (NCD) for 30 weeks, high-fat diet as the obese group (HFD) for 30 weeks, first high-fat diet for 10 weeks then low-fat and high-fiber diet as weight loss for 20 weeks (LFD). The male offspring of these groups were normally fed after weaning, then received a second hit (fasting for 36 hours or high-fat diet for 4 weeks).

Participants/materials, setting, methods: These offspring were divided NCD F1, HFD F1 and LFD F1, respectively. And in the fasting condition, the oil tolerance test and P407 test were carried out to detect the hepatic lipid metabolism function. HE staining and Oil Red O staining were performed on the liver sections of offspring to clarify the accumulation of lipid droplets in the liver. Liver autophagy was detected by western blotting and confocal microscopy.

Main results and the role of chance: Through oil tolerance test, it was found that TG and NEFA of HFD F1 increased at 1 hour and 3 hours after gavage, while LFD F1 significantly decreased. The P407 test further proved that under fasting conditions, the release of TG from the liver of HFD F1 was significantly weakened, and LFD F1 was restored. HE staining and Oil Red O staining of liver sections indicated that lipid droplets accumulated in the liver of HFD F1, while LFD F1 were significantly reduced. Under the second hit, the difference in liver lipid droplets among the three groups was more obvious. In addition, we found the levels of serum C-reactive protein, TNFα, and IL-6 increased in HFD F1 under the second hit, while LFD F1 could restore. It was found that the p62 of HFD F1 was higher and the ratio of LC3II to LC3I was lower than that of NCD F1, which indicated that HFD offspring liver autophagy was weakened through western blotting, and LFD offspring could restore liver autophagy. It was found that the co-localization of lipid droplets and lysosomes in the HFD offspring was reduced, which was more obvious under the second hit, while the LFD offspring could recover.

Limitations, reasons for caution: This study provides phenotypic evidence, however, the genetics of paternal disease remain to be explored.

Wider implications of the findings: The study provides new evidence that the father's lifestyle affects offspring metabolism, but this effect is not permanent and can be eliminated by changing the lifestyle.

Trial registration number: 82088102

Abstract citation ID: dead093.391
P-024 Effect of microsurgical varicocelectomy on fertility potential in patients with low sperm vitality

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Study question: Does microsurgical varicocelectomy improve semen parameters and fertility potential in patients with low sperm vitality?

Summary answer: Micro surgical varicocelectomy in patients with low sperm vitality is beneficial as it results in significant improvement in sperm count and progressive motility.

What is known already: Low sperm vitality (Necrozoospermia) indicates the presence of higher percentage of necrotic/ dead sperm in the ejaculate which can impair fertility potential. Varicocele induced testicular hyperthermia is among the most common causes of impaired semen quality, including necrozoosprmia. While many studies confirmed the beneficial effect of varicocelectomy on different semen parameters, very few have evaluated its role on sperm vitality in particular.

Study design, size, duration: A retrospective study of 1521 patients who underwent microsurgical sub inguinal varicocelectomy in our center from 2011–2021.

A total of 115 patients with clinical varicocele and low sperm vitality (<58%) were included.

Participants/materials, setting, methods: The exclusion criteria were history of genitourinary tract infection, exposure to heat, drugs or toxic compounds, hormonal disturbances or prior infertility related treatments. Patients demographics, and clinical data were collected, in addition to the pre- and post-operative Semen analysis (WHO 5th edition), sperm DNA fragmentation (Halosperm) and hormonal profile (estradiol, FSH, LH, Prolactin, Testosterone). Spearman's correlations and Wilcoxon Signed Ranked Test were used for data analysis.

Main results and the role of chance: Mean age was 34.9 ± 7.6 years. Varicocelectomy was done on left side in 75 patients and bilateral in 40 patients.

Sperm vitality was significantly negatively correlated with sperm DNA fragmentation (-0.618, $p = 0.000$) and abnormal sperm morphology (-0.435, $p = 0.000$, while it was significantly positively correlated with total motility (0.674, $p = 0.000$) & progressive motility (0.272, $p = 0.003$).

The median (IQR) sperm vitality pre-operatively was 41% (30-50) and the level increased post-operatively to 54% (31-63). There was a significant improvement in post-operative sperm count, progressive motility as well as LH and FSH levels. Improvements were also noted in total motility, normal morphology and sperm DNA fragmentation however the result didn't reach statistical significance.

Limitations, reasons for caution: The main limitation is the retrospective design of the study and it did not evaluate the pregnancy outcomes.

Wider implications of the findings: Improvement in semen parameters by microsurgical varicocelectomy in patients with low sperm vitality is important as it may change the management plan for patients and extend their fertility options to natural conception or intrauterine insemination instead of only intracytoplasmic sperm injection.

Trial registration number: not applicable

Abstract citation ID: dead093.392
P-025 Optimising stimulated IUI; a systematic review and network meta-analysis

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Study question: What is the effectiveness and safety of various follicular phase ovarian stimulation protocols for intrauterine insemination (IUI) performed for any indication?

Summary answer: Significantly higher LBR/OPR was noted for gonadotrophins versus oral agents. Clomiphene+estrogen and gonadotrophins+letrozole demonstrated significantly higher OPR/LBR compared to no stimulation/stimulation with oral agents alone.

What is known already: Stimulated IUI is one of the most widespread fertility treatments offered for various indications. The rationale of stimulated IUI cycles follows the concept that more available oocytes for fertilisation would lead to increase chances of conception. Although several small pairwise comparisons are reported between different stimulation agents, there is no overall comparison using a network meta-analysis. Optimising the ovarian stimulation is an important step towards evidence-based guidelines. There is a cost- effect balance which should be kept in mind between achieving multi follicular growth to boost success rates and keeping the intervention safe in relation to multiple pregnancies.

Study design, size, duration: This review has been conducted in accordance with PRISMA guidelines and has been registered in PROSPERO (CRD42022300857). A computerized literature search was performed using EMBASE, MEDLINE and CINAHL as well as the Cochrane Central register of trials from database inception to May 2022. Randomized controlled trials (RCTs) were included. A random effects network meta-analysis within a frequentist setting was performed for the primary outcome live birth rate/ongoing pregnancy rate (LBR)/(OPR) and for multiple pregnancy rate.

Participants/materials, setting, methods: Couples/single women undergoing one or more cycles of stimulated IUI for various indications using partner's or donor sperm were included. The effect sizes of the outcome were estimated as odds ratio (ORs) and presented along with their 95% confidence interval (CIs). We used network plots to illustrate head-to-head comparisons. The superiority of the interventions was assessed; we calculated the probability of being the best, the mean rank, and the surface under cumulative ranking (SUCRA).

Main results and the role of chance: 57 RCTs were identified comparing stimulation protocols. These included oral agents (clomiphene, letrozole, anastrozole, tamoxifene, estrogen), injectable hormones (FSH, hMG +/- GnRH agonists and GnRH antagonists), alone or in combination, compared to other stimulation agent (s). The included trials randomized 11341 participants across 14 countries between 1994 and 2020. Fourteen trials were multi-centre and 43 (75%) were single centre. Comparisons were assessed in relation to LBR/OPR and in relation to multiple pregnancy rate in order to identify which combination is superior in achieving maximum success rates without compromising safety.

The comparison between ovarian stimulation with gonadotrophins and clomiphene had the most trials and participants and showed significantly higher OPR/LBR for ovarian stimulation with gonadotrophins compared to clomiphene (OR: 1.58, CI 1.24-2.02). No stimulation or stimulation with clomiphene or aromatase inhibitors demonstrated significantly lower OPR/LBR compared to clomiphene + estrogen (OR no stimulation: 0.29, CI 0.10-0.83; OR clomiphene: 0.27, CI 0.10-0.69; OR letrozole 0.30, CI 0.11-0.83) and gonadotrophins + letrozole (OR no stimulation: 0.22, CI 0.06-0.82; OR clomiphene: 0.21, CI 0.06-0.73; OR letrozole 0.23, CI 0.06-0.85).The safety of each ovarian stimulation strategy was assessed in terms of risk of multiple pregnancy.

Limitations, reasons for caution: Comparing different stimulation protocols for IUI is challenging in view of heterogeneity amongst included trials. Different add-ons could benefit specific patient groups but based on the available data, safe recommendations cannot be proposed according to subfertility diagnosis. Various add-ons, protocol variations and sperm preparation techniques could also affect outcomes.

Wider implications of the findings: Stimulation strategies aiming to increase success rates should be safe in terms of multiple pregnancy. Strict cancellation criteria and monitoring are required. Cost effectiveness, ease of administration, chances of cycle cancellation and couple's preferences should be taken into consideration. Future trials should take into account subfertility background and patient characteristics.

Trial registration number: not applicable

Abstract citation ID: dead093.393
P-026 Effect of Microsurgical Varicocele Ligation in Patients with Isolated Oligozoospermia

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Study question: Is varicocelectomy effective in improving the semen quality of patients with isolated oligozoospermia (IO)?

Summary answer: Varicocelectomy is a valid option for infertile men with clinical varicocele and documented IO considering the significant improvement in semen concentration.

What is known already: Varicocele is the leading correctable cause of male factor infertility and its surgical treatment has been associated with sound improvement in semen quality and fertility potential. While varicocelectomy is clearly indicated in men with abnormal semen parameters, its effect on men with isolated abnormalities is less commonly assessed.

Study design, size, duration: The charts of 1521 patients who underwent microsurgical subinguinal varicocelectomy for clinically palpable varicocele between 2011-2021 were retrospectively reviewed. Infertile patients with IO were included in the study group, while data from patients with oligoasthenoteratozoospermia (OAT) were utilized for comparison.

Participants/materials, setting, methods: Men with azoospermia, a history of genitourinary infection, and those utilizing peri-operative fertility medications were excluded. The collected data included patient's demographics, varicocele grade and veins' sizes, and their semen (semen analysis, WHO 2010; sperm DNA fragmentation, Halosperm) and hormone parameters before and up to 6 months after varicocelectomy. Data were analyzed using Wilcoxon Signed Ranks test and chi-squared tests. A p value <0.05 was considered significant.

Main results and the role of chance: A total of 96 patients with IO and 123 patients with OAT were included in the study. Patients with IO had a mean age of 32.5 $\pm$ 7 years, and an average testis volume of 15.6ml. The varicocele was of grades 2 and 3 in 58% and 38% of patients. Among the IO group, a significant increase in sperm concentration was identified postoperatively ($p=0.01$). Surprisingly, results revealed a significant decrease in sperm total motility, however, the postoperative median was still within the normal range (45.5%). No significant changes were observed in other semen or hormone parameters. When a comparison was made with the OAT group, 83% of patients with IO had an improvement in sperm concentration compared to 63.9% of patients in OAT group following varicocelectomy ($P=0.044$). However, a higher percentage of patients showed an improvement in total

motility among the OAT group (67.6%) in comparison to the IO group (42.9%) ($p = 0.04$).

P-026 Table I

	Preoperative	Postoperative	p value
Volume	3 (2-4.4)	3.35 (2.15-5)	0.99
Concentration	9.8 (5.5-12)	16 (6.83-24)	0.01
Total Motility	54.5 (46-60)	45.5 (28-62)	0.03
Prog. Motility	12.5 (5-29.75)	10 (5-25.5)	0.54
Normal morphology	30 (6-58.75)	42.5 (15.75-65)	0.87
DNA Fragmantation	20 (15-30)	25 (21-38)	0.11

Limitations, reasons for caution: Results were obtained from a relatively small sample size. However, varicocele treatment is not necessarily considered as the first treatment option in men with mild or isolated derangements in semen quality.

Wider implications of the findings: Results of this study shed the light on the effect of varicocele treatment in men with isolated oligozoospermia and hence may be beneficial for patient counselling in the clinic.

Trial registration number: not applicable

Abstract citation ID: dead093.394

P-027 Absence of perinuclear theca ACTRT1 protein induces sperm head deformation and primary male infertility in humans

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Study question: Given that *Actrt1* knockout induces teratospermia, fertilization failure and severe male infertility in mice, will *ACTRT1* deficiency affect sperm morphology and male fertility in humans?

Summary answer: The ~110 kb microdeletion of X chromosome only including *ACTRT1* gene was identified inducing sperm head deformation and fertilization failure in an infertile Chinese man.

What is known already: The actin-related proteins, ACTRT1, ACTRT2, ACTL7A, and ACTL9, interact to form a multimeric complex in the sperm subacrosomal region and is vital for the attachment of acrosome to nucleus. The disruption of *ACTL7A* or *ACTL9* have been identified to cause acrosomal detachment, total fertilization failure and male infertility in humans and mice. *Actrt1*-knockout mice are severely subfertile also because of malformed sperm heads with detached acrosomes and a partial deficiency in fertilization. Loss/reduced expression and/or abnormal localization of PLCζ, a well-known sperm-borne activating factor, are identified in *ACTL7A/ACTL9* mutant patients as well as *Actrt1*-, *Actl7a*-, and *Actl9*-deficient mice.

Study design, size, duration: We recruited a cohort of 85 infertile men with teratospermia which is characterized by deformed sperm heads in the center for reproductive medicine from August 2019 to June 2022. Genomic DNA (gDNA) of patients was extracted from peripheral blood, then whole-exome sequencing and *in silico* analyses were performed to identify gene mutations. Morphological analysis, functional assays, and assisted reproductive therapy were performed in 2022.

Participants/materials, setting, methods: The *ACTRT1* deficiency was identified by whole-exome sequencing and confirmed by whole-genome sequencing, PCR and qPCR. Family members' gDNA was collected to define the hereditary mode. Papanicolaou-staining, scanning and transmission electronic microscopy were performed to reveal sperm morphologies. Western blot and immunostaining of spermatozoa were conducted to explore the pathological mechanism of the *ACTRT1* deficiency. Intracytoplasmic sperm injection (ICSI) combined with artificial oocyte activation (AOA) was applied for the assisted reproductive therapy of *ACTRT1*-deletion patient.

Main results and the role of chance: We identified a whole-gene deletion mutation of *ACTRT1* on Chromosome X in an infertile male with teratospermia, which was inherited from his mother. Papanicolaou-staining, scanning electronic microscopy, and transmission electronic microscopy showed the sperm head deformation owing to acrosome detachment, which mimicked the previously reported phenotype of *Actrt1*-knockout mice. The results of western blot and immunostaining suggested that ACTRT1 deficiency induced a down-regulated expression of ACTL7A and PLCζ proteins, but not ACTRT2, in human sperm samples. The detached acrosomes induced fertilization failure in assisted reproductive therapy of both *Actrt1*-knockout mice and the *ACTRT1*-deletion patient, which could be effectively rescued by ICSI combined with AOA.

Limitations, reasons for caution: Additional cases are needed to confirm the genetic contribution of *ACTRT1* mutations to male infertility with teratospermia and fertilization failure. In addition, the proband with *ACTRT1* deletion was with severe oligozoospermia and mild asthenozoospermia. The effect of *ACTRT1* mutations on sperm count and motility still need more investigation.

Wider implications of the findings: Our results reveal a gene–disease relationship between *ACTRT1* deficiency and human male infertility owing to teratospermia and fertilization failure. This report also describe a good fertility outcome of assisted reproductive therapy with ICSI and AOA for the *ACTRT1*-deficient patient.

Trial registration number: not applicable

Abstract citation ID: dead093.395

P-028 Loss of the m6A methyltransferase METTL5 impairs spermiogenesis and male fertility

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Study question: Whether and how METTL5 regulates germ cell development during spermatogenesis.

Summary answer: METTL5 is required for spermiogenesis. Loss of METTL5 resulted in teratozoospermia and male infertility via reduced translation of acrosome and flagellum formation proteins.

What is known already: The roles of m^6A modifications on mRNA in spermatogenesis have been extensively studied. It was reported that METTL5 knockout mice showed the brain development defect and sterility of 16-week male mice. However, the detailed mechanism of METTL5 affecting male fertility remains elusive.

Study design, size, duration: Mettl5 KO mice were kindly gifted from Prof. Shuibin Lin of The First Affiliated Hospital of Sun Yat-sen University. Heterozygotes of Mettl5 mice were used to generate Mettl5 homozygous knockout mice. The phenotype of KO mice was assessed. Ribosomal sequencing, proteomics analysis and further validation of protein translation were performed to explore the mechanism.

Participants/materials, setting, methods: Fertility assessment, sperm parameter analyses, sperm nuclear morphology analysis, Transmission electron microscopy (TEM), tissue Collection and histological analysis, protein extraction and western blot analysis, immunofluorescence studies, cDNA synthesis and qRT-PCR, in vitro fertilization (IVF) were used in this study.

Main results and the role of chance: Here we reported that methyltransferase-like 5 (METTL5) is involved in spermiogenesis as a methyltransferase mediating m^6A modification on rRNA. Mettl5 knockout mice were infertile

with teratozoospermia. The acrosome in the sperm head was absent with reduced sperm motility. Furthermore, the fertilization ability of sperm in the IVF experiment failed. Mechanistically, the level of rRNA m^6A modification was significantly decreased in the testes of Mettl5 KO mice. The translational efficiency and protein levels of acrosome and flagellum formation proteins such as FSIP2, ODF2, GK2, PGK2, and AKAP4 were significantly reduced when METTL5 was depleted.

Limitations, reasons for caution: The METTL5 mutation in the patient with teratozoospermia was not examined in the present study

Wider implications of the findings: The rRNA m6A modification is also involved in regulating spermatogenesis by METTL5. This study highlights the critical role of rRNA epigenetic modifications during spermatogenesis and provides novel theoretical explanations for the m6A modifications.

Trial registration number: Not applicable

Abstract citation ID: dead093.396
P-029 Centrifugation-free sperm separation device offers an efficient and standardized protocol to select high quality spermatozoa

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Study question: Can centrifugation-free sperm separation device be used to simplify and standardize the selection of high-quality spermatozoa?

Summary answer: Centrifugation-free CA0 sperm separation device minimizes inter- and intra-operator variability and yields spermatozoa with comparable fertilizing properties in a variety of semen conditions.

What is known already: Centrifugation-based sperm separation methods have been used in assisted reproductive technology (ART) for many decades. However, the conventional methods are criticized for harmful effects due to centrifugation. To overcome the disadvantage, more noninvasive technologies have been developed and attempted to improve the sperm separation process, e.g., the formation of capillary bridge to select motile spermatozoa, migration-sedimentation technique to sort out functional spermatozoa, microfluidic sorting chip to isolate healthy sperm. While these methods provide alternatives for noninvasive sperm separation, the limitations such as inconsistency of semen quality improvement and lack of standardized procedure remain to be resolved.

Study design, size, duration: A randomized controlled trial of 76 men who sought ART treatment (Lee Women's Hospital, Taichung, Taiwan) from June to October 2022 was carried out. Seventy-six neat semen specimens were categorized into 27 normozoospermic specimens, and 49 non-normozoospermic samples (semen quality below any of the WHO 5th Edition lower reference values: concentration <15 million/mL, total motility <40%, or normal morphology <4%).

Participants/materials, setting, methods: Neat semen samples were separated for three replicates (replicate 1-3) using LensHooke® CA0 sperm separation device (Bonraybio, Taichung, Taiwan). Three operators (operators 1–3) performed CA0 procedures on each sample to test inter-technician variability. Interclass-correlation coefficient (ICC) between replicates as well as operators were evaluated. Pre-selection and post-selection semen quality were evaluated. The parameters included total motility, progressive motility, rapid progressive motility, morphology, DNA fragmentation index (DFI), and acrosome reaction rate (AR).

Main results and the role of chance: CA0 selects self-propelling spermatozoa within a microenvironment created by a microporous filter membrane. The procedure involves three pipetting steps: loading semen sample, adding sperm washing medium, and recovering the processed sample. Following the standard procedure, CA0 resulted in a low intra- and inter-operator variability and ICC values between replicates and different operators were all greater than 0.9, indicating an excellent reproducibility of CA0. In addition, significant higher levels of motility and normal morphology were observed in post-selection specimens either of normozoospermic or non-normozoospermic samples (pre-selection vs. post-selection, all $p < 0.0001$). In paired analysis of the advanced semen parameter, our study showed noteworthy results that CA0 significantly improved DFI from 18.2% to 2.6% for normozoospermic samples; such reduction was also found in non-normozoospermic sample processing, from 13.4% to 4.2% (both, $p < 0.0001$). The levels of AR were significantly reduced in normozoospermic samples (from 14.4% to 5.4%) and non-normozoospermic samples as well (from 12.8% to 5.0%) (both groups $p < 0.0001$). In conclusion, CA0 provides an efficient, noninvasive, standardized, and reproducible sperm separation model that CA0 diminishes the variations and ensures sperm quality.

Limitations, reasons for caution: The presented study was a pilot trial examining the sperm quality improvement. Follow-up analysis on IUI/IVF outcomes associated with the improvement in semen quality utilizing CA0 will be assessed in future studies.

Wider implications of the findings: CA0 provides multifaceted benefits covering consistent clinical outcomes, simplified and standardized procedure, user-friendliness, and cost reduction. We believe CA0 not only allows noninvasive sperm separation of clinically usable but also gives the possibility of standardization on sperm separation procedure.

Trial registration number: CS2-22039

Abstract citation ID: dead093.397
P-030 Clinical outcomes of microdissection testicular sperm extraction (micro TESE) and intracytoplasmic sperm injection (ICSI) in non-obstructive azoospermia (NOA) with the history of cryptorchidism

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Study question: What is sperm retrieval rate (SRR) by micro TESE and the clinical outcomes using testicular sperm in couples with the history of cryptorchidism?

Summary answer: NOA couples with the history of cryptorchidism had a higher SRR by micro TESE but lower clinical outcomes by ICSI compared of unexplained NOA.

What is known already: Undescended testis (UT) which is exposed to a higher temperature compared with the scrotal temperature is associated with impairment of germ cell maturation, and progressive Leydig and Sertoli cell atrophy, and subsequent infertility in adulthood. There have been very few studies of ICSI with a focus on, or large enough numbers to examine, the specific outcomes associated with male factor infertility, however improvement in sperm retrieval techniques including micro TESE and micromanipulation techniques, such as ICSI, has led to excellent fertilization and pregnancy outcomes of treatment cycles.

Study design, size, duration: We performed a retrospective study based on two reproduction centers in Japan and evaluated 1521 azoospermic patients in our clinics between September 2013 and December 2022. We investigated SRR by micro TESE in these patients and therefore aimed to evaluate the prevalence and the significance of ICSI outcomes with embryonic development in NOA couples with the history of cryptorchidism.

Participants/materials, setting, methods: We evaluated SRR of micro TESE, two pronuclei (2PN) oocyte rates, blastocyst development, good-quality blastocyst (Grade 3BB and above on day 5 by the Gardner scoring), and clinical pregnancy rates per embryo transfer (ET) in 72 NOA cases with the

history of UT, 953 cases of unexplained NOA, not including after orchidopexy, Klinefelter syndrome, cryptozoospermia, mumps orchitis, and 284 cases of obstructive azoospermia (OA). Statistical analysis was performed using unpaired t-tests and chi-squared tests.

Main results and the role of chance: SRR of first attempt micro TESE in UT (44/56=78.6%) was higher than unexplained NOA (160/748=21.4%) ($p < 0.001$). Spermatozoa were successfully retrieved in 13 of 22 (59.1%) UT group and 36 of 226 (15.9%) unexplained NOA who had previously undergone micro TESE with no sperm found. No correlation was found between serum FSH, LH, and T level with the success of sperm retrieval. Testicular volume and patient age at orchidopexy also did not affect the SRR for micro TESE. 2PN oocytes, blastocysts development, and good-quality blastocysts rates were 45.5%, 47.4%, and 18.9% in UT, 51.2%, 45.0%, and 20.9% in unexplained NOA, and 62.6%, 52.1%, and 23.7% in OA, respectively. Clinical pregnancy rates per ET were 29.3% in UT, 32.3% in unexplained NOA, and 41.6% in OA. Significant differences were only observed in 2PN oocytes between unexplained NOA and OA ($p < 0.05$) and clinical pregnancy between UT and unexplained NOA ($p < 0.05$), and OA and unexplained NOA ($p < 0.01$). Several UT patients showed a very small number of spermatozoa and even only immotile sperm of retrieved by micro TESE.

Limitations, reasons for caution: We included the patients only after a surgery of cryptorchidism, but not a delayed testicular descent without surgery. The cohort size of this study is not small, however, our screened population of azoospermic patients may be biased.

Wider implications of the findings: This study shows a high impact in micro TESE and ICSI outcomes with embryonic development for the NOA couples with the history of cryptorchidism. Our study emphasizes that history of cryptorchidism provides clinically valuable prognostic information to couples considering surgical sperm retrieval.

Trial registration number: not applicable

Abstract citation ID: dead093.398
P-031 Presence of HPV 16 and HPV 18 in spermatozoa and embryos of mice

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Study question: The clarification of Human Pappiloma Virus (HPV) internalization in spermatozoa and early preimplantation embryos.

Summary answer: Spermatozoa are able to internalize constructs of cloned high risk HPV viruses either as integrated or as episomal DNA.

What is known already: Human Pappiloma Virus (HPV) is a non-enveloped, circular, double stranded DNA virus. Persistent infection with oncogenic HPV genotypes triggers HPV DNA integration into the host genome, eventually leading to chromosomal damage accumulation and genome destabilization in infected cells. Living spermatozoa of almost all species are able to take up spontaneously exogenous DNA and internalize a part of it into their nucleus.

Study design, size, duration: Taking into account the capability of sperm cells to integrate exogenous DNA into their genome, we sought to clarify HPV internalization into sperm genome. A total of 26 sperm samples were studied. The control group included 97 embryos. As for the HPV 16 and HPV 18 group, a total of 89 embryos were studied.

Participants/materials, setting, methods: Sperm was incubated with plasmid vectors containing the complete genome of human HPV 16 and HPV 18 tagged with the green fluorescent protein (GFP) gene, to investigate HPV 16 and HPV 18 integration in mouse spermatozoa. Oocytes were in vitro fertilized with preincubated spermatozoa to investigate HPV 16 and HPV 18 potential transfer to mouse embryos.

Main results and the role of chance: Spermatozoa were able to internalize constructs of cloned high-risk HPV either as integrated or as episomal DNA. Constructs of cloned HPV can also be transferred to mouse embryos, through in vitro fertilization of the oocytes by mouse spermatozoa.

Limitations, reasons for caution: Viral DNA integration into the sperm nucleus could not be directly demonstrated, but nevertheless integration is evident by GFP gene expression.

Wider implications of the findings: This study highlights the possibility of viral DNA transmission to the early embryo via sperm, opening new perspectives on the effect of HPV in reproductive cells. HPV persistence may impair sperm parameters, suggesting caution in the use of these cells for assisted reproduction techniques or sperm banking.

Trial registration number: Not applicable

Abstract citation ID: dead093.399
P-032 Exploration of the common genetic landscape of COVID-19 and male infertility

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Study question: What is the cross-talk molecular mechanisms between COVID-19 and male infertility?

Summary answer: The present study identified and validated hub COVID-19-related differentially expressed genes (CORGs) in COVID-19 and male infertility, and systematically explored molecular interactions and regulatory features.

What is known already: COVID-19 has spread widely across continents since 2019, causing serious damage to human health. Accumulative research uncovered that SARS-CoV-2 poses a great threat to male fertility. SARS-CoV-2 has been confirmed to exist in semen specimens and seminiferous tubes in the testes of male COVID-19 patients. COVID-19 had an unfavorable influence on the semen parameters in infertile men, including sperm count, motility, and morphology. Male infertility (MI) is a common comorbidity for the COVID-19 pandemic.

Study design, size, duration: The study included the bioinformatic calculation, participant recruitment, specimen collection, and experimental validation in the university hospital.

Participants/materials, setting, methods: Four transcriptome data regarding COVID-19 and male infertility were downloaded from the GEO repository, and were divided for initial analysis and external validation. Differentially expressed genes analysis, GO and pathway annotation, PPI network, connectivity ranking, ROC analysis, and immune infiltration were performed to gain hub CORGs. We recorded medical information of COVID-19 patients with male infertility and matched controls, and harvested their sperm samples. Expressions of hub CORGs were detected through the qRT-PCR technique.

Main results and the role of chance: We identified 460 overlapped CORGs in both the COVID-19 differentially expressed genes (DEGs) and MI DEGs. CORGs were significantly enriched in DNA damage and repair-associated, cell cycle-associated, ubiquitination-associated, and coronavirus-associated signaling. Module assessment of PPI network revealed that enriched GO functions were closely related to cell cycle and DNA metabolism processes. Pharmacologic agent prediction displayed protein-drug interactions of ascorbic acid, biotin, caffeine, and L-cysteine with CORGs. After connectivity ranking and external validation, three hub CORGs (ENTPD6, CIB1, and EIF3B) showed good diagnostic performance (area under the curve > 0.75). Subsequently, three types of immune cells (CD8+ T cells, monocytes, and macrophages M0) were dominantly enriched, and 24 transcription factor-CORGs interactions and 13 miRNA-CORGs interactions were constructed in the network. Finally, qRT-PCR analysis confirmed that there were significant differences in the expression of hub CORGs (CIB1 and EIF3B) between the patient and control groups.

Limitations, reasons for caution: Diverse sequencing platforms and different ethnic subjects in the genetic data have influence on the accuracy of our analysis to some extent.

Wider implications of the findings: It is the first time for us to mine the genetic interrelationship between COVID-19 and male infertility. Our study provided new clues for the pathogenesis of these two diseases, and shared emerging potential biomarkers and drug targets for COVID-19-associated male infertility.

Trial registration number: National Natural Science Foundation of China (No. 82201758, No. 81700667, and No. 82201775)

Abstract citation ID: dead093.400
P-033 Impact of the sperm reactive oxygen species on Intrauterine Insemination outcome

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Study question: Does sperm reactive oxygen species values affect the pregnancy rate after Intrauterine Insemination (IUI)?

Summary answer: Sperm reactive oxygen species values affect pregnancy rate and can be used as a predictive marker for IUI outcome.

What is known already: Oxidative stress resulting from excessive production of reactive oxygen species (ROS) can profoundly affect the sperm and subsequent functional sperm integrity. Diagnostics to measure semen oxidative stress have been added to the 6th edition of the WHO laboratory manual for examining and processing of human semen, under advanced examinations. Although a semen analysis has classically been used as the gold standard for determining a man's fertility, basic sperm analysis alone cannot accurately predict infertility since between 6 and 27 % of men with normal semen parameters are infertile. Determination of oxidative stress in diagnosing infertility may contribute to better outcomes.

Study design, size, duration: This study was based on semen analyses of 50 male patients whose samples were used for IUI treatment between November 2022 and January 2023. The oxidation-reduction potential was measured using novel galvanostat-based technology, the MiOXSYS System. Briefly, 30 µL of liquefied semen at room temperature were applied to the MiOXSYS sensor. Both absolute ORP (mV) and normalized sORP values (mV/10 ^{6} sperm/ml) based on sperm concentration were calculated.

Participants/materials, setting, methods: Sperm analysis were performed according to the WHO laboratory manual sperm criteria (Sixth edition 2021). All participants followed a period of 3–5 days of sexual abstinence and had a diagnosis of unexplained infertility (normozoospermia). Ovarian stimulation for IUI cycle was initiated on the 5th day of menstruation by using recombinant gonadotropins. Clinical pregnancy was determined based on the Beta-hCG values. For statistical comparison between group values the Mann-Whitney U test was used.

Main results and the role of chance: According to the guidelines of the World Health Organization, all participants included in the study had normal BMI (21.85 ± 3.80 kg/m^2). The average age of the female patients included in the study was 33.9 ± 3.1 years while the average age of the male patients was 37.8 ± 4.8 years. Out of the 50 patients, 32% (N = 16) got pregnant. For the study propose sperm ORP and sORP values were compared between patients based on the pregnancy outcome. Statistical analysis showed a significant difference in ORP values. Patients with a positive pregnancy outcome after IUI had a lower ORP value compared to patients where IUI was not successful [25.65 [6.7-52.6] mV vs. 40.2 [6.3-109.8] mV, respectively, p = 0.02]. Additionally, normalized values according to the sperm concentration (sORP) were also significantly different between those groups [0.37(0.17-1.44) mV/10 ^{6} sperm/ml vs. 0.88(0.13-20.81) mV/10 ^{6} sperm/ml, respectively p = 0.006]. Due to the above-mentioned significant difference logistic regression analysis were performed, and the obtained results confirm that sperm ORP (p = 0.02) as well sORP (p = 0.016) significantly affects the pregnancy rate after the IUI procedure.

Limitations, reasons for caution: The limitation of the presented study was a relatively small patient cohort. Additionally, pregnancy was determined based on the beta hCG values, therefore future research should link ORP values and live birth rate as well.

Wider implications of the findings: In the present study suggests ORP as an additional parameter with diagnostic value to identify the best therapeutic approach concerning IUI in couples that show minimal or no impairments of the WHO semen parameter.

Trial registration number: not applicable

Abstract citation ID: dead093.401
P-034 Clinical experience with expanded carrier screening results on a sperm donor applicant population

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Study question: What is the frequency of clinically significant results from expanded carrier screening (ECS) in a sperm donor applicant population and how should these be managed?

Summary answer: ECS results revealed clinically significant information for approximately 1 in 51 donor applicants, which warrant specific management considerations in the context of third-party reproduction.

What is known already: The American Society of Reproductive Medicine (ASRM) Practice Committee published guidance in January 2021 that outlines the recommended carrier screening approach for gamete donors. However, there is no additional direction related to results management including donor suitability, counseling, and informed consent. Prior studies have illustrated the high frequency of donors who are identified as carriers of one or more recessive disorders on an ECS panel; thus, excluding all prospective gamete donors identified as carriers for recessive conditions is not feasible or appropriate given the availability of reciprocal screening for the reproductive partner.

Study design, size, duration: A retrospective review of donor genetic screening records from July 2017 to December 2021 was performed. Relevant data was extracted and categorized by carrier screening result. Genetic counselors evaluated the health and reproductive risks to the potential donors (PDs) associated with being a carrier of a pathogenic mutation in each gene using published data, reference laboratory interpretations, and the professional health management guidelines.

Participants/materials, setting, methods: ECS was performed on sperm donor applicants as part of the routine donor qualification process. Testing was performed at an outside reference laboratory after participants provided written consent for genetic testing. The genes included on the ECS panel were analyzed using multiple methodologies. Specific methodologies varied based upon laboratory offerings at the time in which the potential donor entered the donor program.

Main results and the role of chance: A total of 966 PDs had ECS during the specified timeframe. Of these applicants, 19 total PDs (1.97%) were identified as having potentially significant health risks based on their ECS results. Of those 19 PDs, eleven were found to be either heterozygous or hemizygous for conditions that may convey significant health risks to carriers. Of these, nine were positive for a variant in a gene typically associated with an AR condition and two carried variants in genes associated with X-linked conditions. Eight additional PDs were found to be either compound heterozygous or homozygous for variants in a gene associated with an autosomal recessive condition.

Limitations, reasons for caution: Donor recruitment was limited to select major metropolitan areas in the U.S.; thus, donor applicants may not represent all ethnic groups or socioeconomic backgrounds. Additionally, this study described results from a single reference laboratory and does not illustrate the variation in ECS panels across genetic testing laboratories.

Wider implications of the findings: While this study examined ECS results in a donor applicant population, findings are also applicable to the general reproductive population and illustrate the necessity of informed consent and post-test counseling. In some cases, ECS may provide insight on potential future health risks and health management opportunities.

Trial registration number: Not applicable

Abstract citation ID: dead093.402

P-035 Effect of Varicocelectomy on normal semen in patients undergoing the procedure for indications other than fertility: evidence needed for counseling

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Study question: Does microsurgical varicocelectomy impact normal semen parameters in fertile patients undergoing the procedure for indications other than infertility?

Summary answer: Micro surgical varicocelectomy does not alter the fertility potential of men with normal semen.

What is known already: Varicocele repair results in improvement in semen parameters and fertility potential in sub fertile patients with clinical varicocele. However, the procedure is also offered to patients with indications other than infertility including testicular pain, testicular size affection, or before army recruitment. Queries about the effect of varicocelectomy on fertility potential in this patient population have been raised but unfortunately not strongly backed by literature.

Study design, size, duration: A retrospective study of 1521 patients who underwent microsurgical sub inguinal varicocelectomy in our center from 2011–2021.

Participants/materials, setting, methods: A total of 435 patients with normal semen parameters and underwent varicocelectomy for indications other than infertility were included. Exclusion criteria were patients with infertility, abnormal semen parameters, and prior varicocelectomy. Patient demographics, clinical and laboratory data were collected. Semen analysis (WHO 5th edition), sperm DNA fragmentation (Halosperm) & hormonal assay were performed pre-operative and up to 6 months post-operative. Data were compared using Wilcoxon Signed Ranked Test. A p value <0.05 was considered significant.

P-035 Table I

	Pre-operative Median (IQR)	Post-operative Median (IQR)	P value
Volume	3 (2 -4.5)	3 (2 -4)	0.134
Concentration	39 (26 -57)	39.5 (23 -61.7)	0.872
Total Motility	59 (50 -65)	60 (48 -67)	0.405
Progressive Motility	22 (10 -37)	28 (10 -42)	0.499
Sperm DNA fragmentation	21.2 (16 -31.5)	24 (14 -38)	0.816
Normal Morphology	26 (8 -52)	37 (11.5 -55.5)	**0.042**
Estradiol	92 (70 -119)	96 (67 -120.5)	0.294
LH	3.7 (2.6 -4.9)	3 (2 -5)	0.827
FSH	3 (2 -4.2)	3 (2 -5)	0.704
Testosterone	16.8 (13.1 -21.6)	16.5 (13.5 -21.5)	0.475
Total Motile Sperm Count	66.5 (38.9 -119.2)	59.3 (30.8 -110.7)	0.140

Main results and the role of chance: The mean age was 32.8 ± 7.7 years. Varicocelectomy was performed on left side in 373 patients and bilateral in 62 patients. The majority of the study population had grade III varicocele on the left side. There were no statistically significant changes in semen volume, concentration, total & progressive motility, sperm DNA fragmentation, and hormonal profile after 6 months post varicocelectomy in comparison to initial baseline parameters (table 1). Only a statistically significant improvement in morphology was observed.

Limitations, reasons for caution: The main limitation is the retrospective design of the study. Long-term effect and conception rate were not evaluated.

Wider implications of the findings: Results of this work are beneficial during preoperative counseling of patients with normal semen undergoing varicocelectomy for non-fertility indications. Patients can be reassured that the procedure would not affect their fertility potentials negatively.

Trial registration number: not applicable

Abstract citation ID: dead093.403

P-036 Relationship between days of male sexual abstinence on the day of oocyte retrieval and clinical outcomes

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Study question: Does male sexual abstinence affect clinical outcomes in oocyte autologous and donation cycles?

Summary answer: A reduced sexual abstinence period significantly improved ongoing pregnancy rates in oocyte donation cycles

What is known already: Among various factors affecting sperm quality, the period of sexual abstinence is often overlooked. The World Health Organization (WHO) has established specific standards for sperm analysis and advocates 2-7 days abstinence prior to ejaculation. Other guidelines (ESHRE and NAFA) limit this time to a shorter interval of 3-4 days. Studies suggest that a shorter abstinence period may reduce oxidative stress and improve motile sperm count. However, there is still no consensus on the optimal period of sexual abstinence for achieving the best clinical outcomes when undergoing assisted reproduction techniques (ART).

Study design, size, duration: Retrospective, single-centre study including 479 ICSI cycles (January 2018 to December 2021). First embryo transfer of the first cycle per patient for both autologous ($n=217$) and donor ($n=38$) oocyte cycles were analysed. Preimplantation Genetic Testing cycles and those employing microfluidic devices for sperm preparation were excluded as well as patients with abnormal karyotypes, uterine abnormalities and evidence of chronic infectious diseases (Hepatitis B, C or HIV).

Participants/materials, setting, methods: All cycles used male partner sperm. Two abstinence periods were compared: short ($\leq$ 2 days, $n=108$ and $n=16$) or long ($\geq$ 3 days, $n=109$ and $n=22$) for autologous and donor oocytes, respectively. Outcomes considered: seminal parameters; fertilized, 1PN, 3PN, >3PN and degenerated oocytes; implantation and ongoing pregnancy rates. Analysis of variance, Chi-square test and student t-test (Mann Whitney) were used to calculate p-values. A p-value of <0.05 was considered significant.

Main results and the role of chance: Mean paternal age was 34.9 and 35.2 years for autologous and oocyte donor cycles, respectively. Regarding sperm parameters, a significant increase in concentration was seen in the long abstinence group ($p=0.009$). There was a trend towards improved fertilization and implantation rates in the short abstinence group and also towards lower rates of 1PN, 3PN, >3PN and degenerated oocytes in oocyte donation cycles. Moreover, oocyte donation cycles linked to short abstinence, showed significantly higher ongoing pregnancy rates compared to those involving a long abstinence period ($p=0.015$). A similar trend was observed in autologous cycles.

Limitations, reasons for caution: The small sample size and retrospective design warrant careful interpretation.

Wider implications of the findings: Our findings suggest that the length of sexual abstinence may have an impact on sperm quality and clinical

outcomes. Reduced abstinence may improve ongoing pregnancy rates, potentially due to reduced levels of sperm DNA fragementation. New criteria for abstinence periods should be considered.

Trial registration number: not applicable

Abstract citation ID: dead093.404

P-037 The extent of paternal effect: the blastocyst matters! A retrospective analysis of 1351 single, frozen-thawed embryo-transfers

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Study question: Is ongoing pregnancy rate (OPR) impaired by total motile count (TMC) values in IVF/ICSI cycles?

Summary answer: TMC does not affect the OPR in IVF/ICSI cycles.

What is known already: Studies exploring the paternal role and specifically the influence of sperm parameters on the IVF/ICSI outcomes led to contradictory results, especially after the blastocysts formation. If a negative impact of severe male infertility on fertilization rate, embryo morphology and blastocyst formation rate have been described by several studies, its effect on implantation rate (IR), OPR and live birth rate (LBR) is still debated. In this context, TMC is an useful and validated tool for sperm evaluation, combining three critical sperm parameters into a single indicator of sperm quality.

Study design, size, duration: Retrospective analysis of 1351 freeze-all IVF/ICSI cycles performed between January 2015 and June 2022 in the Reproductive Sciences Unit of Gynaecology/Obstetrics Department of San Raffaele Hospital in Milan, Italy.

Participants/materials, setting, methods: We included all the first, single, frozen-thawed transfers performed in the study period. Transfers of blastocysts obtained by frozen semen were excluded. Couples' characteristics, semen parameters and controlled ovarian stimulation data were collected. Semen quality was assessed using TMC and grouped into quartiles of TMC for the analyses. The primary outcome of the study was the evaluation of the effect of TMC on the implantation potential of the obtained blastocysts.

Main results and the role of chance: 1351 transfers were analyzed. Performing a logistic regression analysis adjusted for confounding factors (maternal age, number of oocytes retrieved and quality of transferred blastocysts), OPR was not influenced by TMC values [odds ratio (OR) = 1.00; confidence interval (CI) = 0.99-1.00; p = 0.5]. After grouping the male infertility population into quartiles of TMC, no significant differences in OPR were found between extreme quartiles (n = 339, TMC ≤4 million and n = 348, TMC ≥42 million respectively), even when adjusted for confounders [OR = 0.99; CI 0.73-1.35; p = 0.97]. Therefore, the implantation potential of the obtained blastocysts, once formed, seems to be independent from the sperm quality.

Limitations, reasons for caution: The major limitation of the study is the retrospective design. Additionally, although a positive correlation between TMC and pregnancy rate is well described, a TMC threshold for defining severe male infertility has not been yet identified.

Wider implications of the findings: With a larger sample size we confirmed the results of our previous study. Indeed, TMC did not demonstrated to impair OPR of blastocysts from infertile couples submitted to IVF/ICSI. Further data, such as pregnancy and obstetrics outcomes, would be necessary to better define the role of male infertility in reproduction.

Trial registration number: Not applicable

Abstract citation ID: dead093.405

P-038 Correlation between sperm DNA fragmentation index, semen parameters and Human Papillomavirus: an analysis conducted under World Health Organization 2021 guidelines

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Study question: Following 6thWHO (2021), we analysed the correlation between DNA fragmentation index (DFI), Human papillomavirus (HPV), and seminal parameters, highlighting slow and rapid progressive motility alterations.

Summary answer: DFI rates and seminal parameters correlated with rapid, slow, and progressive motility. However, HPV-positivity caused the loss of association between DFI and slow progressive motility.

What is known already: HPV detection in semen samples has long opened an investigation into its influence on male infertility. Some studies indicate that HPV can affect sperm quality and DFI, while others have failed to find any correlation. With reference to 2010 WHO guidelines, our latest work highlighted how HPV positivity significantly impairs progressive motility, morphology, and immotile sperm rate. Since the latest 2021 WHO guidelines included the evaluation of slow and rapid progressive motility and DFI, we analysed if these new parameters and the other conventional parameters could be altered by HPV infection.

Study design, size, duration: From August 2021 to December 2022, 121 semen samples were collected from male partners of HPV-positive women attending in vitro fertilization (IVF). Every specimen underwent DFI evaluation, analysis of seminal parameters, and HPV test.

Participants/materials, setting, methods: Seminal samples were collected by masturbation after 3-5 days of sexual abstinence. The inclusion criteria were as follows: no other sexually transmitted infections, no genetic diseases, and no inflammatory disorders. Sperm concentration, morphology, non-progressive and immotile sperms, and both slow and rapid progressive motility were evaluated according to WHO 2021 guidelines. DFI analysis was assessed by sperm chromatin dispersion test (SCD), while HPV-DNA detection was performed using InnoLipa HPV Genotyping Extra II (Fujirebio, Tokyo, Japan).

Main results and the role of chance: Of the 121 semen samples tested, 60 (49.6%) were HPV-positive and 61 (50.4%) were HPV-negative. DFI rates showed a significant negative correlation with rapid progressive motility in both groups and a positive correlation with slow progressive motility in the HPV-negative group. Conversely, the significance of the correlation between DFI and slow progressive motility was completely lost in HPV-positive patients. Sperm concentration, normal forms and immotile spermatozoa percentages were correlated with both motility parameters in the HPV-negative group. Similar results were observed in HPV-positive samples, except for the normal form rate, which was not associated with slow progressive motility. In addition, the same samples displayed a negative correlation between non-progressive motility and rapid progressive motility, absent in HPV-negative samples. Significant associations were found also for the derived parameter of progressive motility, which was correlated with DFI, sperm concentration, immotile sperm, and normal forms rate in both groups. The results suggest how high DFI rates, in the presence or absence of HPV infection, could affect reproductive health through a consistent impairment of spermatozoa motility. In

particular, the distinction of slow and rapid progressive motility by WHO 2021 allows a deeper understanding of the possible correlations between DFI, semen parameters and HPV infection.

Limitations, reasons for caution: This is a preliminary study characterized by a small number of samples. Therefore, confirmation of these findings requires the enlargement of the patient cohort, which is already taking place.

Wider implications of the findings: Our results highlight how the introduction of the new WHO 2021 evaluation criteria, i.e. DFI, and slow and rapid progressive motility, provides additional information about sperm quality and the impact of HPV infection on it.

Trial registration number: Not applicable

Abstract citation ID: dead093.406
P-039 Pregnancy outcomes and genetic analysis of globozoospermic patients: A case series and review of the literature

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Study question: How should men with globozoospermia be managed in Assisted Reproductive Technologies (ART)?

Summary answer: IMSI (intracytoplasmic morphologically selected sperm injection), without oocyte activators (OA) allows to obtain a live birth, even when there is a mutation of DPY19L2 gene.

What is known already: Globozoospermia is a rare syndrome found in less than 0.1% of the infertile population. It is characterized by the presence of spermatozoa with round heads devoid of acrosomes. Two main genes are involved in the transmission of the disease: SPATA 16 and DPY19L2. Only Assisted Reproductive Technologies (ART) such as ICSI (intra-cytoplasmic sperm injection) and IMSI, with or without OA allow these couples to obtain pregnancies. There are no live births described after IMSI without OA in patients with a DPY19L2 mutation.

Study design, size, duration: Four infertile couples followed in the Department of ART at the Poissy Hospital Center, were studied between 2011 and 2022.All patients had total globozoospermia. Three patients were tested for the DPY19L2 gene mutation. Patients were managed in ART with the IMSI technique without the use of OA.

Participants/materials, setting, methods: The first patient had a homozygous mutation of the DPY19L2 gene. Two live births were obtained after IMSI.

The second patient was not DPY19L2 mutated. 2 IMSI were performed, resulting in a biochemical pregnancy.

The third patient had a homozygous mutation of the DPY19L2 gene. An IMSI was performed without obtaining a pregnancy, a second attempt is scheduled soon.

The fourth patient refused genetic testing and IMSI management. Sperm donation was performed without achieving pregnancy.

Main results and the role of chance: In this case series, we present the management of four globozoosperm couples in the Reproductive Biology and Andrology Department at the Poissy Hospital Center. We also present the first two live births after performing an IMSI, without OA, in a globozoospermic patient with a homozygous mutation of DPY19L2 gene. In fact, according to our review of the literature, 53 case reports or articles of patients having benefited from ART with or without OA have been reported in globozoospermic patients. In these couples, 60 children were born. However, no live births had been reported until now in couples managed in IMSI without OA, where men had globozoospermia with DPY19L2 mutation.

Limitations, reasons for caution: The use of OA to increase the membrane permeability of oocyte to calcium and increase fertilization rates. The highest number of live births in the globozoosperm population occurs after ICSI or IMSI with OA. There is no described live birth without the use of OA in a DPY19L2 mutated patient.

Wider implications of the findings: The use of OA remains debated and is not authorized in some countries. Although our study found low fertilization rates after IMSI, it's possible to obtain live births. The selection of spermatozoa with an acrosome outline at high magnification is thus an interesting alternative to the use of oocyte activators.

Trial registration number: not applicable

Abstract citation ID: dead093.407
P-040 The Role of ACE2 and DNA fragmentation index on covid-19 post infected, healthy and infertile men

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Study question: to investigate the relationship between ACE2 and DNA fragmentation index and their impact on men's reproductive system in covid-19 post-infected patients.

Summary answer: the drastic effect of covid-19 on the male reproductive system might be due to elevated DFI and ACE2 levels.

What is known already: In the last two years, many studies had been devoted to uncovering the depraved impact of covid-19 by measuring ACE2 concentrations or sperm DNA fragmentation but no study was applied to measure both parameters, this study performed on both parameters to figure out the role of angiotensin-converting enzyme (ACE2) with seminal fluid parameters and DNA fragmentation index in covid-19 post infected men and infertile patient in comparison to healthy fertile men.

Study design, size, duration: This prospective cohort study was conducted from November 2021 to June 2022. one hundred and nine men who participated in this study.

Participants/materials, setting, methods: The participants were divided into four groups: post-infected men with Covid-19 who recovered from Covid-19 infection with a recovery interval of no more than three months from the time of symptoms onset and all were fertile married men, healthy fertile men who haven't been infected with Covid-19 in the last three months obtain normal seminal fluid parameters as control, The third group is composed of oligospermia, while the fourth group is constituted of azoospermia volunteers

Main results and the role of chance: In this study, the result showed that the seminal fluid parameters for post-infected men with covid-19; were demotion in sperm concentration, total count, motility, and morphology with a p-value < 0.001 when compared with healthy men. DNA fragmentation in post-infected men was 4 times higher than in non-infected and it was found that post-infected men had 10 times more ACE2 concentrations in comparison with healthy men. The study showed that in oligospermia and azoospermia volunteers own a high ACE2 level compared to healthy volunteers and that the oligospermia subjects have a significantly high sperm DNA fragmentation index.

In conclusion, the drastic effect of covid-19 on the male reproductive system might be due to elevated DNA fragmentation and ACE2 levels, and there is a statistically significant positive correlation between the DNA fragmentation index and ACE2 levels.

Limitations, reasons for caution: number of post-infected men with covid-19

Wider implications of the findings: 1- A positive relationship exists between DNA fragmentation index percentage (DFI) and angiotensin-converting enzyme level (ACE 2).

2- Covid-19 infection alters sperm parameters such as concentrations, total sperm count, motility, and morphology.

3- The percentage of DNA fragmentation rose significantly after covid-19 infection.

Trial registration number: self funding

Abstract citation ID: dead093.408
P-041 Enhancing Embryos of the Desired Sex in Couples Undergoing PGT-A while Minimizing Embryo Wastage

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Study question: Can we enhance the proportion of euploid embryos of the desired sex for couples undergoing ICSI and PGT-A with sex selection while reducing embryo wastage?

Summary answer: Using a novel sperm selection technique, we generated higher proportions of embryos and offspring of the desired sex without impairing clinical outcomes or offspring health.

What is known already: Although controversial, the wish for offspring of a specific sex is common in the U.S. Many couples, particularly those requiring IVF with PGT-A, request a euploid embryo of a specific sex. This is often associated with the generation of more conceptuses of the unwanted sex, resulting in embryo wastage. We propose utilizing a simple and safe sex selection technique (SST) to skew spermatozoa toward the desired sex so that concurrent embryos can be generated in higher proportions.

Study design, size, duration: Over a 7-year period, ejaculates from male partners of couples (n = 144) undergoing ICSI with PGT-A were processed using SST to enrich spermatozoa for their preferred sex. Standard sperm processing was carried out for couples undergoing ICSI exclusively to assess conceptus aneuploidy, comprising the control group (n = 1,440). The proportion of male and female spermatozoa in the initial and selected specimens, PGT-A results, and ICSI outcomes following frozen embryo transfer (FET) were compared between the two groups.

Participants/materials, setting, methods: A total of 1,584 couples were treated in 2,723 ICSI cycles. Standard sperm processing was performed for 1,440 couples who did not have offspring sex preferences, comprising the control. For 144 consenting couples, SST was used to enrich spermatozoa for either sex (IRB 1306014043). To confirm sex enrichment and ploidy, ≥1,000 sperm cells were screened by fluorescent in-situ hybridization (FISH) for nine chromosomes. Embryology and PGT-A outcomes were compared between the control and SST cohorts.

Main results and the role of chance: Couples (n = 1,440) from the control cohort (maternal age, 37.1 ± 4yrs; paternal age, 39.1 ± 6yrs) underwent 2,541 ICSI cycles, yielding an 80.1% fertilization rate (15,901/19,859). PGT-A confirmed that 46.6%(n = 4,479) of conceptuses were female and 53.4%(n = 5,132) were male. These couples achieved 76.2%(866/1136) implantation and 64.9%(737/1136) clinical pregnancy rates, resulting in 624 healthy deliveries (48% female, 52% male).

From the study cohort (n = 144), 79 desiring female offspring (maternal age, 37.9 ± 4yrs; paternal age, 40.8 ± 6yrs) obtained an 81.6% sperm sex enrichment. They underwent 98 ICSI cycles, achieving a 76.7%(792/1032) fertilization rate, resulting in a greater proportion of female embryos (79.1%, 351/444) than the control ($P<0.05$), of which 78.6% (276/351) were euploid. Following FET, 39 couples obtained a 79.5% (31/39) implantation rate, yielding 27 clinical pregnancies with deliveries, thus far, of 16 female singletons, all developing normally.

The remaining 65 couples (maternal age, 37.6 ± 3yrs; paternal age, 40.8 ± 5yrs) preferring male offspring obtained 80.8% male sperm enrichment and underwent 84 ICSI cycles, achieving a 74.9%(723/965) fertilization rate with a greater proportion of 79.3%(249/314) male embryos compared with the control ($P<0.05$), of which 66.3%(165/249) were euploid. Following FET, their implantation rate was 90.0%(36/40), yielding 25 clinical pregnancies that resulted in the deliveries, thus far, of 20 male singletons, all developing normally.

Limitations, reasons for caution: Although SST does not absolutely grant offspring of a specific sex, it allowed couples to obtain a greater proportion of conceptuses of their preferred genotype. This method does not replace PGT-A for sex selection, but rather enhances the generation of embryos of the desired sex, thereby reducing embryo wastage.

Wider implications of the findings: We confirmed a significantly higher proportion of conceptuses of the desired sex. Thus far, embryo developmental competence, aneuploidy rates, delivery rates, and offspring health were not impaired by SST-processed spermatozoa. This supports the safety of SST, rendering it effective and ethically palatable.

Trial registration number: n/a

Abstract citation ID: dead093.409
P-042 Sperm Selection Based on Speed Using Rheotaxis in a Parallel Array of Microfluidic Apertures

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Study question: Does selecting human spermatozoa using a dynamic microfluidic system impact semen parameters and genomic characteristics?

Summary answer: Flow rate adjustment in a dynamic microfluidic device selects spermatozoa with superior DNA integrity. It also appears to select a higher proportion of Y-chromosome-bearing spermatozoa.

What is known already: Mammalian spermatozoa demonstrate rheotactic motion against the flow. This is important in order to overcome the fluid drag force in the female genital tract to reach the oocyte. Sperm processing methods have been developed based on these principles; more recently, static and dynamic microfluidic devices have been tested due to the association between high progressive motility and greater genomic integrity. These devices also appear to have an impact on sperm sex selection.

Study design, size, duration: Human ejaculates (n = 7) were evaluated and simultaneously processed by density gradient centrifugation (DGC) or a novel microfluidic sperm sorter (MFSS) with an adjustable flow rate inspired by the constriction of the uterotubal junction. Concentration, motility, morphology, and SCF were assessed and compared between raw, DGC-, and MFSS-processed specimens. To identify differences in the SCF and the proportion of X- and Y-spermatozoa, spermatozoa isolated at different flow rates (150, 250, 350, and 450 µl/h) were evaluated.

Participants/materials, setting, methods: Ejaculates were evaluated by standard semen analysis according to WHO 2021 criteria. Following complete liquefaction, 1 mL of sample was used for DGC, and 0.8 mL of semen was used for the prototype MFSS at 0.2 mL for each flow rate. SCF was assessed by TUNEL assay with a normal threshold of ≤ 15%. Fluorescent in situ hybridization (FISH) was performed using X- and Y-chromosome probes.

Main results and the role of chance: A total of 7 men (35.4 ± 5 years) had the following semen parameters: volume of 3.3 ± 2 mL, concentration of 76.7 ± 33 $x10^6$/mL, 45.5 ± 1% motility, and a normal morphology of 3.2 ± 0%. When comparing the post-processing parameters between the two methods, DGC yielded a concentration of 49.9 ± 25 $x10^6$/mL and MFSS yielded a reduced concentration of 4.5 ± 5$x10^6$/mL ($P<0.0001$). DGC yielded 91.1 ± 1% motility and normal morphology of 3.2 ± 0.4%. Albeit at a cost of concentration, MFSS yielded spermatozoa with significantly higher motility (96.3 ± 3%; $P<0.0001$) and a higher proportion with normal morphology (4.0 ± 0.6%; $P<0.0001$). Moreover, the SCF of the raw sample was 9.4 ± 2.0%; although the SCF was 8.1 ± 3.6% with DGC, it was 4.5 ± 2% ($P<0.001$) with MFSS.

An analysis was performed within the different flow rates of the MFSS; a 250 µl/h flow rate yielded the highest recovery rate (42.0 ± 6%; $P<0.001$), highest concentration (5.6 ± 4 $x10^6$/mL; $P<0.01$), highest motility (97.0 ± 2.2%; $P<0.05$), and a normal morphology rate (4.3 ± 1%). The SCF for this flow rate was <3%. Surprisingly, we noticed that the proportion of Y-chromosome–bearing spermatozoa positively correlated to the flow rate,

suggesting that there may be a subtle difference in the kinetics of male and female spermatozoa.

Limitations, reasons for caution: Although our novel MFSS platform is a promising technique, it is costly and requires specific expertise and training. This is a limited observation and requires integration of clinical outcomes to prove its benefits.

Wider implications of the findings: A microfluidic device with a controlled flow rate allowed the fine-tuned selection of spermatozoa with superior hydrodynamic and kinetic characteristics and a higher genomic integrity. Once automated, this device may help to select spermatozoa with greater embryo developmental competence and be useful to isolate spermatozoa of the desired sex.

Trial registration number: N/A

Abstract citation ID: dead093.410
P-043 Increased paternal age adversely affects live birth rates in oocyte recipient cycles

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Study question: To investigate the influence of paternal age on the live birth rates in oocyte recipient cycles

Summary answer: Increased paternal age appears to have a negative impact on oocyte recipient cycles when adjusted for sperm quality.

What is known already: While the effect of maternal age on ART outcomes is well established, the effect of paternal age is unclear. Even systematic reviews assessing the impact of paternal age in oocyte recipient cycles have yielded conflicting results. While Morris et al., 2020 could not demonstrate changes in miscarriage rates or live birth rates with increased paternal age, Murugesu et al., 2022 showed an adverse effect of increased paternal age on miscarriages but did not comment on live birth rates. The challenges of systematic reviews are the heterogeneity of the studies and the possible effect of laboratory and clinical practices.

Study design, size, duration: This retrospective cohort study was conducted at a single centre. A total of 449 IVF/ICSI oocyte recipient cycles performed between January 2015 and June 2022 were examined. Total of 328 cycles resulting in either fresh or frozen-thawed embryo transfers and meeting the inclusion criteria was analysed. The inclusion criteria were donor age $\leq$35 years, endometrial thickness $\geq$ 6 mm and a normal endometrial cavity on aqua scan. We excluded cycles with donor sperm or PGT-A.

Participants/materials, setting, methods: The couples who used donor oocytes because of diminished ovarian reserve, premature ovarian insufficiency, recurrent implantation failure with autologous oocytes or hereditary disease participated. Paternal and recipient ages were grouped according to previous literature to allow for comparability. The primary outcome was the live birth rate and secondary outcomes were clinical pregnancy and miscarriage rates. Simple and multivariate logistic regression analyses were performed.

Main results and the role of chance: The median recipient and paternal ages were 42 years (interquartile range [IQR] 19–50) and 42 years (IQR 19–76), respectively. The mean age of the donors was 26 years (IQR 18-35).

55.5% [N = 182] of the cycles corresponded to fresh embryo transfers and 44.5% [N = 146] to frozen embryo transfers. Indications for using donor oocytes were diminished ovarian reserve 54.39%, premature ovarian insufficiency 5.7%, recurrent implantation failure with autologous oocytes 37.05% and hereditary disease 2.85%.

The overall live birth rate was 155/328 (45.7%). Live birth outcome appeared to be significantly reduced in paternal age over 46 years after adjusting for semen parameters according to WHO 2010 criteria and for recipient age, with an OR 1.5 (95%,0.85 to 1.98, $p < 0.002$). Clinical pregnancy outcome was significantly reduced in paternal age over 51 years with OR 0.62 (95%, 0.31 to 1.89, $p < 0.0001$). The overall miscarriage rate was 15/328 (4.57%) and it was not possible to perform subgroup analysis by paternal age group due to the small numbers.

When both maternal and paternal age were retained in the multivariate model, the probability of live birth decreased with paternal age over 51 years and maternal age $\geq$40 years with OR 0.88 [0.40 to 1.38 [N = 37]).

Limitations, reasons for caution: This study has some limitations. This is a retrospective study and its relatively small subgroup sizes affected the statistical analysis of miscarriage results. In addition, we did not include any effect of sperm DNA fragmentation in the analysis.

Wider implications of the findings: The overall live birth outcome was positive. Whilst the study can be used to counsel couples with increased paternal age, it should not be used to preclude patients from treatment.

Trial registration number: IRB-003C02-09-21

Abstract citation ID: dead093.411
P-044 Outcomes of spermatogenesis induction in hypogonadal men over a 5-year period at a UK Male Fertility Centre

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Study question: Assess the effectiveness of treatments at a Male Endocrine Fertility Clinic, over a five-year period, through spermatogenesis rates and fertility outcomes via a patient-centred questionnaire

Summary answer: Rate of successful spermatogenesis was 68% and live birth (LB) 36%. Trend towards lower odds ratio for LB was noted, if partners reported subfertility.

What is known already: Approximately half of infertility cases are due to male factor, with up to 70% being idiopathic. The remaining 30% of male factor infertility is attributed to primary testicular or central endocrine disorders. Identifying the aetiology of male hypogonadism is important to determine treatment strategies, such as gonadotrophin therapy and surgical testicular sperm extraction (TESE). Although treatment pathways for hypogonadal men with infertility have been established over the last thirty years, the effectiveness of such pathways is diverse and data from large studies are scanty.

Study design, size, duration: A questionnaire, covering basic demographic data about both partners, as well as health conditions affecting fertility, was prospectively sent out to 168 men who had received gonadotrophins for induction of spermatogenesis between 2017 and 2021. Men presented with azoospemia or severe oligospermia (<5million/ml). Eighty-four questionnaires (50%) were completed. Medical records were reviewed, to validate participant responses, identify baseline endocrine biochemistry from the start of spermatogenesis and seminal parameters at the end of the process.

Participants/materials, setting, methods: Mean age of participants was 38 years (range 21-63). Twenty-two men had primary (26%) and the remaining 74% central hypogonadism. The questionnaire was sent via email and responses were anonymised with each participant given a unique study identification number. All statistical analysis was performed using Graph Pad Prism. All hypothesis testing was two-tailed; $p < 0.05$ was considered statistically significant.

Main results and the role of chance: 58% of men (49/84) had sperm seen in their ejaculate and 10% of men (8/84) after microTESE. Men with persistent azoospermia were more likely to have a diagnosis of primary hypogonadism (PH) and small testicular size. 28% (13/47) had partners, who conceived spontaneously and delivered healthy babies. 9% (4/47) had a live birth after ART. Only 1 of 2 men with PH seeking fertility, had a partner with

a positive pregnancy test, which resulted in a miscarriage. Age, body mass index, alcohol intake, baseline LH, FSH and testosterone were not significantly different between men with or without a live birth.

Men without a live birth trended towards having partners with subfertility (pelvic inflammatory disease, endometriosis or polycystic ovarian syndrome), though this did not reach statistically significance (Odds ratio 6.9, 95%CI: 0.8 – 59.9, p = 0.07). Also, LBR was lower for female partners above 35 years, compared to LBR for younger female partners; 35% (6/17) for couples with partners above 35 years, versus 65% (11/17) for couples with partners below 35 years. Seminal parameters were not statistically different between men with or without live births, which supports the possibility that factors other than male reproductive status affected fertility outcomes.

Limitations, reasons for caution: The 50% response rate to our questionnaire-based study means outcomes may not be representative of all patients attending our clinic and can introduce bias with differences between responders and non-responders. Furthermore, the questionnaire used was not externally validated and its data may not be representative of outcomes in other centers.

Wider implications of the findings: Our study highlighted successful spermatogenesis and comparable live birth rates to other male fertility services worldwide. It is the first to compare the clinical, biochemical and seminal characteristics between men with live births versus those without a live birth. Ensuring couples are well informed is essential to further successful outcomes.

Trial registration number: not applicable

Abstract citation ID: dead093.412
P-046 Sperm mitochondrial DNA copy number and mitochondrial function in normozoospermic men may predict fertilization rates after intracytoplasmic sperm injection

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Study question: Do sperm mitochondrial DNA copy number (mtDNAcn) and mitochondrial function correlate with fertilization rates after intracytoplasmic sperm injection (ICSI)?

Summary answer: We reveal a negative correlation between sperm mtDNAcn and fertilization rates after ICSI, while higher mitochondrial function corresponded to an increase in sperm fertilization capacity.

What is known already: Over 40% of male-factor infertility is of unknown origin (unexplained and idiopathic in nature). As current diagnoses remain largely descriptive, the identification of markers predicting the fertilization capacity of sperm will hold the key towards improved treatment. Mitochondria are fundamental for supporting spermatogenesis and fertilization. A recent study showed that altered levels of sperm mitochondrial proteins led to oocyte-activation deficiency, resulting in fertilization failure after ICSI. Poor semen parameters have also been linked to high mtDNAcn, however the relationship between mtDNAcn in sperm and oocyte-activation capacity remains unexplored. Further characterization may uncover prognostic markers associated with sperm-related fertilization failure.

Study design, size, duration: Twenty normozoospermic semen samples were included in the study. These were obtained from oocyte donation ICSI cycles, performed between March 2021 and November 2022. Both fresh and cryopreserved sperm samples were analysed. Five million sperm were collected from the fresh ejaculate for genomic DNA (gDNA) isolation and mtDNAcn quantification, while the remainder of the sample was used for mitochondrial functional analysis. These analyses were also performed on washed samples, cryopreserved after swim-up.

Participants/materials, setting, methods: Mean paternal age and BMI were 40 ± 5.8 years and 25 ± 2.3 kg/m^2, respectively. We evaluated mtDNAcn using qPCR, by assessing the ratio of mitochondrial-to-nuclear genes (NT-ND4/B2M). For our functional studies, we quantified mitochondrial membrane potential (MMP) in individual spermatozoa by JC-1 staining and flow cytometry. Flow cytometry analysis was performed on ≥ 10 million sperm treated with a mitochondrial uncoupler for 15 minutes (1 μM trifluoromethoxy-carbonylcyanide-phenylhydrazone, FCCP) and controls. Spearman correlation was applied.

Main results and the role of chance: We observed variable fertilization rates in our cohort (69.0 ± 24.9%). One sperm sample resulted in total fertilization failure (TFF) after ICSI and was analyzed independently. In fresh samples, mtDNAcn (5.8 AU ± 2.61) correlated negatively with progressive motility (rs = -0.65) and fertilization rate (rs = -0.32). Notably, the sample resulting in TFF presented with the highest mtDNAcn value (13.9 AU ± 2.77). Flow cytometry analysis revealed two distinct sperm populations, corresponding to motile and immotile sperm. While immotile sperm presented with low or undetectable JC-1 aggregates (red signal), motile sperm showed different levels of MMP (ratio INTred/INTgreen) (2.3 AU ± 1.83). In motile sperm, our results revealed a positive correlation between MMP and fertilization rate (rs = 0.59). As with raw semen, we observed a negative association between MMP and mtDNAcn in motile sperm (rs = -0.53).

Limitations, reasons for caution: Cryopreservation is known to affect sperm motility and active mitochondria in sperm, however its effect on MMP intensity remains to be established. Our limited sample size warrants careful interpretation.

Wider implications of the findings: Our findings suggest a negative correlation between sperm mtDNAcn and fertilization rates after ICSI, while fertilization capacity improved with increasing MMP. Sperm mtDNAcn and MMP may have prognostic value for male fertility and ICSI outcomes. Further validation may deliver clinically useful markers, aiding infertility diagnosis, treatment and counselling.

Trial registration number: NA

Abstract citation ID: dead093.413
P-048 Vitrification of human semen impairs the mobility of spermatozoa when compared to the conventional slow-freezing

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Study question: Does vitrification provide a better method for human sperm cryopreservation than slow freezing in terms of survival, mobility, maturity, and DNA fragmentation?

Summary answer: Vitrification of human semen yields similar outcome as slow-freezing considering survival, mobility, DNA fragmentation, and maturity but the mobility after thawing is lower.

What is known already: Slow freezing is the most common technique for cryopreservation of human sperm. Unfortunately, many sperm cells die during the process or lose their mobility and are thereafter unable of fertilization. Meanwhile, vitrification of animal sperm showed good outcomes in terms of sperm survival and motility while being easier to perform than slow freezing. So far, many studies focused on survival and mobility of human sperm after vitrification compared to slow freezing, with results still not unified. They also neglected the importance of sperm maturity and DNA fragmentation caused by the process. DNA damage can affect embryo development or cause abortion.

Study design, size, duration: The study was performed from March January to July 2022. It included 69 male participants who underwent a

diagnostic spermiogram at the Division of gynaecology and obstetrics of University Medical Centre Ljubljana. All participants gave written consent of using the remaining of sample, after performing the spermiogram, for our study. Sperm samples were divided in three aliquots, one saved as native sample, one intended for vitrification, and one intended for slow freezing.

Participants/materials, setting, methods: Participants in the study had normal sperm quality parameters according to 5th edition of WHO manual. Slow freezing was performed using Sperm Freezing medium (Origio). For vitrification sperm was diluted in solution consisting of Sperm preparation medium (Origio), sucrose, and HSA in 1:1 (v/v) ratio, incubated for five minutes and then vitrified by adding 30 microliter droplets directly into liquid nitrogen. After thawing sperm survival, mobility, maturity, and DNA fragmentation were assessed.

Main results and the role of chance: The data are presented as mean values (± standard deviation) when normally distributed and as medians (IQR) when not normally distributed. The patient's age was 35.0 ± 5.1 years. In native sperm sample, sperm concentration was 69.6 ± 59.0x106 cells per millilitre and total motility was assessed to be 60.0% (50.0%-70.0%). After thawing we noticed significantly lower mobility ($p < 0.001$), while also the mobility was significantly lower after vitrification when compared to slow-freezing (17.6% (12.4%-25.3%) and 14.5% (9.0%-22.6%), $p = 0.007$). Interestingly, there was no difference in survival after thawing the samples (20.5 ± 10.7 % after slow freezing and 23.7 ± 13.7 % after vitrification). Also there was no difference in maturity of sperm when checked with protamine staining (native vs. slow-freezing vs. vitrification; 53.6 ± 15.2 % vs. 53.9 ± 16.0 % vs. 52.4 ± 15.2 %, $p = 0.905$) and also when checked with hyaluronan binding assay (78.0% (67.5%-82.4%) vs. 79.4% (71.7%-82.95%) vs. 75.3% (67.5%-81.1%), $p = 0.607$) . While sperm maturity was not affected with cryopreservation, DNA fragmentation was significantly higher after cryopreservation (native vs. slow-freezing vs. vitrification; 19.7 ± 9.5%, vs. 24.7 ± 11.6 vs. 27.1 ± 15. %, $p = 0.049$).

Limitations, reasons for caution: We believe our study would be better by obtaining higher number of participants and by being performed over longer time period. We would also like to further optimise vitrification protocol. More knowledge is also needed in poor sperm samples, since we only included sperm samples of good quality.

Wider implications of the findings: We believe our finding are of great importance for the field of andrology. Our results show vitrification of human sperm can be comparable to slow freezing in most of parameters analysed, but lower mobility after vitrification is reason for caution.

Trial registration number: 20210024

Abstract citation ID: dead093.414

P-049 Verteporfin suppresses cell proliferation, survival and migration of TCam-2 Human Seminoma Cells via inhibits the YAP-TEAD complex

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Study question: Does verteporfin administration effect on proliferation, survival and migration of TCam-2 human seminoma cells?

Summary answer: Verteporfin may be a promising anticancer agent for TCam-2 human seminoma cells via inhibition of cell viability, proliferation, migration and stimulation of apoptosis.

What is known already: Seminoma is germ cell tumor of the testis and TCam-2 cells is the first seminoma-derived cell line that retains many of the characteristic traits of seminoma. The Hippo signalling is a evolutionarily highly conserved pathway that controls organ size, tissue homeostasis, and cancer development. In mammals, the Hippo pathway consists of the serine/threonine kinases Mammalian Sterile 20-protein Kinase 1 and 2 (MST1/2), Large Tumor Suppressor Homologues 1 and 2 (LATS1/2), Yes-Associated Protein (YAP), and its transcriptional cofactor TAZ. *Verteporfin* inhibits YAP–TEAD complex, blocking cell proliferation and survival.

Study design, size, duration: TCam-2 cells were treated with different concentrations of verteporfin (1, 5, 10, 20 and 40 μM) for 24, 48 and 72 hours (h).

Participants/materials, setting, methods: TCam-2 cells were treated with verteporfin at different doses and incubation times. Afterwards, protein expression of Hippo pathway proteins was determined by western blot, mRNA levels were analyzed by qRT-PCR and protein localizations were evaluated by immunoflourescence staining. Cell migration was determinated with wound-healing scratch assay; cell viability, early-late apoptosis and necrosis rates were evaluated by flow cytometry.

Main results and the role of chance: We detected that 40 μM verteporfin decreased LATS1/2 and p-LATS1/2 protein expression after 48h and 72h treatment ($p < 0.05$, $^{**}p<0.01$). MST1/2 and p-MST1/2 was significantly decreased in groups treated with 20 and 40 μM verteporfin after 48 and 72 h of treatment ($p < 0.05$, $^{****}p<0.0001$). YAP expression decreased at 5, 20 and 40 μM verteporfin treatment after 48h and all verteporfin concentrations after 72h ($^{**}p<0.01$, $^{***}p<0.001$, $^{****}p<0.0001$). p-YAP expression significantly decreased at all verteporfin concentrations after 48h and only at 40 μM concentrations after 72h ($^{**}p<0.01$). TEAD4 expression decreased at all verteporfin concentrations after 48 and 72h ($^{****}p<0.0001$). After 48 and 72h of verteporfin treatment, no significant difference was observed in the expression of the *MST1, MST2, LATS1, LATS2* and *YAP1* mRNA levels in TCam-2 cells, while *TEAD4* mRNA expression level was significantly decreased at 20 and 40 μM compared to control ($^{**}p<0.01$, $^{***}p<0.001$). We also showed that Hippo signaling pathway proteins localized in the cell nucleus especially YAP1 and TEAD4, were translocated to cytoplasm depending on the increasing dose of verteporfin in TCam-2 cells. Verteporfin decreased cell proliferation ($^{****}p<0.0001$), migration and cell viability ($^{****}p<0.0001$), as well as increased the early-late apoptosis ($^{**}p<0.01$, $^{***}p<0.001$, $^{****}p<0.0001$) in TCam-2 human seminoma cells dose-dependent manner.

Limitations, reasons for caution: The TCam-2 cell line was gifted by Prof. Dr. Hubert Schorle (Department of Developmental Pathology and Institute of Molecular Diagnostic Pathology, Faculty of Medicine, Germany, Bonn) to our laboratory. There is no limitation in the scope of the study.

Wider implications of the findings: We determined for the first time the effect of verteporfin treatment on the protein expression and mRNA levels of Hippo signaling pathway in TCam-2 human seminoma cells. We suggested that Hippo pathway and verteporfin relationship may be a therapeutic target for seminoma by affecting cell proliferation, survival, migration and apoptosis.

Trial registration number: not applicable

Abstract citation ID: dead093.415

P-050 Using a second sample of ejaculate for IVF treatment

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Study question: Is it beneficial to use the second sample of ejaculate collected within 2 hours from the previous sample to fertilize the oocytes in IVF treatments?

Summary answer: A significantly higher clinical pregnancy rate was achieved using the second sample of ejaculate.

What is known already: The WHO recommends abstinence of 2 to 7 days prior to semen collection for standard evaluation. Although semen volume and sperm concentration may increase with prolonged abstinence, such abstinence may have a negative impact on sperm motility and viability. Previous studies have shown that a second ejaculate sample following a short period of abstinence exhibited improved sperm concentration, progressive motility, vitality, and decreased DNA fragmentation. It was suggested that a second ejaculate collected within 1 h might be preferable for ART procedures.

To the best of our knowledge it has not yet been clinically implemented in routine IVF treatment cycles.

Study design, size, duration: A retrospective study of couples treated at our IVF unit between Dec 2020 and Dec 2022. All male partners were asked to give a second sample of ejaculate within 2-3 hours after the first. In all cases we used the second sample for fertilization whether it was better or worse than the first one.

Participants/materials, setting, methods: The study included 422 male partners of couples treated for IVF. Patients were divided into 2 groups. Group 1- 223 male partners that provided a second sample of ejaculate and Group 2- 199 patients unwilling to be detained that refused to provide an additional sample. Sperm count, motility percentile and sperm volume were recorded for each sample of ejaculate. Fertilization, cleavage, positive beta-hCG and clinical pregnancy rates were adjusted according to maternal and paternal age.

Main results and the role of chance: Of the 223 samples in Group 1 the second sample showed improved sperm quality in 123 samples, identical sperm parameters in 31samples and inferior sperm quality in 69 samples compared with the first sample. Regardless of sperm quality, only the second sample was used in all Group 1 participants. Demographic characteristics such as maternal age, cause of infertility, semen concentration and motility were similar in both groups. Paternal age was higher in Group 2 compared with Group 1 (37.2 ± 7.0 and 35.2 ± 6.3, $p = 0.002$ respectively). Clinical pregnancy rates were 36.9% in Group 1 and 21.8% in Group 2 ($p < 0.003$). Higher clinical pregnancy rates using the second sample remained after adjustment for maternal and paternal age OR = 1.92; 95% (1.15-3.19) and even when the second sperm sample was inferior in quality compared with the first.

Limitations, reasons for caution: The study limitation stems from the retrospective nature of the study and the fact that the groups might have a selection bias we were unaware of.

Wider implications of the findings: These findings suggest that it may be beneficial to routinely ask male partners, among couples undergoing IVF, for a second ejaculate 2-3 hours following the first. Further studies showing higher clinical pregnancy rates following the use of the second ejaculate for fertilization are needed to validate these findings.

Trial registration number: CMC-0017-22

Abstract citation ID: dead093.416

P-051 Male cancer patient sperm cryopreservation for fertility preservation: 11-year multicenter experience: 16 regions of the mainland China national sperm

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Study question: What is the current status of fertility preservation (FP) of male cancer utilization, efficacy and safety in China and what national trends could be observed?

Summary answer: In this national report on FP status of male cancer over a 11-year period, data on population and trends, region development of FP are analyzed.

What is known already: Cryopreservation of sperm, the current standard option to preserve male patient's fertility, is very advantageous for men suffering from cancer. Unfortunately, the data on population and trends, region development of fertility preservation (FP)of male cancer in mainland China is scarce.

Study design, size, duration: A multicenter retrospective study of male cancer patients who performed sperm cryopreservation from 16 regions of a national network of sperm banks over a 11-year period from 2010 to 2020. A total of 2270 men with cancer were referred to 16 human sperm banks for fertility preservation.

Participants/materials, setting, methods: This national survey was conducted through 16 provincial administrative sectors to sperm cryopreservation for Chinese male cancer. Sperm cryopreservation for male cancer is conducted in 16 human sperm banks, which done well in Sperm cryopreservation for male cancer. Cancer diagnoses were classified according to types, as Testicular tumors, Lymphomas, Leukemia, Colorectal tumors, Nasopharyngeal carcinomas, Sarcomas, Brain tumors, Extragonadal germ cell tumors, and with other cancers grouped together.

Main results and the role of chance: The number of male cancer patients with sperm cryopreservation showed an overall upward trend. Statistically significant mean yearly increases were observed for three most frequent types of cancer (testicular cancers, lymphoma, leukemia). The development of male cancer FP in the eastern, central, and western regions of Chinese mainland displayed imbalance. There are seven tumor types for sperm preservation in the top incidence ten tumor types, including lymphoma, leukemia, nasopharyngeal carcinoma, sarcoma, thyroid cancer and brain tumor. Moreover, nasopharyngeal carcinoma is a high incidence rate in China, which is related to high sperm preservation rate, different from others countries. The most percentage of males receiving sperm cryopreservation in the testicular cancers (15-39 years old) of China in 2020 was 5.55%, 1.29% in the lymphoma and 0.39% in the Leukemia. According to type of cancer, a statistically significant lower pre-sperm density, total sperm output and post-sperm density was observed in Testicular cancers. It's worth noting that the prevalence of azoospermia 22.2 % in Leukemia patients. Disposition of cryopreserved sperm categories included continued storage (47.2%), discarded (9%), death (0.9%), and use (3.7%). Up to 2020, 70 patients (3.7%) who underwent assisted reproductive technology (ART) used their cryopreserved semen.

Limitations, reasons for caution: The limitations of our study are the inability to follow-up couples who were naturally conceived. Another limitation is that not all clinical outcomes have been gated.

Wider implications of the findings: This study provides the most comprehensive national statistical census and review of fertility preservation in men cancer patients trends, quantity and cancer types. Our findings regarding patients' characteristics and analysis of semen parameters and sperm usage rate.

Trial registration number: not applicable

Abstract citation ID: dead093.417

P-052 A novel variant in CFAP69 gene causes morphological abnormalities of human sperm flagella with good ART outcomes

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Study question: Is there any novel variant in *CFAP69* gene related to multiple morphological abnormalities of sperm flagella (MMAF) in humans?

Summary answer: We identified a loss-of-function variant of *CFAP69* in a Chinese man with MMAF and intracytoplasmic sperm injection (ICSI) is favorable for the affected couple.

What is known already: MMAF is a specific type of asthenoteratozoospermia characterized by immotile and malformed spermatozoa. Genetic defects are the major cause of MMAF, and more than 20 genes have been related to approximately 70% MMAF-affected cases. *CFAP69*, a cilia- and flagella-associated gene, is abundantly expressed in the testis and associated with infraglabellar transport. To date, four variants in *CFAP69* were related to four MMAF patients in the literatures, and the male *Cfap69*-knockout mice mimicked the infertility and profound flagellum morphology defects phenotype.

Study design, size, duration: A cohort of 35 infertile Chinese men with MMAF were recruited in the center for reproductive medicine from August

2019 to October 2022. Genetic testing was based on a targeted-sequencing panel of 22 MMAF-related genes. Morphological, ultrastructural, and immunostaining analyses were conducted in 2022. We also performed assisted reproductive technology (ART) with ICSI to explore the treatment strategies.

Participants/materials, setting, methods: Peripheral blood and semen samples were collected from every participant. The *CFAP69* variant was identified by genetic testing with targeted-panel sequencing of 22 MMAF-associated genes and validated through Sanger sequencing, pedigree analysis and *in silico* analysis. Papanicolaou staining, SEM, immunofluorescence, and TEM were performed to explore the effect of the variant. ICSI was carried out for the affected couples to get their own progenies.

Main results and the role of chance: We identified a novel frameshift variant in *CFAP69* (c.2061dup, p. Pro688Thrfs*5) from a MMAF-affected infertile male with low sperm motility and malformed morphology of sperm. Furthermore, immunofluorescence staining, and transmission electronic microscopy revealed that the variant induced reduction of CFAP69 expression and the aberrant ultrastructure including the absence of the central pair and disorder of the microtubule doublets in the patient's spermatozoa. Moreover, the partner of the proband was able to conceive through ICSI and birthed a healthy girl.

Limitations, reasons for caution: A limitation of this study is the low number of variants identified in *CFAP69* and the small number of *CFAP69*-mutant patients available. Additional *CFAP69* variants and cases are needed to better characterize the genetic etiology of the MMAF phenotype and to improve the management of MMAF patients with *CFAP69* variants.

Wider implications of the findings: This study extended the mutant spectrum of the *CFAP69* gene and could facilitate researchers and clinicians to understand the genetic etiology of MMAF better, thus improving counseling of infertile men with MMAF in the future.

Trial registration number: not applicable

Abstract citation ID: dead093.418

P-053 Sperm aneuploidy and DNA integrity pre- and post-chemotherapy in men with testicular cancer

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Study question: Are fragmentation levels (single-double stranded DNA breaks) or aneuploidy frequencies in sperm increased in patients with testicular cancer who recovered spermatogenesis after chemotherapy (CT)?

Summary answer: Similar levels of sperm DNA fragmentation and sperm aneuploidy frequency are found in men with testicular cancer pre- and post-chemotherapy.

What is known already: Testicular cancer is the most frequent solid tumor among young men. Therefore, the restoration of fertility and achievement of fatherhood in survivors have become important concerns. The integrity of genetic material in sperm is crucial since some paternal genes are essential for early embryo development. Although previous studies analyzed the effect of CT on sperm DNA integrity, they did not differentiate single vs. double-stranded DNA breaks. Few data are available on aneuploidy in sperm from testicular cancer survivors, so it remains to be elucidated whether cancer treatments may lead to an increased risk of aneuploidy syndromes in offspring.

Study design, size, duration: Prospective multicenter study including 31 males with a history of testicular cancer who requested semen cryopreservation between 2007-2021. All patients included underwent CT and recovered spermatogenesis once treatment ended. Post-CT samples were collected and seminal parameters, single-double stranded DNA breaks and the proportion of sperm aneuploidies were compared with one aliquot of the initial ejaculate. The project was approved by an Institutional Review Board and written informed consent for participation was obtained from each subject.

Participants/materials, setting, methods: Patient's age prior CT was 31.23yrs (IC 95%: 29.29-33.17). Mean interval time between 1st and 2nd samples was 4.53yrs (IC 95%: 3.42-5.64). TUNEL quantified overall sperm DNA fragmentation by flow cytometry analyzing at least 20,000 cells, while γH2AX staining was applied for double DNA fragmentation assessment. Sperm aneuploidies were calculated by using fluorescence in situ hybridization (FISH) probes for chromosomes 13, 18, 21, X, Y. A paired t-test was used to compare variables pre- and post-CT.

Main results and the role of chance: Similar results were found when sperm quality parameters were compared. Mean sperm concentration pre-CT was 33.03M/mL (IC 95%: 23.98-42.07) and 36.41 M/mL (IC 95%: 27.85-44.96) in post-CT ejaculates (N.S.). A comparable rate of motile spermatozoa was observed in pre-CT vs. post-CT samples [(43.81% (IC 95%: 38.42-49.19) vs. 44.73% (IC 95%: 38.95-50.51), N.S.].

Regarding TUNEL assay results, the sperm DNA fragmentation rate was 21.65% (IC 95%: 17.66-25.63) in pre-CT samples and 23.00% (IC 95%: 19.90-26.61) in post-CT samples (N.S.). Furthermore, similar results were obtained when double-stranded fragmentation levels (H2AX) were compared between pre and post-CT [10.2% (IC 95%: 6.45-13.96) vs. 12.44% (IC 95%: 8.75-16.13) N.S.]. Comparable sperm disomic proportions were found in PRE and POST-CT samples: [(Cr.13 =0.02%, Cr.18 =0.01%, Cr.21=0.30%, Cr.XY=0.84%) vs. (Cr.13 =0%, Cr.18 =0%, Cr.21=0%, Cr.XY=0.33%), N.S]. Results were also evaluated according to the time elapsed between the PRE and POST -CT samples. No significant differences were found for seminal values (sperm concentration, $p = 0.26$; motility, $p = 0.68$), overall sperm DNA fragmentation ($p = 0.68$), double-strand DNA fragmentation ($p = 0.90$) and the frequency of sperm disomies (Cr. 13 $p = 0.79$, Cr.18 $p = 0.46$, Cr. 21 $p = 0.85$, Cr.XY $p = 0.05$).

Limitations, reasons for caution: The main limitations of the present study are that we only recruited patients who recovered spermatogenesis and that our data evaluated a long-term effect of CT.

Wider implications of the findings: All men wishing to have their own children after gonadotoxic treatment should receive adequate counseling about the side effects of the therapy. Based on our results on sperm DNA integrity and euploidy, patients who recovered spermatogenesis should not be advised to use samples that were preserved before undergoing CT.

Trial registration number: na

Abstract citation ID: dead093.419

P-054 Significant differences of micro RNA expression pattern in extracellular vesicles between fertile and infertile individuals: new markers of male reproductive status?

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Study question: Is the miRNAs cargo of the extracellular vesicles (EVs) present in seminal plasma different between healthy semen donors and infertile patients?

Summary answer: There are significant differences in the miRNAs expression patterns in the load of seminal EVs between infertile patients and healthy semen donors

What is known already: Extracellular vesicles (EVs) present in seminal plasma contain a wide range of different proteins, lipids and nucleic acids, such as microRNA. These microRNA can execute their action by regulating gene expression of target cells triggering an important role in different reproductive processes. It is well stablished in different pathologies that the EVs cargo is representative for the functional status of the producer cell. In this sense, the alteration in microRNAs expression patterns in seminal plasma has been associated with spermatogenic impairments, subfertility and

azoospermia, indicating that microRNAs in seminal EVs could work as biomarkers of the male fertility status

Study design, size, duration: This prospective study, performed between March 2021 to December 2022, included 30 semen samples from infertile males seeking infertility treatment (study group), and 7 semen samples from healthy semen donors (control group) from our donor semen bank. All the semen samples from patients showed abnormal seminal parameters (oligo- and/or asthenozoospermia). Men under drug treatment and/or with infectious or chronic diseases were excluded from the study

Participants/materials, setting, methods: After conventional semen analysis, EVs were isolated from seminal plasma by ultracentrifugation. To confirm EVs isolation, an aliquot of each sample was used for nanoparticle tracking analysis (NTA). Then, microRNAs from EVs were extracted and miRNAseq was performed. Raw data were standardized using counts per millions and ANOVA was performed to assess differences in the miRNAs expression between groups.Target genes were annotated using miRTarBase and functional enrichment analysis was performed by MIENTURNET and GeneCards

Main results and the role of chance: The presence of EVs after ultracentrifugation of seminal plasma was confirmed by NTA, showing a range size within 40-140 nm. miRNAseq generated reads for 914 annotated miRNAs and significant differences in the expression of 17 of them were found between patient and control samples. These miRNAs target a total of 1310 genes and functional enrichment analysis indicated that they participate in several processes related to cell differentiation, cell proliferation, immunomodulation, cellular adhesion and apoptosis. Specifically, patient group showed significant higher expression of some members of the hsa-let-7 family ($p < 0.001$ for let-7i-5p, let-7f-5p, let-7c-5p and let-7a-5p; $p < 0.05$ for let-7b-5p), which are implicated in adaptative immune system activation and differentiation by gene modulation. Results also found significant ($p < 0.01$) higher expression in patient group of hsa-miR-92a-3p, hsa-miR-200c-3p and hsa-miR-888-5p, which target genes are related to apoptotic processes. Finally, patient group showed significant ($p < 0.001$) lower expression of hsa-miR-320b and miR-320a-3p, which interact with transcription factor sequences (DLX5, MYC) implicated in cell proliferation and differentiation; and also lower expression in hsa-miR-423-5p ($p < 0.01$) and miR-423-3p ($p < 0.05$) that interact with the sequence of transcription factors as p21, a regulator of cell cycle progression at G1, and with RNA-binding proteins involved in ribosome assembly.

Limitations, reasons for caution: This study included males with dissimilar seminal disorders (astheno, oligo, and teratozoospermia). Therefore, further studies are needed lowering the heterogeneity of the study group. At present, no data about reproductive outcome is available

Wider implications of the findings: This work suggests that the significant differences found in the expression pattern in microRNAs in seminal EVs between infertile and fertile men may be related with gene modulation in the reproductive processes and could be used as novel biomarkers of male fertility status.

Trial registration number: 1904-MAD-045-AP

Abstract citation ID: dead093.420
P-055 Four decades of sperm donation: Motivation and attitudes among donors

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Study question: How has the attitudes and motivation towards donation among sperm donors developed through the years from 1992, 2002, and 2012 to 2022?

Summary answer: Altruism and economic compensation remain the primary motivations, but there are significant changes in responses for certain life and attitude-questions from current to previous years.

What is known already: The growing application of medically-assisted reproduction (MAR) has made treatment with donor sperm commonplace, and in recent years more people have been seeking pregnancy using donor sperm. With the global shortage of sperm donors, it is important to seek the opinion of the donor population on the perspectives of anonymity, motivations, information-sharing, and feelings towards the donor-conceived children, as well as the psychological factors related to being a donor. Previous studies have shown that altruism is the primary motivation and changing aspects of the donor population is of interest to all who seek to maintain the application of sperm donation.

Study design, size, duration: The study was based on the same questionnaire from the three preceding decades with a few updated modifications in the 2022-setup. Active sperm donors from the Danish sperm bank Cryos International were invited by email to participate in the study in the period June 9th to July 1st 2022. This is one of the largest studies of its kind to seek the opinion of sperm donors.

Participants/materials, setting, methods: Results from 173 donors were compared with previous answers from donors in the same sperm bank: 1992 ($n = 39$), 2002 ($n = 58$), and 2012 ($n = 91$). In 2012 and 2022, donors could choose between being ID-release (formerly known as non-anonymous) and non-ID-release (anonymous). Anonymized answers provided from the questionnaires were analyzed statistically to compare ID-groups and investigate changes in responses from previous surveys with the use of Chi-Squared tests and logistic regression.

Main results and the role of chance: There was a significant increase in the proportion of donors being ID-release from 29% in 2012 to 54% in 2022. The altruistic motivation of helping childless people was the most important factor to both ID-release donors and non-ID-release donors. Still, economic compensation was an invariable term of condition. In general in 2022, donors were willing to donate sperm to same-sex couples (80%) and single women (68%), and 55% were positive towards donation to transgender males. Logistic regression showed that ID-release donors were more likely to want information about donor offspring (OR = 1.80, [95% CI 1.02, 3.16]), while there was no association with having a partner. Also, a positive association between ID-release status and intending to inform current or future children about their sperm donation (OR = 1.73, 95% CI 1.10, 6.85) was observed. Having a partner was not significantly associated with the chance of donors wanting to inform current or future children about donations (OR = 0.65, 95% CI 0.90, 1.05). The findings of this study presented evidence that the donor population was not homogeneous in relation to several aspects of donation and had diverse opinions towards mutual information sharing.

Limitations, reasons for caution: The number of participants increased from 39 in 1992 to 173 in 2022. In 1992, donors who were not yet approved for donation were included, unlike the following years. All donors were anonymous in 1992 and 2002 due to legislation. Only in 2012 and 2022, donors could differentiate between ID-types.

Wider implications of the findings: The questionnaire clearly shows that the needs and attitudes of donors have developed across years. Different attitudes between ID-release and non-ID-release donors suggest that donor profiles fit people with different requirements. It is advisable to continually update provided services to fit the donors' needs and opinions concerning mutual information.

Trial registration number: Not applicable

Abstract citation ID: dead093.421
P-056 Oxidative stress and human semen quality: new results for diagnostic process of male infertility

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Study question: Is oxidative stress (OS) evaluated in human spermatozoa a predictive marker of better semen quality?

Summary answer: CellROX®Orange is a new fluorescent probe able to detect Reactive Oxygen Species (ROS) identifying a viable oxidized sperm fraction related to a better sperm performance.

What is known already: OS, defined as an unbalance between ROS production and antioxidant defences, is considered one of the causes of male infertility. OS evaluation in spermatozoa represents an important goal of research in this field because this parameter could be helpful to predict sperm fertilization ability to improve Assisted Reproductive Technology outcomes. By using different probes and methods for OS evaluation in spermatozoa or in semen, several studies were performed demonstrating a negative role of ROS on sperm functions. Such studies were not conclusive for the small number of included subjects, the high variability in the cohorts and the lack of validation.

Study design, size, duration: An observational study was conducted on 121 semen samples from patients undergoing routine semen analysis for couple infertility in the Andrology Laboratory of Careggi University Hospital of Florence from September 2021 to March 2022. Washed and Swim-up selected spermatozoa were incubated with two fluorescent probes, CellROX® Orange and Dihydroethidium (DHE), and revealed by flow cytometry.

Participants/materials, setting, methods: After routine semen analysis, the percentage of oxidized spermatozoa was evaluated with CellROX® Orange and DHE, and then correlated with standard semen parameters and sperm DNA fragmentation (sDF, evaluated by TUNEL/PI). Sperm kinematic parameters and hyperactivated motility were also assessed by C.A.S.A. system. Furthermore, CellROX® Orange positivity and phosphatidylserine membrane exposure (determined by Annexin V staining) or Caspase 3 and 7 activities (measured by FLICA™) were concomitantly evaluated.

Main results and the role of chance: We demonstrated that CellROX® Orange is able to detect hydrogen peroxide only in viable spermatozoa and the percentage of CellROX® Orange positive spermatozoa were positively associated with semen quality and negatively with sDF. To confirm these results, we performed the same experiments by using DHE, another probe which reveals hydrogen peroxide and superoxide anion in both viable and unviable cells. Similarly to CellROX® Orange, a positive correlation with semen quality and a negative one with sDF were found in viable spermatozoa. Conversely, in unviable spermatozoa, opposite associations were observed. These results indicate that oxidative status revealed by the two probes is related to a better sperm quality. To further investigate this possibility, we double labelled spermatozoa with CellROX® Orange and Annexin V (a marker of early signs of apoptosis) as well as with CellROX® Orange and FLICA™ (that detects caspase activity, a late sign of apoptosis). We found that CellROX® Orange mostly identifies spermatozoa without apoptotic features. Furthermore, we evaluated CellROX® Orange positivity in Swim-up selected spermatozoa, finding significantly higher levels in these samples respect to unselected samples, demonstrating once again that this probe identifies spermatozoa with better quality.

Limitations, reasons for caution: Our results indicate that within the viable oxidized spermatozoa there are cells with physiological and non-physiological intracellular ROS levels, however, they do not explain at what levels ROS cease to be functional and become deleterious for spermatozoa.

Wider implications of the findings: ROS evaluation in viable spermatozoa with the commercially available probes CellROX® Orange and DHE, allows the identification of the oxidized sperm fraction related to a sperm better performance. Therefore, the two probes may be useful to determine such fraction, likely improving the diagnostic process of male infertility.

Trial registration number: not applicable

Abstract citation ID: dead093.422
P-057 Does sperm cryopreservation affect the clinical outcome? A retrospective study in an oocyte donation program

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Study question: Does sperm cryopreservation have a negative impact on pregnancy and live birth rate (LBR)?

Summary answer: The use of frozen sperm does not affect pregnancy rates and LBR significantly compared to fresh sperm.

What is known already: The use of frozen sperm, especially in oocyte donation cycles, is a common strategy in organizing a couple's treatment. By now, contradictory data has been published concerning the impact of fresh sperm versus frozen on pregnancy and LBR. There is data indicating reduction of the LBR with the use of frozen sperm in a donation program, while in other studies no significant difference between fresh and frozen sperm was observed in the LBR.

Study design, size, duration: This is a retrospective cohort study conducted in Embryolab Fertility Clinic, Greece, from January 2019 to March 2020. Only oocyte donation cycles were included in the study. On the day of donor's oocyte retrieval ICSI was performed, either with fresh (group A) or frozen sperm (group B)All embryos were cultured to blastocyst stage, assessed for their development, vitrified and two were transferred post warming on a subsequent HRT cycle.

Participants/materials, setting, methods: For the present study, 135 cases were analyzed: in 28 cases fresh sperm was used, while frozen sperm was used in 107 cases[pd1] . The mean men's age was 44.4 years, while all oocyte-donors were below 35 years old. The main outcome measure was LBR, while fertilization rate, blastulation rate as well as positive β-HCG were also analyzed. All patients with uterine factor, severe male factor or surgically obtained sperm were excluded from the study.

Main results and the role of chance: There was no significant difference between LBR of fresh vs frozen sperm (53.57% ± 50.78 vs 50.47% ± 50.23, p=0.086). Although positive β-CG reached a higher percentage in fresh sperm samples, there was no significant difference between two groups (85.71% ± 35.63 in fresh vs 77.78% ± 41.76 in frozen sperm, p=0.857). Similarly, both blastulation rate (68.93% ± 25.22 in fresh vs 67.97% ± 28.72 in frozen sperm, p=0.873) and fertilization rate (85.36% ± 16.92 in fresh vs 83.27% ± 17.01 in frozen sperm, p=0.565) did not differ significantly between fresh and frozen sperm samples. [pd1] Regression analysis adjusting for relevant confounders (endometrial thickness the day of the embryo transfer, BMI, donor's age) showed no association between LBR and sperm type (fresh vs cryopreserved).

Limitations, reasons for caution: This is a retrospective study, including sperm samples irrespectively to quality parameters (sperm count, motility, morphology). The number of previous treatments per couple was not included in the study.

Wider implications of the findings: The outcome of the study indicates that the use of frozen sperm is a viable choice in a donation program, as it does not induce any negative effects in LBR. However, specific subgroups might benefit from fresh sperm especially when the sperm parameters are low.

Trial registration number: N/A

Abstract citation ID: dead093.423
P-058 Defects in phospholipase C zeta cause polyspermy and low fertilization after conventional IVF: not just ICSI failure

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Study question: How does sperm phospholipase C zeta (PLCζ) affect fertilization in conventional *in vitro* fertilization (cIVF)?

Summary answer: Absence of PLCζ causes polyspermy, and a decline in the proportion of sperm expressing PLCζ is correlated with a low fertilization rate (FR) after cIVF.

What is known already: PLCζ is a key sperm-borne factor that triggers Ca^{2+} oscillations and the subsequent oocyte activation following gamete fusion. Mutations in *PLCZ1*, the gene encoding PLCζ, cause male infertility and intra-cytoplasmic sperm injection (ICSI) fertilization failure; and PLCζ expression and localization patterns are significantly correlated with ICSI FR. However, few studies have been published on the relationship between PLCζ and cIVF, an insemination procedure involving several key events that are bypassed in ICSI, e.g., sperm-zona binding and penetration, as well as polyspermy blocking. It is possible that sperm PLCζ may affect fertilization in a different manner from that in ICSI.

Study design, size, duration: Sixty couples who underwent cIVF treatments were recruited from the Reproductive and Genetic Hospital of CITIC-Xiangya between February 2019 and January 2022.

Participants/materials, setting, methods: We performed whole-exome sequencing in two unrelated males who exhibited infertility and polyspermy in cIVF. Immunofluorescence staining and oocyte-activation testing were employed to evaluate the effects of the candidate variants on PLCζ protein. Subsequent PLCζ analysis was performed in additional 58 males who underwent cIVF. The Pearson correlation coefficient was performed to evaluate the relationships between PLCζ and FRs. The receiver operating characteristic curve and Youden's index analysis were used to indicate the cutoff value.

Main results and the role of chance: We identified one previously reported and two novel *PLCZ1* variants in two males that were associated with male infertility and polyspermy characterized by excessive sperm–zona binding and a delay in pronuclear (PN) formation. *In vitro* functional analyses revealed that virtually all sperm from patients lacked functional PLCζ and were thus unable to evoke the physiologic pattern of Ca^{2+} oscillations. ICSI with an artificial oocyte-activation treatment successfully rescued the polyspermic phenotype and resulted in a live birth. Subsequent PLCζ analysis were performed in additional 58 males, who were allocated to three groups according to fertilization outcomes: a multiple pronuclear (MPN) group (no. of PN $\geq$ 3, MPN rate $\geq$ 50%, n = 9) and low fertilization (LF, total FR $\leq$ 30%, n = 18) and normal fertilization groups (NF, 2PN $\geq$ 50%, 1PN + MPN < 30%, n = 31). We found that the proportion of sperm that expressed PLCζ was positively correlated with both 2PN rate ($P = 0.0005$) and total FR ($P = 0.0008$) after cIVF, and that the optimal cutoff value below which males were likely to experience low FR (total FR $\leq$ 30%) after cIVF was 56.7% (area under the ROC curve, 0.705; $P = 0.0176$).

Limitations, reasons for caution: Precise mechanisms that underly the roles of PLCζ in cIVF outcomes are still uncovered. Follow-up studies with larger numbers of patients are required to validate the sensitivity, specificity, and accuracy of PLCζ as a diagnostic biomarker in cIVF outcomes.

Wider implications of the findings: Our study identified *PLCZ1* as a novel causative gene of polyspermy in humans,highlighting the potential value of PLCζ as a fertility marker for cIVF outcomes.

Trial registration number: nor applicable

Abstract citation ID: dead093.424

P-059 Sperm DNA fragmentation (SDF) after cryopreservation and sperm selection: implications for clinical pregnancies and live births after intrauterine insemination with donor sperm

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Study question: Can SDF assessment pre-cryopreservation, post-thaw and after post-thaw sperm selection predict clinical outcome in a heterologous intra-uterine donor insemination program (IUI)?

Summary answer: SDF increased post-cryopreservation in donor sperm and after post-thaw density gradient without affecting clinical pregnancy, live birth and miscarriage rates.

What is known already: Sperm cryopreservation is effective for preservation of male fertility and facilitates the storage of donor semen, while infectious disease screening can be completed and confirmed negative. Sperm viability and motility are the most vulnerable parameters during the freeze-thaw process. Inherent reactive oxygen species may induce SDF via oxidative stress. Controversial results are reported regarding induction of SDF after cryopreservation due to differences in cryo tolerance, methods of cryopreservation and methods used to evaluate SDF. Sperm selection post-thaw is indispensable to select a normal motile fraction for insemination. Again, results are controversial regarding influence on SDF levels after sperm selection post-thaw.

Study design, size, duration: This was a prospective, observational study. Between March 2015 and March 2019, eighteen potential sperm donors were screened via a three step plan – semen assessment, medical assessment and serological blood tests. In accordance with the Belgian legislation (2007), a sperm donor was matched to obtain a maximum of 6 pregnancies in acceptor women/couples, allowing for more than one offspring/woman or couple. A total of 106 acceptors were matched for heterologous intra-uterine insemination.

Participants/materials, setting, methods: Semen samples were collected after 2-7 days abstinence and standard semen parameters analyzed within 60 mins after ejaculation. Within the same time frame SDF test was performed using TUNEL assay both before and after cryopreservation and after a two-step discontinuous density gradient centrifugation post-thaw. Samples were cryopreserved by the conventional slow freezing method using sperm freeze solution. A yield of $\geq$ 2 M progressive spermatozoa post-thaw after selection was sufficient to contemplate IUI.

Main results and the role of chance: Mean female age at first cycle was 33.9 ± 4.1 years and donor age 28.5 ± 5.6 years. Out of 429 cycles, 100 (23.3%) resulted in clinical pregnancy. Excluding three ongoing pregnancies, we counted 75 live births (17.6% of cycles or 77.3% of pregnancies), while 21 pregnancies ended in miscarriage (4.9% of cycles or 21.6% of pregnancies) and 1 resulted in stillbirth.

Progressive motility (59.3 ± 12.5%) decreased significantly after cryopreservation (30.0 ± 13.2%; $p < 0.001$), but increased post-thaw after density gradient centrifugation (61.6 ± 16.0%; $p < 0.001$). On the contrary, SDF (12.0 ± 5.9%) increased post-cryopreservation (26.3 ± 14.5%; $p < 0.001$) and further increased after sperm selection (34.9 ± 22.1%; $p = 0.04$).

Using multilevel mixed-effects logistic regression (Odds ratio [95%CI]), we found that female age significantly influenced clinical pregnancy (0.911 [0.847-0.981]; $p = 0.01$), live birth (0.894 [0.834-0.959]; $p = 0.002$) and miscarriage rates (1.180 [1.033-1.347]; $p = 0.015$). There was no significant age-adjusted effect of SDF post-thaw after density gradient selection on clinical pregnancy (1.007 [0.994-1.021]; $p = 0.30$), live birth (1.001 [0.988-1.014]; $p = 0.84$) or miscarriage (1.021 [0.997-1.046]; $p = 0.08$). Median time-to-live birth was 4 cycles. Analyses of time-to-live birth with mixed-effects Cox models revealed no significant association with progressive motility; SDF before or after cryopreservation and after sperm selection post-thaw.

Limitations, reasons for caution: Results concern a small donor population, with high normal semen parameters and should therefore be extrapolated cautiously to subfertile men with normal and subnormal semen parameters.

Wider implications of the findings: In a donor IUI program with strict selection on conventional sperm parameters, differences in SDF before and after cryopreservation and gradient centrifugation do not change IUI outcomes, although cryopreservation and centrifugation increase SDF. Findings could be useful for the optimization of sperm freezing and selection in different assisted reproductive procedures.

Trial registration number: not applicable

Abstract citation ID: dead093.425

P-060 how a learning model based on Artificial Intelligence can predict the pregnancy in relation to DNA spermatozoa damage

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Study question: The DNA spermatozoa integrity impacts on the male fertility, we aim to define if the damage status can predict the pregnancy.

Summary answer: The relation between the spermatozoa DNA damage and the pregnancy can support the doctor to recommend the couples to proceed in vivo or vitro conception

What is known already: The DNA spermatozoa is getting more and more important in the male fertility. Studies show that spermatozoa of infertile males have higher level of DNA damage than fertile males. DNA damages are often connected to poor seminal parameters such as sperm count, motility and morphology even if the 8% of males with normal seminal parameters show the same diagnosis. We are also skeptical about using the spermatozoa with damaged DNA in the ICSI, indeed there could be some consequences. The WHO suggests to evaluate the DNA damage in the routine exams.

Study design, size, duration: The aim of the study is to develop an Artificial Intelligence model that it can predict the pregnancies related on DNA quality of spermatozoa. During 2015 and 2017, we evaluated the DNA damage of 268 male after their partners had a natural pregnancy. The DNA spermatozoa damage is evaluated with p53 protein dosage so we developed a preliminary "learning model".

Participants/materials, setting, methods: It has been enrolled 356 male partners (pm) of infertile couples, divided into:

group A, 123 pm with 2-9 million spermatozoa/ejaculate (ICSI);
group B, 108 pm with 10-38 million spermatozoa/ejaculate (IUI);
group C, 125 pm with >39 million spermatozoa/ejaculate (natural conception).

p53 dosage, with ELISA method, was performed for the groups A and B preliminary to the treatment ICSI and IUI; for group C, it was performed from 10 to 30 days afterwards pregnancy (betaHCG > 400 miU/mL)

Main results and the role of chance: Group A: 29 pregnancies (23,6 %) occurred out of 123 ICSI performed.

According to our learning model:

- group A1: 42 pm (p53 < 1.65), 26 pregnancies were assumed against the 25 obtained, forecast percentage of 96.2 %.
- group A2: 81 pm (p53 > 1.66), 6 pregnancies were assumed against the 4 obtained with a forecast percentage of 66.6 %.

Group B: 16 pregnancies (14,8 %) occurred out of 108 IUI performed.

According to our learning model:

- group B1: 38 pm (p53 < 1.65), 16 pregnancies were assumed against the 14 obtained, forecast percentage of 87.5 %.
- group B2: 70 pm (p53 > 1.66), 2 pregnancies were assumed against the 2 obtained with a forecast percentage of 100.0 %.

Group C: out of 125 couples, with natural conception, 28 pregnancies (22,4 %).

According to our prediction model:

- group C1: 71 pm (p53 < 1.65), 25 pregnancies were assumed against the 24 obtained, predicted rate of 96.0 %.
- group C2: 54 pm (p53 > 1,66), 4 pregnancies were assumed against the 3 obtained with a 75,0 % forecast percentage.

The forecast is accurate (AUC 0.70).

Limitations, reasons for caution: The comparison between the control "learning model" and the expectation is not totally comparable. It is necessary to add a detailed case history of the couple to minimize other factors that may interfere with conception. For this reason, it is still necessary to be cautious to adopt this model.

Wider implications of the findings: A preventive examination, such as evaluating sperm DNA damage, could minimize in vivo and in vitro conception failures.

This "learning model" seems to have an excellent ability to predict a pregnancy. To achieve greater sensitivity, greater numbers are needed through its diffusion.

Trial registration number: Not Applicable

Abstract citation ID: dead093.426
P-061 In situ microfluidics of fluidic walls: a novel deviceless and cost-effective approach for sperm selection in the same ICSI-dish

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Study question: Is our novel deviceless method based on *in-situ* microfluidics a valuable strategy to select suitable sperm for ICSI?

Summary answer: The novel protocol allows selecting sperm for ICSI within 15 minutes, in the same ICSI dish and disregarding fungible supplies, centrifugation and washing steps.

What is known already: Microfluidics is an innovative operation in ART which integrates sperm guidance biomimicry during the *in vitro* sperm selection process. In contrast to other sperm preparation methods such as Wash-Swim-Up and Density- Gradients-Centrifugation, microfluidics disregards washing and centrifugation steps along the procedure. In addition, microfluidics are time-efficient methods which reduce risks associated with handling, gamete mix-up and ROS production. However, microfluidics devices are costly and likely to fail to select motile sperm in dispermic samples. With the aim of outlining the application of microfluidics in ART, we conceived a lab-on-a-chip approach for sperm selection by *in-situ handmade* microfluidics of fluidic walls.

Study design, size, duration: The microfluidics circuit conforms to Laplace and hydrostatic pressure laws and integrates the sperm guidance mechanisms of rheotaxis and boundary-following behavior. System's efficacy is based on the volume differences, the distances between microdoplets, and their arrangement. It divides into Section 1 (dispersion and seminal plasma removal) and Section 2 (sperm sorting in response to positive rheotaxis). The system was adjusted to recover at least 20 selected progressive sperm for ICSI in less than 15 minutes.

Participants/materials, setting, methods: We prepared the system on a conventional IVF polystyrene round dish (15 mm diameter) using MOPS buffer, PVP and mineral oil only. We verified the flow-driven dynamics by registering the horizontal displacement (direction) and flow velocity (μm/s) using eosin-Y and methylene blue dyes. We confirmed the removal of the seminal plasma in a PSA assay. The proof-of-concept analysis was performed using fresh semen samples from 150 patients (Caucasian; mean age: 33,53 ± 8,19).

Main results and the role of chance: Our design includes three main points to allow sperm separation: 1 (initial), 2 (rebalancing), 3 (final) with a total distance of 1,5 cm between 1 and 3. Our system assures a continuous microfluidic flow, with two opposed directions (from point 1 to point 2 and from point 3 to point 2). PSA assay showed a significant decrease in PSA levels >200000 ng/ml in the fresh sample to undeterminable levels (<0,0091 ng/ml) at point 3. To assess the utility to select a suitable number of sperm for ICSI we used sperm samples with the following characteristics: volume 3,6 (1,0-7,6) ml, concentration 68 (0,29-150) $\times 10^6$/ml, progressive motility 24 (2-41) % and morphology 6 (1-15) % normal. The minimum and maximum times for sperm recovery at point 3 were recorded at 5 and 35 minutes, respectively. Normozoospermic samples required lower recovery times compared to dispermic ($p < 0,001$), although according to the median, samples with at least 1×10^6/ml of motile sperm need less than ten minutes recovery time. Sperm parameters: concentration, motility and morphology were inversely correlated with the recovery time ($p < 0,0001$), being morphology the parameter with the lesser influence on the outcome.

Limitations, reasons for caution: The present protocol depends on a free-hand preparation. Although the use of templates and the training reduces inter and intra-operator variability, a ready-to-use surface would benefit the operability. Non-inferiority studies are required to compare our method with the currently used ones (Wash-Swim-Up or Density-Gradients-Centrifugation) before clinical implementation.

Wider implications of the findings: Our deviceless methodology streamlines sperm selection for ICSI and reduces risks along the procedure. This novel approach represents a paradigm shift in sperm preparation, since it dispenses with centrifugation, washing and plasticware. The present protocol can be implemented to the clinic in the near-term, particularly due to its simplicity.

Trial registration number: na

Abstract citation ID: dead093.427

P-062 The influence of the ejaculatory abstinence period on semen parameters from the first diagnostic sample – results from analysis of 14000 samples

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Study question: Is ejaculatory abstinence period (EAP) associated with semen parameters in males undergoing preliminary fertility investigation?

Summary answer: Short EAP is associated with increased progressive motility (%), vitality but with reduced volume, concentration, total sperm count. No association was indentified with morphology, DNA fragmentation.

What is known already: It is known that semen parameters may present important intra-individual variations. At present, the last edition of the WHO Manual recommends ejaculatory abstinence from two to seven days for semen analysis.

Nonetheless, this period is not supported by some relevant bodies in the reproductive field guidelines that limit abstinence period to three or four days. Recently published reviews on this matter support that the relationship between the abstinence time and sperm quality is not straightforward. Therefore the aim of this study is to analyze correlation between the ejaculatory abstinence time and sperm parameters in patients having initial work-up in our clinic.

Study design, size, duration: We conducted a retrospective cross-sectional study on the diagnostic semen samples from individuals having their initial fertility investigation in the Fertility Department of a University Affiliated Hospital between the beginning of 1997 and the end of 2022. Basic semen parameters and sperm deoxyribonucleic acid (DNA) fragmentation were registered in 14067 and 784 samples respectively. Sperm morphology was analysed in 4812 samples.

Participants/materials, setting, methods: We included exclusively results from the first diagnostic semen sample from males of any age undergoing preliminary infertility work-up. All samples were produced on-site and analysed "*ad hoc*" for basic semen parameters (according to 6th Edition of WHO Manual) or/and sperm DNA fragmentation (Sperm Chromatin Dispersion test). Samples were divided according to EAP: ≤2(**A**-group); 2-7(**B**-group); >7 days (**C**-group) and compared with ANOVA. Additionally, Spearman Correlation was used to confirm correlation between EAP and sperm parameters.

Main results and the role of chance: Semen analysis was performed either completely manually (23.4%) or with the use of Computer Assisted Semen Analysis (CASA) system (76.6%) with the exception of morphology which was always assessed manually.

Overall, 661 samples were produced after ≤2 days of abstinence, 12734 after 2 to maximum 7 days and 690 after >7 days of which ones 330(50%), 6798(53%) and 336(49%) were normozoospermic, respectively. Mean male age was 39 years (SD 6.5) and EAP 4.5 days (SD 1.9).

We found the following parameters positively correlated with EAP in both normozoospermic and abnormal samples: volume (A-2.6ml; B-3.6ml; C-4ml, $p < 0.05$), concentration (A-72M/ml; B-93M/ml; C-127M/ml, $p < 0.05$), total sperm count (A-161M; B-285M; C-472M, $p < 0.05$), total motile count (A-111M; B-186M; C-270M, $p < 0.05$), straightness coefficient (STR) (A-73.1; B-75.7; C-77.8%, $p < 0.05$) and Zinc concentration (A-14277; B-17375; C-22538µg/dl, $p < 0.05$).

The following parameters were negatively correlated with EAP in both normozoospermic and abnormal samples: progressive motility (A-46; B-43.6; C-36.6%, $p < 0.05$), vitality (A-60.5; B-60.3; C-53.7%, $p < 0.05$).

We did not find any correlation with leucocytes, fructose, morphology or DNA fragmentation in unselected patients. However, in the group of abnormal samples there was a negative correlation between EAP and morphology (A-3.7; B-3.1; C-2.8%, $p < 0.05$) and DNA fragmentation, in generalized additive model (Spearman-Correlation, R^2 0.01, $p < 0.05$).

Limitations, reasons for caution: This is a cross-sectional study which demonstrates only association and does not allow establishment of a cause-effect relationship between EAP and sperm parameters. Additionally, despite significant correlation between EAP and some sperm parameters, its clinical usefulness remains unclear.

Wider implications of the findings: To our knowledge this is first study correlating EAP with semen parameters only from diagnostic semen samples produced on-site, which confirms that the abstinence time should be seriously considered when analysing ejaculates, especially when borderline results are obtained. Additionally, different clinical strategies may be considered for normo and non-normozoospermic patients.

Trial registration number: not applicable

Abstract citation ID: dead093.428

P-063 Microfluidic technology is highly effective in selecting a sperm population with high progressive motility and low DNA fragmentation index

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Study question: Which sperm preparation procedure is able to select the best population of sperm?

Summary answer: Microfluidic sperm sorting using Fertile Plus device isolates sperm with the highest motility and lowest levels of DNA fragmentation.

What is known already: The sperm selection in Medically Assisted Reproduction should lead to fully functional spermatozoa able to reach the fertilization site and allow optimal fertilization and embryo development. These abilities are dependent on specific sperm parameters such as, but not limited to, motility, morphology, Acrosome Index (AI), and DNA fragmentation Index (DFI). At present, the routine sperm selection is suboptimal, does not replicates the physiological processes observed in nature and consequently may induce sperm damage. The new Microfluidic Sperm Sorting technology allows a physiological selection of sperm. However, no comparison with other selection procedures regarding all the above-mentioned parameters was reported.

Study design, size, duration: Prospective-observational study performed at an University Hospital-IVF Centre between April-November 2022 comparing the effect of four different sperm selection procedures on various semen parameters. Fifty-two samples with minimum $20x10^6$ sperm/ml and progressive motility ≥30% were included.

Descriptive statistics reported the mean ± SD [minimum-maximum] values for each variable. Interclass correlation coefficient (ICC) was calculated for the absolute agreement in between the four different procedures, where DGC was considered the standard-of-care. An ICC-value <0.4 indicates a low agreement.

Participants/materials, setting, methods: Unused raw semen after routine diagnostic analysis were individually split in 4 fractions and processed immediately by different sperm preparation methods: 1) sperm wash (SW), 2) Density Gradient Centrifugation (DGC), 3) Magnetic Activated Cell Sorting (MACS, (Miltenyi Biotec)), and 4) Microfluidic Sperm Sorting (MSS) using Fertile Plus Device (Koek Biotechnology).

Each fraction was analyzed for progressive motility, morphology, Acrosome Index (AI) and DNA fragmentation Index (DFI; TUNEL assay protocol).

Main results and the role of chance: The mean age of the patients was 35.9 years (SD ± 6.8). The mean value of days of abstinence was 4.1 (SD ± 6.8).

Mean progressive motility rates were: 54.3 ± 10.6% [23-86%], 74.3 ± 11.8% [38-90%], 77.2 ± 12.5% [37-92%] and 88.6 ± 4.2% [73-96%] for SW, DGC, MACS, and MSS, respectively.

Mean percentages of normal morphology were: 3.3 ± 2.9% [0-13%], 4.1 ± 3.1% [0-13%], 4.2 ± 3.7% [0-18%], and 5.1 ± 3.9% [0-16%] for SW, DGC, MACS, and MSS, respectively.

Mean AI were: 8.5 ± 4.9% [1-20%], 9.7 ± 6.0% [1-30%], 8.7 ± 4.9% [0-19%], and 10.8 ± 6.8% [1-30%] for SW, DGC, MACS, and MSS, respectively.

Mean DFI were: 6.2 ± 4.6% [0.8-26.1%], 2.7 ± 3.2% [0.2-14%], 2.1 ± 4.3% [0.9-20.8%], and 0.2 ± 0.4% [0.0-2.3%] for SW, DGC, MACS, and MSS, respectively.

Values referring to ICC absolute agreement between the DGC and the other methods indicate MSS as the only procedure in strong disagreement regarding progressive motility [0.29, 95%CI (-0.2-0.58)] and DFI [0.17; 95%CI (-0.19-0.45)]. No disagreement regarding morphology and AI was observed between all techniques.

A further paired comparison confirmed the strong disagreement between MSS and MACS [0.2; 95%CI (-0.17-0.49) and 0.21; 95%CI (-0.25-0.52)] and between MSS and SW [0.06; 95%CI (-0.04-0.25) and 0.06; 95%CI (-0.15-0.28)] regarding progressive motility and DFI, respectively.

Limitations, reasons for caution: Differences in DNA-damage may have been smaller between techniques if abstinence period was shorter. Due to the design of the experiment, data on clinical outcomes are not available.

Wider implications of the findings: The selection of a population of highly motile spermatozoa with undamaged DNA from unprocessed semen is ideally performed with MSS using Fertile Plus. Question remains how these results compare with a shorter abstinence period and whether this improves the embryological outcomes in the IVF laboratory.

Trial registration number: NCT05383599

Abstract citation ID: dead093.429

P-064 MicroRNAs as potential biomarkers for male infertility related to Testicular Germ Cell Tumors

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Study question: Could microRNAs represent efficient biomarkers to evaluate male infertility and the tendency of develop testicular germ cell tumors (TGCTs)?

Summary answer: The up-regulation of miR-193a-5p, miR-93-5p and let-7c-5p in TGCT patients with impaired spermiogram could represent a molecular signature of male infertility related to TGCTs.

What is known already: Testicular germ cell tumors (TGCTs) are the most recurrent tumors in young men with the highest incidence between the ages of 20 and 40 years and represent more than 60% of all cancers diagnosed in this age range. Several studies have shown a correlation between TGCTs and infertility, not only because infertility could be a consequence of testicular damage due to TGCTs but also because infertility may represent a risk factor for TGCTs. Therefore, it is important to find valid biomarkers that could be used to specifically identify infertility related to an increased risk of developing TGCTs.

Study design, size, duration: From March 2021 to January 2023, we collected 44 seminal plasma samples: 17 from TGCT patients who were undergoing sperm cryopreservation prior to chemotherapy, 14 with impaired spermiogram (IS) and 3 with normal spermiogram (NS) and 27 from control patients, 16 with IS and 11 with NS, undergoing assisted fertilization techniques. We evaluated the potential role of miRNAs as non-invasive biomarkers, analyzing the differential expression of 84 miRNAs in relation to cancer, infertility and both.

Participants/materials, setting, methods: Semen samples were placed 30'/37°C, seminal plasma was purified using density-gradient centrifugation and stored at -80°C. RNA was purified by Qiagen miRNeasy Serum/Plasma Kit and analyzed by miRCURY LNA miRNA SYBR®Green PCR_SerumPlasma, 96-well plate. We applied the $2^{-\Delta\Delta CT}$ method and statistical significance was evaluated by Significance Analysis of Microarrays, screened by p-values ≤ 0.05. We performed a Pearson correlation analysis applying two-sided p-values. miRTarBASE, MIENTURNET and Cytoscape were used for bioinformatics analysis.

Main results and the role of chance: In the four comparisons, we found 9 differentially expressed microRNAs. In particular, miR-221-3p, miR-222-3p, miR-204-5p and miR-205-5p down regulation would appear to be related to infertility, regardless to the cancer. Conversely, all TGCT patients shown an up-regulation of miR-376c-3p. Interestingly, up-regulation of miR-193a-5p, miR-93-5p and let-7c-5p specifically discriminates the infertile cancer patients *vs* other categories. Moreover, these three miRNAs showed a significant positive correlation both in cancer patients with impaired spermiogram and in infertile controls. This correlation demonstrates that their expression changes in the same way in the single samples and strongly suggests their role as early biomarkers of TGCT patients with impaired spermiogram. From the KEGG pathway analysis has emerged that DE miRNAs are involved in several signaling pathway, such as MAPK, mTOR, p53, PI3K-Akt, AMPK, FoxO, JAK-STAT, Ras, Estrogen signaling pathways, as well as cell cycle and cellular senescence. Moreover, from the Diseases Ontology analysis we found than those miRNAs are involved in germ cell tumor, male reproductive organ cancer, prostate cancer, male infertility and azoospermia.

Limitations, reasons for caution: The data must be validated by single assays in higher number of samples. These experiments are currently ongoing.

Wider implications of the findings: Three of the DE miRNAs are overexpressed in TGCT with impaired spermiogram patients. Specifically, they are over-expressed in TGCT with impaired spermiogram compared to infertile controls, so they may represent specific biomarkers able to discriminate, among the young men with infertility, those with higher risk of develop testicular cancer.

Trial registration number: Not applicable

Abstract citation ID: dead093.430

P-065 A systematic review of the accuracy of laboratory semen analysis as a test of fertility

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Study question: Which semen parameters are predictive of treatment-dependent and treatment-independent reproductive outcomes?

Summary answer: Progressive motility predicted treatment-independent pregnancy, total motile sperm count (TMSC) predicted failed fertilisation with IVF and pregnancy following IUI, and morphology predicted pregnancy after IUI.

What is known already: Semen analysis, an essential component of any fertility work-up, is useful in identifying azoospermia and oligozoospermia, but its

discriminatory capacity to identify fertile and infertile men has been questioned, including by the World Health Organisation (WHO). It has also been suggested that a combination of semen parameters has better predictive value than a single parameter. We conducted a systematic review of the literature to determine the association between different semen parameters and spontaneous (treatment-independent) pregnancy outcomes, as well as pregnancy outcomes following various fertility treatments (treatment-dependent outcomes).

Study design, size, duration: A protocol was registered prospectively with PROSPERO (CRD42021251988). A systematic search of MEDLINE, EMBASE, Web of Science, and the Cochrane Central Register of Controlled Trials was undertaken to include papers published from 1st January 2000 to 25th May 2021. Studies were not limited by language. Two reviewers independently screened the search results and undertook data extraction. Risk of bias assessment was undertaken with the QUADAS-2 or QUIPS tool, depending on study design.

Participants/materials, setting, methods: Studies were eligible if they included men undergoing semen analysis for any indication and reported the accuracy of one or more semen analysis parameters in predicting treatment-dependent or treatment-independent reproductive outcomes – these included fertilisation rate, clinical pregnancy, live birth, and time to pregnancy. Studies were excluded if semen analysis was not the primary test being assessed or no reproductive outcomes were reported.

Main results and the role of chance: Of 5236 publications and 139 full-text articles, 62 studies were included. 52 studies reported treatment-dependent outcomes and 10 reported treatment-independent outcomes. Most treatment-dependent studies lacked key information and were rated as unclear risk of bias in at least one domain (79.3% overall). Half of the treatment-independent studies were high risk of bias in at least one domain.

Of all semen parameters, progressive motility and sperm concentration predicted treatment-independent pregnancy outcomes. Three studies concluded progressive motility predicted pregnancy rates at a range of thresholds (threshold 24%: area under the curve (AUC) 0.909; $<32\%$ vs $\geq 32\%$: adjusted odds ratio (aOR) 4.2 (95% CI 1.1-15) and $<50\%$ vs $\geq 50\%$: aOR 2.8 (95% CI 1.3-6.1); per 10% reduction in progressive motility, pregnancy chances reduced by 11%: hazard ratio (HR) 0.89 (95% CI 0.85-0.93)). Sperm concentration predicted pregnancy within 6 months (≥ 20 vs < 20 million/ml, adjusted rate ratio 0.68 (95% CI 0.52-0.91)) and 12 months (adjusted HR 1.35 (95% CI 1.09-1.66)).

In those undergoing IUI, pre-/post-wash TMSC and morphology predicted pregnancy, but with no clear threshold values. TMSC predicted "total fertilisation failure" in an IVF population (AUC 0.631-0.75). In an ICSI population, no semen parameter predicted fertilisation rate, pregnancy, or live birth rate.

Limitations, reasons for caution: Meta-analysis was not possible due to a wide range of cut-off values used for each semen parameter. Due to the low quality of the evidence included, poorly detailed female populations, and WHO manual methods often not being used for reporting, the findings of this review should be viewed with caution.

Wider implications of the findings: Whilst this review has identified certain semen parameters in isolation are predictive of pregnancy in treatment-dependent and treatment-independent scenarios, clear threshold values have not been identified. This questions the reporting of morphology as it is labour intensive to assess and is only predictive of pregnancy in a population having IUI.

Trial registration number: Not applicable

Abstract citation ID: dead093.431
P-066 Testis expressed 44 is essential for spermatogenesis and male fertility

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Study question: Dose Testis expressed 44 (TEX44) gene associated with spermatogenesis and TEX44 mutations required for male infertility ?

Summary answer: TEX44 is expressed in testis and participated in spermatogenesis. And mutations of TEX44 may be involved in risk of asthenozoospermia and lead to subfertile.

What is known already: The World Health Organization estimates that 7-9 % of male population worldwide struggle with infertility. Asthenoteratozoospermia is one of the major factors for male infertility, whereas the causes of large numbers of cases are still unknown.TEX44 is a protein coding gene, which is specifically expressed in male germ cells. However, the expression, localization pattern and precise underlying mechanisms of TEX44 in mouse and human have not been clearly during spermatogenesis and risk of asthenozoospermia.

Study design, size, duration: This study was an analysis of 120 Han Chinese men with asthenoteratozoospermia by whole-exome sequencing(WES), at the Reproductive Medical Center of Peking University Third Hospital between January 2018 and January 2022.All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and national research committees of Peking University Third Hospital and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Participants/materials, setting, methods: Real-time polymerase chain reaction (Q-PCR) and western blot assays (WT) were used to investigate the mRNA and protein levels of TEX44 in testes. Immunofluorescent analysis was performed on testis sections by TEX44 antibodies during the spermatogenesis. TEX44 knockout mice were generated by CRISPR/Cas9 gene-editing. Computer-assisted sperm analysis (CASA) and fertility testing were also carried out to evaluate the effect of TEX44 on spermatogenesis and male reproduction.TEX44 mutation was an analysis of asthenozoospermia patients using WES.

Main results and the role of chance: TEX44 mRNA and protein were found to be expressed at the highest level in mouse testis by Q-PCR and WT. Immunohistochemical results revealed TEX44 was located in the cytoplasm of elongating spermatids and exhibited specific localization to the flagellum and manchette during spermiogenesis. Tex44 knockout mice presented no detectable difference in testis-to-body weight ratios when compared with wild-type mice. However, gross testis morphology shows reduction of testis size in TEX44 knockout mice compared wild-type after 12 weeks old. Tex44(-/-) males are subfertile because of abnormal sperm movement and reduced motility.Proportions of motile and progressive movement showing spermatozoa of TEX44 knockout mice were always 30–50% lower than that in controls using CASA.Tex44-/- mice are fertile despite a significant reduction in sperm motility. But the average total number of mouses born of Tex44-/- mice were decreased ($P < 0.01$). In addition, WES analysis revealed two novel frameshite point mutation patients in TEX44 gene during asthenozoospermia patients. Of these missense mutations, patients with asthenozoospermia have abnormal sperm movement and reduced motility. Fertility could be rescued by the use of intra-cytoplasmic sperm injections (ICSIs).

Limitations, reasons for caution: Additional cases are in need of study, especially with TEX44 mutation patients from only a small number of asthenozoospermia patients. Protein analysis and function were limited. In future investigations, a larger sample size should be used and the role of the interaction proteins with TEX44 should be analyzed.

Wider implications of the findings: TEX44 was validated as spermatids-essential gene could play important roles in spermatogenesis. The identification and characterization of TEX44 enhances our understanding of the molecular mechanisms of spermatids differentiation and provides insight into its potential role in the asthenozoospermia.These found will provide important guidance for genetic counseling and assisted reproduction treatments.

Trial registration number: This study was supported by the National Natural Science Foundation of China (NO.81671513) and Beijing Natural Science Foundation (NO.7172236).

Abstract citation ID: dead093.432

P-067 Characterization of seminal microbiota in patients with asthenozoospermic and normozoospermic using next-generation sequencing

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Study question: How are the semen microbiota compositions of patients with asthenozoospermic and normozoospermic different, According to data from 16S rRNA gene-specific Next-generation sequencing (NGS)?

Summary answer: Large numbers of microbes can be present in seminal fluid, and there are differences in the semen microbiota between asthenozoospermic and normozoospermic semen samples.

What is known already: Male factor is attributable in up to 50% of cases of infertility. Most studies on normal and abnormal sperm microbiota are based on 16S rRNA gene-specific next-generation sequencing (NGS). Microbiome analysis based on subunit 16S rRNA sequencing is a fast tool that can enable the identification of all the pathogenic microorganisms associated with semen in clinical pathology. Studies on the impact of semen micro-biomes in asthenozoospermic and normozoospermic samples could improve the results of assisted reproductive technologies. The major bacterial diversity in asthenozoospermic and normozoospermic samples belongs to the genera *Lactobacillus*, *Prevotella, Staphylococcus*, and *Anaerococcus.*

Study design, size, duration: Two eighty patients with their own semen samples were included in the study. The study population consists of patients attending to the "Pacific IVF Center" Pacific Medical University and Hospital (Udaipur, India) from November 2020 to October 2022. Depending on the spermiogram results, they were divided into two groups. Group 1 (n = 127) was asthenozoospermic, and Group 2 (n = 153) had normozoospermic semen samples, Patients were aged between (20-45 years). Ethical approval was obtained from the institute.

Participants/materials, setting, methods: Genomic DNA was extracted from samples using commercially available (Qiagen, DNeasy Power Soil kit). The amount of extracted DNA was measured using a Nanodrop spectrophotometer (Thermo Scientific). The microbiota of semen was analyzed using 16S ribosomal RNA (rRNA) gene amplification (MinION) Oxford Nanopore Ltd. Bioinformatics analysis was performed using QIIME2 and Microbiome Analyst packages. Alpha, beta diversity, and taxonomic characterization were compared for the seminal microbiome in asthenozoospermic and normozoospermic semen samples.

Main results and the role of chance: Different bacterial communities were detected when asthenozoospermic and normozoospermic semen samples were analyzed. In patients with asthenozoospermic parameters, a higher alpha diversity index tendency was found. In normozoospermic semen samples, a p-value of bacterial diversity was non-significant ($p = 0.14$ for the Shannon index), and significant differences in asthenozoospermic semen samples ($p = 0.01$ for the Shannon index). In the beta diversity analysis, no significant differences were observed between both groups

Relative abundance analysis identified the bacterial communities, *Lactobacillus, Streptococcus*, and *Ureaplasma* in normozoospermic semen samples, and Anaerococcus and *Gardnerella* in asthenozoospermic samples, Comparison of asthenozoospermic and normozoospermic samples with alpha diversity ($p = 0.06$ for the Shannon index and $p = 0.08$ for the Simpson index) and beta diversity ($p < 0.001$) showed significant differences. Relative abundance in normozoospermic semen samples identified the bacterial communities, *Lactobacillus* ($p = 3.70E\text{-}4$), Prevotella ($p = 8.11E\text{-}4$), and in asthenozoospermic semen samples *Anaerococcus* ($p = 0.004$) and *Gardnerella* ($p = 0.004$).

Limitations, reasons for caution: The limited sample size is the primary constraint of this study. Larger studies with a larger sample size are needed to confirm the distinct microbiome patterns identified in asthenozoospermic and normozoospermic samples in connection to male infertility.

Wider implications of the findings: Further research could determine the detection rate of the described bacterial diversity in semen with other pathologies. Determining the relationship between sperm microbiota characteristics and infertility could enable the development of new algorithms for treating patients with reproductive disorders, An abnormal seminal microbiome appears to be strongly correlated with infertility.

Trial registration number: N/A

Abstract citation ID: dead093.433

P-068 Real-world data shows that frozen-thawed ejaculates used for ICSI with autologous oocytes have lower but clinically negligible cumulative live birth rates compared to fresh samples

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Study question: Does frozen-thawed sperm show an impact on success rates in ICSI with autologous oocyte cycles compared to fresh samples?

Summary answer: The slight decrease in cumulative live birth rates (CLBR) compared to using fresh samples should not overshadow the benefits offered by sperm freezing.

What is known already: Sperm freezing offers many advantages for cycle planning, logistics, sample safekeeping and patient comfort, becoming a reliable daily procedure in infertility clinics worldwide. Nevertheless, there is a lack of agreement amongst the published literature on the potential effect of using frozen-thawed sperm samples for ICSI cycles. The present study focused on outcomes of ICSI cycles using autologous oocytes and, in addition to expressing results as classical success rates, it assessed CLBR, which accounts for the contribution of all used oocytes and embryos from the same insemination procedure towards achieving a live birth.

Study design, size, duration: This is a retrospective multicenter observational cohort study involving 84,371 ICSI procedures with patients' autologous oocytes (81,504 with fresh ejaculate semen samples and 2,856 with frozen ejaculate samples) from January 2008 to November 2021. The fresh sample group included real-world data from the clinical records of these patients and cycles from a cohort of 654,019 inseminated oocytes and 117,443 embryos transferred; whereas the frozen sample group considered 22,518 injected oocytes and 3,847 embryos replaced.

Participants/materials, setting, methods: Classical outcomes such as pregnancy rates and live birth rates (LBR) per embryo transfer (ET) were compared between the two study groups. Generalized linear models were used to obtain adjusted odds ratios (adjOR) and p-values for the comparisons. CLBR were expressed per ET, per embryo transferred and used oocyte, and were plotted as Kaplan-Meier curves. These were adjusted to the female patients' age using Cox regression models to obtain adjusted hazard ratios and p-values.

Main results and the role of chance: There were statistically significant differences between both groups in terms of clinical and ongoing pregnancy rates per ET, and LBR per ET. However, after being adjusted to female and male patients' age and BMI, total progressive motile sperm, semen capacitation, transfer before or at the blastocyst stage, and use of PGT, these differences were no longer statistically significant. Once three embryos were replaced, CLBR was 50.50% (49.91-51.08) in the fresh sample group, versus a 45.12% (41.77-48.28) in the frozen sample group. After five embryos transferred, these were 68.21% (67.27-69.13) and 64.62% (57.81-70.33) respectively ($p > 0.001$). The Cox regression resulted in an adjusted hazard ratio (adjHR) of 1.166 (1.076-1.264). The CLBR in the fresh sample group was 33.15% (32.69-33.61) after using 10 oocytes and 49.93% (49.31-50.54) after 14. In the frozen sample group, these were 28.73% (26.24-31.13) and 43.13% (39.78-46.30) ($p > 0.001$). The adjHR for this comparison was 1.220 (1.125-1.322).

Limitations, reasons for caution: The multivariate analyses aim to control to the best of our abilities the main limitation of the study, which is the

potential biases that could be introduced due to the heterogeneity of the data and the retrospective nature of the present study.

Wider implications of the findings: Although frozen samples had slightly decreased CLBR per used oocyte and ET, there were no statistically significant differences in pregnancy and LBR per ET after the multivariate analysis. The extensive real-world data provided shows that slight declines in CLBR should not offset the many advantages that semen freezing offers.

Trial registration number: Not Applicable

Abstract citation ID: dead093.434
P-069 A male factor panel of NGS can identify the genetic cause of more than half patients with seminal alterations

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Study question: Is Next Generation Sequencing (NGS) an effective diagnostic tool for sperm abnormalities?

Summary answer: NGS male factor gene panel can be a potent diagnostic tool for discovering pathogenic variants linked to sperm abnormalities.

What is known already: Male infertility, responsible for half of infertility cases, often shows as absence or decreased sperm count (azoospermia, criptozoospermia or oligozoospermia), poor motility (asthenozoospermia) or a higher proportion of morphologically abnormal sperm (teratozoospermia).

While the spermiogram remains the gold standard for evaluating male fertility, the potential to identify the molecular basis of sperm abnormalities may make NGS a valuable approach for such patients.

The aim of our study was to assess NGS panel's effectiveness in diagnosing patients with abnormal sperm count, motility and morphology.

Study design, size, duration: A prospective study was conducted from May 2021 to May 2022 in 82 patients including 20 patients with oligozoospermia, 19 with asthenozoospermia and 23 with teratozoospermia. No other sperm alterations were taken into account in each group. The control group consisted of 20 normozoospermic healthy donors selected on the basis of normal sperm parameters according to the WHO criteria (2010).

Patients carrying Y-chromosome microdeletions or abnormal karyotype were excluded.

Participants/materials, setting, methods: Genomic DNA extraction from blood-EDTA of the patients was performed using the commercial MagMax DNA MultiSample Ultra kit and the King-Fisher automated extractor (ThermoFisher®). Next Generation Sequencing (NGS) was done using a panel with 426 genes involved in the spermatogenesis process.

Panel sequencing for identification of genetic variants was performed using Nextera Enrichment technology (Illumina®).

FASTAQ data were processed using BWA and GATK algorithms. VCF files were analysed using Variant Interpreter software and in silico predictors.

Main results and the role of chance: Data analysis showed that thirty-seven of the sixty-two patients were carriers of pathogenic mutations in at least one of the genes included in the panel (37/62,59.6%).

In the oligozoospermic group, eleven patients (11/20,55%) were carriers of pathogenic mutations in:*CFTR, CEP290, WDR66, ESR1, DNAI2, POLG, PIWIL3, GNRHR, MSH5* and *CTNS*.

In the asthenozoospermic group, ten out of nineteen patients (10/19,52.6%) were carriers of pathogenic mutations in:*HS6ST1, PMS2, CYP19A1, DNAI2, POLG, LRRC6, G6PD, CCDC39* and *PIWIL3*.

In the teratozoospermic group, six of the twenty-three patients (6/23,26%) were carriers of pathogenic mutations in:*CFTR, PIWIL3, CYP21A2, SRD5A*.

No pathogenic mutations were detected in the control group.

The analysis showed comparable results among the various spermiogram alterations with no significant difference ($p > 0.05$).

Each variant was found in a single patient per group, except *POLG* and *CCDC39*, which were found in two cases in the oligozoospermic and asthenozoospermic group, respectively, also *CFTR* and *PIWIL3* in two teratozoospermic patients. Besides, it is remarkable that some of the pathogenic variants were present in genes in more than one group:*CFTR* (3/62), *DNAI2* (2/62), *POLG* (3/62) and *PIWIL3* (4/62).

All identified variants were linked with processes of spermatogenesis such as dynein assembly and DNA integrity.

Limitations, reasons for caution: The main limitation of this study is the limited number of patients included.

Functional studies with a larger cohort of males with seminal disorders is warranted to confidently correlate the genetic variants identified in this analysis with spermatogenic failure.

Wider implications of the findings: The gene list included in our panel represents a step-forward in the screening of males with altered sperm parameters.

Our results may add in the knowledge of male factor infertility to provide etiologic factors towards a personalized treatment and adequate genetic counselling.

Trial registration number: Not applicable

Abstract citation ID: dead093.435
P-070 SARS-CoV2 antibody isotypes in seminal fluid of vaccinated men

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Study question: Are SARS-CoV2 antibody isotypes detectable in seminal fluid of vaccinated men

Summary answer: Systemic circulating SARS-CoV2 antibodies are likely to be precluded from transport to seminal plasma following vaccination in males

What is known already: Expedited development of SARS-CoV-2 vaccines led to public concerns regarding impacts of the novel vaccine on gametes in patients seeking assisted reproduction.

Recent studies on ovarian follicular fluid from women post SARS-CoV2 infection or BNT162b2 mRNA vaccination show similar levels of SARS-CoV2 IgG antibodies in follicular fluid as those found in serum. To our knowledge, no study to date has examined if SARS-CoV2 antibody isotypes are detectable in seminal fluid of vaccinated men.

Study design, size, duration: This was a longitudinal cohort study of 17 normospermic male patients attending a fertility clinic associated with a tertiary university maternity hospital. Semen and matched peripheral blood samples were collected prior to vaccination ($t = 0$), within $46 + 18.9$ hours of vaccine completion (acute; $t = 1$) and at $88.4 + 12$ days (3 months; $t = 2$) post vaccination.

Participants/materials, setting, methods: Serum and seminal plasma anti-SARS-CoV-2 spike isotypes (IgA, IgM and IgG1) and immune factors (IL-6, IL-8, IL-10, IFN-γ, TNF-α, IP-10; CXCL10, MCP-1, CCL2) were analysed using ELISA-based approaches at three time points. To rule out potential factors present in seminal plasma that could interfere with IgG detection, a

commercial positive anti-Spike control was used to confirm detection in the ELISA assay. Self-reported symptoms and sperm parameters including count, motility, morphology, DNA damage were also quantified.

Main results and the role of chance: All semen samples were found to be negative for anti-SARS-CoV2 spike antibodies at all three time points indicating that systemic antibodies are likely precluded from transport to seminal plasma. Four out of 17 (23.5%) serum samples were negative for all three isotypes (IgM, IgA, IgG1) at < 72 hours post-vaccine completion, while one patient remained negative for all antibodies at 3 months post-vaccine. No global change from baseline was seen in reported symptoms, mean semen volume, semen pH, sperm concentration, motility, morphology or DNA damage in the acute or long phase. Two men showed a clinically relevant reduction in sperm motility alone in the acute phase that returned to normal by 3 months. Seminal plasma MCP-1 levels showed an acute but transient elevation post-vaccine, while IL-8 was marginally increased 3 months after completion of vaccination. Our findings also indicate a modest, positive correlation between serum levels of the anti-inflammatory cytokine IL-10 and self-reported symptoms in the acute post-vaccine period, with no correlation between serum IL-10 levels and change in sperm parameters from t = 0 to t = 1.

Limitations, reasons for caution: This study included a small population of only 17 men, all of whom were normospermic. Responses in men with subnormal baseline sperm parameters may differ and thus would be helpful to explore.

Wider implications of the findings: Our results show no significant adverse effect of vaccination. There may be temporary decline in sperm motility which could be more significant in men with poor baseline parameters. Further larger studies with inclusion of men with abnormal baseline parameters would be valuable to support timing of vaccination and treatment.

Trial registration number: not applicable

Abstract citation ID: dead093.436
P-071 L-proline enhances sperm quality and chromatin condensation of normozoospermic men during in vitro incubation

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Study question: Does supplementation of human sperm media with L-Proline improve sperm function and chromatin quality during in Vitro Incubation?

Summary answer: The inclusion of L-proline as an antioxidant in human sperm media improves sperm function and chromatin integrity probably by modulating oxidative stress

What is known already: Infertility affects millions of people of reproductive age globally. The quality of gametes is one of the most critical aspects that could affect the success rate of infertility therapy. The storage of spermatozoa at the incubator to achieve capacitation could rise sperm DNA fragmentation as a result of prolonged incubation. Although mild and low levels of Reactive Oxygen Species (ROS) could boost fertilization capacity by improving hyperactivation, and capacitation, oxidative stress is one of the major contributors to low sperm quality. L-Proline plays a pivotal role in many aspects of cell metabolism and physiology, including osmotic protection and oxidative stress

Study design, size, duration: The study was conducted on thirty-five healthy men attending the Motazedi Hospital, Kermanshah of the Division of Andrology, from December 2020 to June 2021. Each semen sample was equally divided into 4 groups: one group as control and three as treatment groups. The control group contained only sperm media (Hams, F10 medium supplemented with 5 mg/ml HSA). In treatment groups, different concentrations of Proline (1, 2, and 4 mmol/L) were also added.

Participants/materials, setting, methods: 35 normozoospermic men with healthy lifestyles were included. Sperm parameters including motility, viability, and morphology were evaluated. The levels of ROS, lipid peroxidation (LPO), and total antioxidant capacity (TAC) were determined in sperm media. Sperm chromatin integrity was analyzed by Aniline blue (AB), Toluidine blue (TB), and Chromomycin A3 (CMA3) staining tests. DNA fragmentation was measured by the Sperm chromatin dispersion (SCD) test. All assessments were conducted after 1, 4, and 24 hours of incubation.

Main results and the role of chance: The inclusion of Proline (2 mmol/L) resulted in noticeably improved maintenance of motility and viability after 24 hr of incubation compared to the control group ($P < 0.05$). Also, 2 mmol/L of Proline was able to significantly maintain normal morphology compared to other groups ($P < 0.01$). However, the level of ROS production non-significantly diminished in the L-proline groups compared to the control group ($P > 0.05$). In terms of lipid peroxidation, the presence of 2 mmol/L of Proline kept the MDA production of spermatozoa at lower levels after 4 hr of incubation compared with the control group ($P < 0.05$). The supplementation of sperm media with 2 mmol/L of Proline led to improved maintenance of TAC level, compared to any other group ($P < 0.01$). Additionally, the results of AB and TB tests indicated that chromatin quality after supplementation with 2 mmol/L L-proline significantly improved compared to the control group ($P < 0.05$). In terms of CMA3 assessment, however, there was a minor improvement between the 2 mmol/L Proline group and the control group. Moreover, 2 mmol/L Proline significantly reduced the level of fragmented DNA by comparison to the control group after 24 hr of incubation ($P < 0.05$).

Limitations, reasons for caution: Our team was unable to carry out additional studies to verify the impact of L-proline on mitochondrial activity, membrane potential, and apoptosis and to validate the efficiency and safety of L-proline using additional parameters such as the in vitro embryonic development and live birth rate.

Wider implications of the findings: While further studies are required to uncover the mechanisms underlying the antioxidant properties of Proline, these findings extend our knowledge of human male fertility and pave the way to improving the human sperm culture and in vitro procedure routinely used in infertility clinics for sperm incubation.

Trial registration number: IR.KUMS.REC.1399.625

Abstract citation ID: dead093.437
P-072 Efficacy of a microfluidic chip technology for sperm selection in intrauterine insemination cycles

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Study question: Is there an advantage of using a new sperm selection method by microfluidics (MFSS) over standard density gradient (DG) for couples undergoing intrauterine insemination (IUI)?

Summary answer: MFSS reduced sperm processing time and selected spermatozoa with greater progressive motility and superior genomic integrity, suggesting a better clinical outcome than DG.

What is known already: The most conservative, cost-effective reproductive treatments for infertile couples are timed intercourse and IUI. The latter is generally performed using DG to enrich and purify the most motile spermatozoa; it requires technician effort and skills as well adequate processing time. Moreover, during DG centrifugation, spermatozoa are exposed to potentially harmful silica gel particles and eventual reactive oxygen species (ROS). The recent availability of a microfluidic chamber has been proposed as a method to safely process spermatozoa in an expedited manner while reducing human intervention. Therefore, we decided to test MFSS in a laboratory that executes over 2,000 IUI cycles/year.

Study design, size, duration: Since September 2022, 93 couples underwent 103 IUI-MFSS. Post-processing semen parameters and clinical outcomes were compared with those of 103 age-matched couples who underwent 103

IUI-DG during the same period. To confirm findings, 45 couples undergoing their first IUI-MFSS had post-processing semen parameters and clinical outcomes compared with 45 first-time IUI-DG couples, serving as a matched control. Finally, within the same 66 couples, outcomes were compared between their initial IUI-DG cycle and a subsequent IUI-MFSS.

Participants/materials, setting, methods: Men had normal raw semen parameters according to the most recent WHO criteria. DG was performed according to WHO21 guidelines, while MFSS as per manufacturer protocol (ZyMōt® Multi 850µL). Stimulation protocols were similar between all patients. Before and after processing, volume, concentration, and motility were compared between the two processing methods. Sperm chromatin fragmentation (SCF) was assessed by TUNEL (In Situ Cell Death Detection Kit; normal threshold,≤15%). For all comparisons, +bHCG and clinical pregnancy (+FHB) were compared retrospectively.

Main results and the role of chance: Ninety-three couples (maternal age,37.3 ± 4; paternal age,39.2 ± 6) underwent 103 IUI-MFSS cycles and were matched for age and abstinence days (1–4d) to 103 IUI-DG patient/cycles. IUI-DG and IUI-MFSS yielded 0.5 ± 0mL volume, 76.8 ± 40x10^6/mL and 41.3 ± 26x10^6/mL concentrations, and 89.4 ± 3% and 98.3 ± 1% motility, respectively (P<0.001). IUI-DG resulted in a 20.4% +bHCG (21/103) and 15.5% +FHB (16/103). IUI-MFSS yielded a 19.4% +bHCG (20/103) and 16.5% +FHB (17/103).

Forty-five couples (maternal age,36.8 ± 4; paternal age,37.8 ± 6) who underwent their first IUI with MFSSwere matched to a first-time IUI-DG cohort with similar semen parameters and abstinence. IUI-DG led to a 0.5 ± 0 mL volume, 77.3 ± 43x10^6/mL concentration, and 90 ± 2% motility. IUI-MFSS yielded a lower concentration (45.6 ± 27x10^6/mL), but a remarkable increase in motility (98.3 ± 1%) (P<0.001). IUI-DG yielded a 13.3% +bHCG (6/45) and an 8.9% +FHB (4/45). IUI-MFSS yielded a 15.6%(7/45) +bHCG and an 11.1% +FHB (5/45).

Finally, we assessed 66 couples who underwent IUI-DG and subsequently IUI-MFSS. IUI-DG resulted in a 77.5 ± 39x10^6/mL concentration and 89.0 ± 3% motility, while IUI-MFSS resulted in a lower concentration (36.9 ± 21x10^6/mL), but an enhanced motility (98.3 ± 1%)(P<0.001). IUI-DG yielded a 15.2% +bHCG (10/66) and an 8.9% +FHB (5/66), while IUI-MFSS yielded a 15.2% +bHCG (10/66); all became clinically pregnant.

In all comparisons, SCF was 6.5 ± 3% in raw samples, became 4.7 ± 2% after DG, and negligible after MFSS at 0.8 ± 0.6%(P<0.001).

Limitations, reasons for caution: IUI-MFSS cycles yielded a higher number of progressively motile spermatozoa with superior genomic integrity and a comparable clinical outcome to IUI-DG. This study came at a higher cost due to the device and offered only preliminary results. Therefore, it needs to be repeated in a larger study population.

Wider implications of the findings: DG is time consuming, labor intensive, and its outcome is technician-dependent. While reducing exposure to chemicals and ROS, MFSS grants higher motility and superior genomic integrity. If a greater clinical outcome is confirmed in a larger cohort, MFSS may prove to be a superior sperm processing method for IUI.

Trial registration number: not applicable

Abstract citation ID: dead093.438

P-073 Comparison of human preimplantation embryo development after sperm selection for ICSI using the microfluidic ZyMōt device versus density gradient centrifugation

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Study question: Does microfluidic sperm selection using ZyMōt device has a significant effect on blastocyst development, ploidy, and improves pregnancy outcomes when compared with density-gradient centrifugation.

Summary answer: The study demonstrated that sperm selection using a microfluidic device had no impact on euploid blastocyst rates or clinical pregnancy outcomes.

What is known already: Selecting competent spermatozoa with the highest genomic integrity for ICSI is a key to achieving normal embryo development and a healthy live birth. Several sperm preparation techniques have long been scrutinized with varied success. The novel microfluidic ZyMōt device appears promising for improving the quality of sperm used in Assisted Reproductive Technologies. Preliminary tests have shown that ZyMōt may increase sperm motility and considerably decrease DNA fragmentation, which could potentially enhance euploid blastocyst development rates. However, the impact of this sperm selection method on clinical outcomes has not been fully investigated.

Study design, size, duration: This is a retrospective cohort study performed in a single academic IVF centre (**IRB # 16367**). Data were collected from 1286 ICSI cycles in 2020-2021 (730 with ZyMōt and 556 with DGC), including 867 cycles with PGT-A outcomes. Morphological grading of 6640 blastocysts cultured in a time lapse imaging incubator (Embryoscope™) and ploidy of 4783 blastocysts using high resolution NGS (Illumina platform and Blue Gnome) were compared between the two study groups.

Participants/materials, setting, methods: All male partners were normo-spermic according to standard semen analysis. ICSI results for a total of 7277 mature oocytes injected with spermatozoa processed with ZyMōt device and 5977 oocytes injected with spermatozoa processed by gradient-density centrifugation were compared. For the analysis of 1478 euploid embryo transfer outcomes, all cases included a single blastocyst transferred in a frozen-thaw cycle. Associated patient characteristics, including age and fertility diagnosis were collected.

Main results and the role of chance: A significantly higher fertilization rate was observed with ZyMōt compared to the gradient prepared sperm (83.4% vs 81.0%, $P = 0.0004$, respectively). The blastocyst rates (61.3%, vs 60.4%, $P = 0.34$) as well as the proportion of high-quality blastocysts were similar in both groups ($P = 0.20$). Euploidy rates were not different between all female age groups, including young egg donors (≤ 30y of age) to patients with advanced maternal age (>40 y of age). Microfluidic sperm selection did not improve clinical pregnancy rates after euploid embryo transfer (42.9% vs 41.4%; $P = 0.58$).

Limitations, reasons for caution: Although this is the largest study to date evaluating application of the ZyMōt device, the retrospective nature and cohort design limited the interpretation of our results. The patient population did not include male factor cases and sperm DFI data was not available. Embryo transfer outcomes were limited to clinical pregnancies.

Wider implications of the findings: The ZyMōt device offers a convenient alternative to standard sperm processing by density gradient method. Further studies are needed to fully determined the impact of ZyMōt on embryo quality and clinical outcomes in different age and diagnosis groups of patients; particularly for those with high DFI and poor IVF/ICSI outcomes.

Trial registration number: not applicable

Abstract citation ID: dead093.439

P-074 In-vitro vitamin D treatment on human sperm improves sperm motility, capacitation, and oocyte activation

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Study question: Does *in-vitro* vitamin D treatment on fertile and infertile human sperm improves sperm motility and alter protein markers involved in capacitation and oocyte-activation.

Summary answer: *In-vitro* vitamin D treatment enhance functionality of sperm by increase intracellular calcium ion, thus increasing sperm motility and capacitation status.

What is known already: Many studies have been done to investigate the relationship between vitamin D and male infertility. It is known that low serum vitamin D in human contribute to male infertility. However, the effect of *in-vitro* vitamin D treatment on sperm remains unclear, particularly on the effect of vitamin D following ejaculation into female reproductive tract. This includes processes involved in fertilisation such as capacitation, hyperpolarization, oocyte activation and sperm-oocyte fusion. Therefore, we examined whether *in-vitro* vitamin D treatment on sperm can improve the sperm parameters, hence will then improve fertilisation and pregnancy.

Study design, size, duration: Randomised controlled trial with prospective interventions (*in-vitro* vitamin D treatment). Based on power analysis, 40 subjects both fertile and infertile men who came for semen analysis were recruited at University Malaya Medical Centre from January 2022 to January 2023. All men were consented to participate in this study. Each washed semen sample were aliquoted to serve as control and receive *in-vitro* vitamin D treatment.

Participants/materials, setting, methods: Total of 40 samples were included for final analysis. All semen samples were washed and treated with 1nM vitamin D *in-vitro* for 45 minutes at 37°C. Motility, intracellular calcium released, capacitation status, mitochondria membrane potential and other basic semen parameters were measured after *in-vitro* vitamin D treatment for fertile and infertile samples. Furthermore, immunofluorescence of sperm origin oocyte activation factor, Phospholipase C Zeta (PLCζ), was done to investigate fluorescence intensity per cell using Image J.

Main results and the role of chance: In both fertile and infertile sample group, total motility is increased after *in-vitro* vitamin D treatment (MD = 8.727%, $P < 0.001$). Fluo-4 AM stain shows there are increase of intracellular calcium release after *in-vitro* vitamin D, with mean difference of Corrected Total Cell Fluorescent (CTCF) for Fluo-4 AM (MD = 25043, $P = 0.0228$. Chlortetracyclin (CTC) test for capacitation status and immunofluorescence of phosphotyrosine shows there are increase CTCF (MD = 0.02595, $P = 0.0142$) after *in-vitro* vitamin D treatment. Staining of JC-1 shows there are more hyperpolarised mitochondria membrane in *in-vitro* vitamin D treatment group compare to the control group. Ratio of red to green fluorescent is 0.639 in control group and 2.22 in *in-vitro* vitamin D treatment group. Moreover, immunofluorescence of sperm origin oocyte activation factor, Phospholipase C Zeta (PLCζ) show *in-vitro* vitamin D improve the expression of these protein. Mean difference of CTCF between control and vitamin D treatment group for PLCζ is 8.013×10^{-3}, $P = 0.0226$. Thus, *in-vitro* vitamin D enhance functionality of sperm by increase intracellular calcium ion, thus increasing sperm capacitation status and sperm membrane hyperpolarization.

Limitations, reasons for caution: Although power analysis is used to calculate sample size, however bigger sample size always needed to increase the accuracy. Moreover, patients' sample are examined and treated after 3 hours due to limited collection room in the facility.

Wider implications of the findings: Vitamin D can be used as a tool in *in-vitro* fertilisation (IVF) procedure and can be added to sperm preparation medium due to its implication in increasing capacitation and hyperpolarised sperm membrane to increase chance of successful IVF.

Trial registration number: not applicable

Abstract citation ID: dead093.440
P-075 Artificial intelligence for sperm analysis: A KNIME-Based automated analysis of sperm morphology

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Study question: To evaluate an automated sperm morphology assessment method using the KNIME Analytics Platform compared to manual sperm morphology analysis performed as per WHO guidelines.

Summary answer: Our method emphasizes how artificial intelligence technologies have the potential to foster standardization of sperm morphology assessment with comparable precision and reliability.

What is known already: Manual sperm morphology assessment is considered the most difficult parameter to standardize due to its subjective nature, strongly linked to the operator's level of expertise. Indeed, there is a high degree of inter and intra-laboratory variability. Manual examination is time-consuming and laborious. There were many attempts to automate sperm morphology analysis, especially with CASA (Computer Assisted Sperm Analysis) systems, but their performance is still disputable. One of the difficulties in this field of study is the lack of publicly available datasets. Besides, the available databases are only focused on the sperm head morphology.

Study design, size, duration: A total of 37 semen samples from men attending our laboratory for infertility investigation were included, over a period of one year. For each sample, semen smears were fixed and stained by the Spermoscan® kit.

Participants/materials, setting, methods: A total of 1000 images of individual spermatozoa were obtained using the MMC® CASA system. The number of images per sample depended on its quality. Three experts have classified these spermatozoa according to modified David classification for sperm morphology. The results were then processed and an algorithm created using teh KNIME Analytics Platform, trained and tested to classify spermatozoa. This workflow uses CNN (convolutional neural network) to perform image classification on our dataset.

Main results and the role of chance: Of the 1000 images analyzed, we counted : 116 Normal sperm morphology, 67 abnormal post-acrosomal region, 128 abnormal acrosomes, 8 elongated heads, 6 thin heads,10 microcephalic,7 multiple heads, 27 coiled tails, 7 cytoplasmic residues, 17 angulated tails, 6 short tails, 4 multiple tails, and 697 associated abnormalities. For each image, a notebook file containing sperm abnormalities as assessed by the three experts was created, in addition to sperm head, mid-piece and tail dimensions obtained by the CASA system.

Our dataset was randomly partitioned into 2 groups: 80% of data formed the training set, and

The remaining 20% formed the fully independent test set.

The best performance with the KNIME-Based algorithm was achieved for post-acrosomal abnormalities (97 % True positive rate), and the worst for multiple tail abnormalities (69 % True positive rate). Our machine learning model classifies sperm morphology at high accuracy (99.5 %). The overall process occurs in less than 10 seconds.

Limitations, reasons for caution: Our database included various head, midpiece and tail anomalies. However, the number of images in each category is unequal due to the limited occurrence of some morphological abnormalities. Thus, it is important to increase the number of images in these categories to obtain better results.

Wider implications of the findings: Our goal is to expand our modified David's classification-based database. We aim to improve the performance of our model, and to put it at the service of learning and routine use in laboratories.

Trial registration number: not applicable

Abstract citation ID: dead093.441
P-076 Sperm selection by thermotaxis: a novel technique to enhance assisted reproductive technologies' outcomes

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Study question: Does sperm selection by thermotaxis increase competent spermatozoa count?

Summary answer: When compared to capacitation alone, our findings suggest an improvement in the availability of high quality spermatozoa after thermotaxis in terms of motility and morphology.

What is known already: Thermotaxis is a natural mechanism that guides competent spermatozoa through the female genital tract. It has been previously shown that, in the mice model, thermotaxis may improve pregnancy rates following *in vitro* fertilization (IVF). However, thus far, there is little information to support these findings with human spermatozoa.

Study design, size, duration: A prospective single-center paired pilot analysis was performed between April 2021 and April 2022 including 21 male individuals, 12 of whom were sperm donors and 9 who were patients with asthenozoospermia (defined as < 32% progressive sperm motility).

Participants/materials, setting, methods: Only otherwise healthy men between 18 and 45 years old were included. All subjects performed a sperm collection at the clinic after a maximum of 3 days following the last ejaculation. Sperm concentration, motility, morphology (all using the WHO 2010 criteria) and DNA fragmentation (TUNEL) were assessed within the same sample at three time-points: fresh sample, after capacitation and following the extra thermotaxis procedure.

Main results and the role of chance: The average age of the participants was 34.9 ± 6.6 years old. The mean sperm concentration was 66.9 ± 39.1 million/mL (91.4 ± 26.1 in the donor subset (group A) versus 34.2 ± 28.0 in the asthenozoospermia patient (group B) subgroup). The median sperm motility was 62.0% (66.5% in group A versus 24.0% in group B). The mean sperm morphology was 7.2% (7.7% in the group A versus 6.7% in the group B). When compared to the sample in which only capacitation was performed, a statistically significant increase in spermatozoa motility (52.10% vs 82.14%, $p < 0.05$) and normal morphology (11% vs 16%, $p < 0.05$) was observed following capacitation plus thermotaxis. Conversely, DNA fragmentation did not vary significantly within the capacitated samples before and after thermotaxis (13% vs 14%).

Limitations, reasons for caution: The present pilot study is based on a limited number of participants and should be extended further.

Wider implications of the findings: The combination improvement in terms of morphology and progressive motility following thermotaxis may optimize selection of sperm cells and ultimately increase pregnancy rates after IVF. In the light of our experience, it is possible that this technique, which does not require high-end equipment, may increase the usable competent spermatozoa count.

Trial registration number: NCT04849156

Abstract citation ID: dead093.442
P-077 The clues underneath the purple head: sperm fragmentation correlation with seminal quality parameters after swim-up techniques and sperm cryopreservation

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Study question: Does the culture media and the protocols used for sperm processing and cryopreservation significantly affect DNA integrity? How does it correlate to other sperm parameters?

Summary answer: In one of the analyzed media, the DNA damage was significantly higher after swim-up. DNA integrity is negatively correlated with concentration, vitality and motility.

What is known already: DNA fragmentation index (DFI) has proven to be a valuable tool in the diagnosis of infertility since men evaluated as normozoospermic in the seminogram may have high levels of sperm DNA fragmentation.There is already evidence of sperm DNA fragmentation affecting fertility, though the use of this information in ART is limited. The study of DNA fragmentation is relevant in fresh samples but also in processed and/or cryopreserved samples, since these procedures are likely to alter the sperm DNA integrity and change the treatment's outcome. Extended culture times, media composition or even the performed protocol may induce those changes.

Study design, size, duration: The sperm sample collection was performed at CETI (Porto, Portugal) for 4 years. During this time, 207 seminogram samples were evaluated for DNA integrity using the Toluidine Blue test (TB) and Pearson's correlation was applied. From that pool, 30 samples were selected to evaluate the impact of time and media used for swim-up protocols. Other 30 samples were analyzed for comparison after cryopreservation with two different media. In both cases an ANOVA test was applied.

Participants/materials, setting, methods: Men aged between 17 to 62 years old, collected a sperm sample for seminogram analysis according to the WHO and ESHRE criteria. After their informed consent was given, the same sample was used to test three different culture media. Swim-up technique was applied and the upper portion was removed for motility, concentration and DFI evaluation. Additionally, two distinct cryoprotectants (glycerol or egg yolk) were compared for sperm motility and DNA integrity before and after cryopreservation.

Main results and the role of chance: In this study, DNA integrity was evaluated using TB staining, a simple and affordable method for indirect assessment of DFI. DNA integrity was negatively correlated ($p < 0.0001$) with concentration ($r = -0.301$), vitality ($r = -0.421$), morphology ($r = -0.278$) and total motility ($r = -0.280$). Moreover, normozoospermic samples showed higher DNA integrity than samples with at least one impaired parameter ($p < 0.0001$). ROC curve analysis was used to establish a diagnostic threshold, above which samples are potentially damaged and consequently may lead to infertility situations. This value is around 21%, which could be an advance for the clinical application of this method, making the evaluation of DFI fair to all ART laboratories. Also, it was found that the incubation at room temperature may be beneficial since it improved sperm motility after 1h and 2h (respectively $p = 0{,}0016$ and $p = 0{,}0130$) compared to incubation at 37°C with the same medium. All media recorded an increase in DNA damage over time. There were no significant statistical differences between cryopreservation media. However, the results suggest that the medium with egg yolk cryoprotectant may promote greater stability and membrane fluidity, decreasing the impact on sperm motility and DNA damage caused by the cryopreservation process.

Limitations, reasons for caution: These results should be taken with caution and, in order to strengthen them, additional samples should be analyzed. Also, the correlation between different sperm parameters is based on comparisons between several individuals who exhibit some interpersonal variability and was based on traditional semen analysis, which is slightly subjective.

Wider implications of the findings: The establishment of a threshold for DFI may be useful for daily practice. We observed that media rich in aminoacids and proteins promote sperm motility after swim-up and that room-temperature incubation is also beneficial. We concluded that media with egg yolk may promote greater sperm motility and less DNA damage.

Trial registration number: Not applicable

Abstract citation ID: dead093.443

P-078 In vitro effects of polycarbophil vaginal moisturizing gel on sperm motility and vitality

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Study question: The aim of this study was to evaluate the possible effects of intravaginal polycarbophil gel in sperm quality in an *in vitro* model.

Summary answer: Incubation of human sperm samples with a vaginal moisturizer containing polycarbophil gel 10% resulted in important reduction of sperm motility and vitality.

What is known already: The possible effect of lubricants on sperm quality has been a matter of debate for over 50 years. Despite the publication of a few studies, the real impact of lubricants on male gametes is remains unknown. Some commercial lubricants have been tested but so far there is a lack of studies evaluating the impact of vaginal moisturizers on seminal quality. Vaginal dryness is a recurring and significant complaint affecting couples trying to conceive who may use lubricants, vaginal moisturizers or even natural oils that could advsersely interfere with seminal quality.

Study design, size, duration: This paired prospective study involved seminal samples obtained fom 14 men undergoing fertility treatment at a private IVF clinic in Brazil from August/2020 to July/2022. The study was approved by the local review board and each participant signed an informed consent. Each sample was divided into two aliquots: 20% polycarbophil gel (final dilution 10%) or medium only. Statistical analysis was performed using D'Agostino/ Pearson, General Linear Model, Wilcoxon, Friedman's. P values <0.05 were considered significant.

Participants/materials, setting, methods: Normozoospermic samples of 14 patients (alpha 0.05, power 0.8)were collected by masturbation, 2 to 5 days after the last ejaculation. After liquefaction ,10 µmL were analyzed using a Makler chamber according to World Helath Organization (WHO) guidelines. Samples were mixed 1:1 v/v with 20% policarbophil gel or with culture medium only, then incubated for 24h at 34°c. Analysis were performed at 0, 15, 30 min and 24h to determine progressive motility and vitality (eosin-nigrosin test)

Main results and the role of chance: There was a significant decrease of sperm motility according to the WHO classification in both experimental conditions over time, but the reduction was more evident in the samples treated with polycarbophil gel ($p<0.0001$ group x time, General LinearModel for Repeated Measure).

The proportion of progressively motile sperm reduced immediately upon contact of the seminal sample with polycarbophil gel (time point 0 min) and decreased throughout the experiment, contrasting to incubation with culture medium (control), which only affected substantially sperm motility after 24h. In the validation cohort, the proportion of progressively motile sperm decreased from a median of 52% in fresh samples to 11% after 24h incubation with culture medium and 0% after 24h incubation with polycarbophil gel ($p<0.0001$).

Sperm vitality at the end of the experiment also differed significantly between treatments, with medians of 62.5% after exposure to culture medium and 47% after exposure to polycarbophil gel ($p=0.020$, Wilcoxon's test)

Limitations, reasons for caution: Results represent samples from a selected group of men undergoing fertility treatment at a private center in Brazil. The effect of polycarbophil gel in fertile males may be different. Moreover, the *in vitro* setting used here does not accurately mimic the vaginal environment which could interfere with the results.

Wider implications of the findings: Polycarbophil-based gels and similar products may harm sperm vitality and motility, therefore woment trying to conceive should avoid the use of such products until more studies prove otherwise.

Trial registration number: Not applicable

Abstract citation ID: dead093.444

P-079 Transcriptomic Analysis of Seminal Plasma in NOA Men as a Predictor of Successful Testicular Sperm Extraction

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Study question: Could transcriptomic profiling of semen ejaculate be used to predict successful testicular sperm retrieval for men with idiopathic non-obstructive azoospermia (iNOA)?

Summary answer: Transcriptomic analysis of seminal plasma RNA in iNOA men revealed gene imbalances that consistently correlated with the success of retrieving spermatozoa via a testicular biopsy.

What is known already: Although microdissection testicular sperm extraction (m-TESE) in NOA men results in successful spermatozoa retrieval in about 60% of the cases, it is difficult to predict which cases will fail to recover sperm. While several algorithms have been proposed to make this prediction, such as age, serum FSH, inhibin B, and genetics, there is no one factor that can do so reliably. Even histopathology fails to be more accurate, and it is equally invasive. Recently, epigenetic analysis on testicular biopsy specimens has shown a differential gene expression in relation to the origin of azoospermia and spermatogenic function.

Study design, size, duration: Over a 3-year period, 23 men diagnosed with iNOA underwent repeated extensive semen analyses and were deemed azoospermic. These patients were categorized based on whether spermatozoa were subsequently retrieved (+Sperm) or not (-Sperm) at micro-TESE. Transcriptomic analysis was performed by RNAseq, and significant differentially expressed gene (DEG) profiles were assessed and compared between the two cohorts. DNAseq was then used to confirm our findings.

Participants/materials, setting, methods: RNA and DNA were isolated from seminal plasma using a commercially available spin column kit and sequenced by Illumina HiSeq 3000/4000 platform at 2x150bp. Transcriptomic profiling was carried out in comparison to a fertile donor control and among the two cohorts. An absolute log2fold change of >1 and a P-value of <0.0005 were considered statistically significant.

Main results and the role of chance: Transcriptomic analyses of ejaculates from 23 azoospermic men were assessed for a total of 21,855 genes against a fertile donor control. Subsequently, 11/23 (47.8%) men (39.0 ± 12yrs) underwent successful testicular sperm retrievals. Their DEG profiles revealed 1,409 imbalanced genes. However, 13 were consistently imbalanced among them: 8 involved in spermatogenesis and 5 in sperm function.

Alternatively, for the 12 men in the –Sperm cohort (34.3 ± 5yrs), 1,265 imbalanced genes were identified with 12 common imbalanced genes associated with spermatogenesis ($n=5$), sperm function ($n=3$), sperm maturation ($n=1$), and cell cycle regulation ($n=3$).

When comparing the DEG profiles between the + and –Sperm cohorts, 8 commonly shared imbalanced genes were identified. IGSF11-AS1, a gene expressed in the testis and implicated in spermatid development, was consistently underexpressed in all –Sperm men. TPTE2, a testis-specific gene regulating spermatogenesis, was overexpressed in 81.8% (9/11) of the individuals in the +Sperm cohort and conversely underexpressed in the -Sperm group.

Most interestingly, NEU1, involved in acrosome development and fertilization, was consistently overexpressed in all individuals of the +Sperm group, yet clearly underexpressed in the entire –Sperm group. DNAseq showed that the NEU1 gene exhibited a synonymous mutation for the +Sperm group and a frameshift mutation in the –Sperm group.

Limitations, reasons for caution: Using noninvasive RNAseq and DNAseq on the seminal plasma has allowed us to identify DEGs that may be used to predict whether a patient with iNOA will have a successful or failed retrieval with micro-TESE. However, these results are preliminary and should be further validated in a larger study cohort.

Wider implications of the findings: Transcriptomics on the ejaculates of men with iNOA represents a noninvasive tool to detect the presence of residual spermatogenesis. Once confirmed, these findings may help guide

patients in weighing their exposure to anesthesia and surgical risks. This would help to mitigate patient emotional and financial distress.

Trial registration number: N/A

Abstract citation ID: dead093.445

P-080 Enzymatic tissue processing after testicular biopsy in non-obstructive azoospermia enhances sperm retrieval and cumulative live birth rates

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Study question: What is the added value of enzymatic processing of testicular biopsies on testicular sperm retrieval rates in patients with non-obstructive azoospermia (NOA)?

Summary answer: In addition to mechanical mincing, enzymatic digestion increased sperm retrieval rates in testicular biopsies of NOA patients with 48.9%.

What is known already: Many studies focus on the surgical approach to optimize recovery of testicular sperm in NOA, and in spite of that, there is still controversy whether the type of surgery makes any difference as long as multiple biopsies are taken. Few studies, however, focus on the role of the IVF laboratory and the benefit of additional lab procedures, like enzymatic digestion, in order to optimize sperm retrieval rates and CLB rate per TESE.

Study design, size, duration: A retrospective single-center cohort study including all patients who underwent their first TESE by open multiple-biopsy method from January 2004 till July 2022. Only patients with a normal karyotype, absence of Y-q deletions and a strict diagnosis of NOA based on histology were included. Primary outcome was sperm retrieval after mincing or enzymes for intracytoplasmic sperm injection (ICSI). Secondary outcome was CLB after ICSI with fresh TESE, and subsequent ICSI cycles with frozen TESE.

Participants/materials, setting, methods: Multiple biopsies were obtained from the testis, unilateral or bilateral, on the day of oocyte retrieval. Upon mechanical mincing, dishes were searched for 30 min; when no or insufficient numbers of spermatozoa were observed, enzymatic treatment was performed using collagenase type IV.

Multivariable regression analysis was performed to predict CLB by adjusting for the following confounding factors: male age, male FSH level, cryptorchidism, enzymatic digestion, number of oocytes and female age.

Main results and the role of chance: Hundred-eighteen patients were included of which 61.0% had successful sperm retrieval. Spermatozoa were retrieved after mechanical mincing (23.7%; 28/118) or after additional enzymatic digestion of the remaining 90 patients (48.9%; 44/90).

Mean male characteristics were not different between patients with sperm retrieval after mincing or enzymes: age (34.5 vs 34.5 y), testis volume (10.2 vs 10.6 ml), FSH (17.8 vs 16.9 IU/l), cryptorchidism (21.4 vs 34.1%) and histological diagnosis (Sertoli Cell Only 53.6 vs 47.7%, maturation arrest 21.4 vs 38.6%, sclerosis/atrophy 25.0 vs 13.6%), respectively.

Of the 72 patients with sperm available for ICSI, 23/72 (31.9%) obtained a LB after the injection with fresh testicular sperm (fresh and frozen embryo transfers). Forty-nine patients remained without LB, of which 34 had supernumerary testicular sperm frozen. Of these, 9/47 (47.4%) had a LB after ICSI with frozen testicular sperm, giving rise to a total CLB per TESE of 32/118 (27.1%) or 32/72 (44.4%) CLB per TESE with sperm.

Of the female characteristics (couples with sperm available), only female age (30.3 vs 32.7 y – p = 0,042) was significantly lower in the group with a live birth.

Multivariable logistic regression analysis showed that enzymatic digestion was associated with significant decrease of CLB per TESE.

Limitations, reasons for caution: Limitations of the study are related to the retrospective design. The selection of only NOA patients with specific characteristics (normal karyotype and absence Y-q deletion) and having their first TESE ever strengthens our findings. Whether enzymatic digestion after a failed TESE without digestion may improve retrieval rate remains undecided.

Wider implications of the findings: Enzymatic processing increases the sperm retrieval rate from testicular biopsies of NOA patients compared to mechanical mincing, demonstrating the importance of an appropriate laboratory protocol. NOA patients should be counselled that if sperm has been found after enzymatic digestion, their chances to father a genetically own child will be lower.

Trial registration number: not applicable

Abstract citation ID: dead093.446

P-081 In utero exposure to diisopentyl phthalate (DiPeP) disrupts the expression of steroidogenesis-related genes and induces multinucleated gonocytes in the fetal rat testis

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Study question: Can the plasticizer diisopentyl phthalate (DiPeP) disrupt testosterone biosynthesis and alter fetal rat testis histology similarly to other common toxic phthalates?

Summary answer: DiPeP can dose-dependently suppress mRNA levels of steroidogenic genes and induce multinucleated gonocytes in the fetal rat testis, resembling the effects of other toxic phthalates.

What is known already: DiPeP is an uncommon phthalate, whose metabolites have been ubiquitously detected in urinary samples from Brazilian pregnant women and children. These results, which contrast with the undetected levels of DiPeP metabolites in other human biomonitoring studies worldwide, led us to investigate DiPeP reproductive and developmental toxicity in rats. Our preliminary data indicate that DiPeP is a potent antiandrogenic phthalate, but no studies have been conducted to assess the impact of DiPeP exposures on the incidence of multinucleated gonocytes in the rat fetal testis, a common feature of the rat phthalate syndrome.

Study design, size, duration: Pregnant Wistar rats (n = 7-9 dams/group) were treated orally (gavage) with DiPeP 0 (control), 11, 33, and 100 mg/kg/day between gestation days 14-20. Dose levels were based on prior rat phthalate toxicity studies and canola oil was used as a vehicle. On gestation day 20, dams were euthanized and up to three male fetuses from each litter were removed for the collection of fetal testes and the assessment of the study endpoints.

Participants/materials, setting, methods: Fetal rat testes were used for enzyme immunoassay quantification of testosterone production following *ex vivo* incubation of testes in culture media, analysis of mRNA levels of steroidogenic genes and insulin-like factor 3 (*Insl3*) by quantitative polymerase chain reaction (qPCR), and histological assessment of multinucleated gonocytes by optical microscopy.

Main results and the role of chance: No signs of systemic toxicity were observed as indicated by the lack of alterations in maternal body weights and the unchanged number, viability, and weight of fetuses. There were no significant changes in the *ex vivo* testosterone production at any DiPeP dose. On the other hand, DiPeP induced significant reductions in the mRNA expression of key steroidogenic genes, including suppressed mRNA levels of the steroidogenic acute regulatory protein (*Star*) and cytochrome P450 family 11 subfamily A member 1 (*Cyp11a1*) at 100 mg/kg/day and of cytochrome P450 family 17 subfamily A member 1 (*Cyp17a1*) at 33 and 100 mg/kg/day. Gene expression of *Insl3*, which is essential for the process of testis descent, was reduced at the highest dose by 58% in relation to control, but this change was not significant (p = 0.07). The number of multinucleated gonocytes, corrected for the number of testicular cord sections or the total area of analyzed testicular cords was significantly increased in the groups exposed to 33 and 100 mg/kg/day. Chance is an unlikely explanation for our results considering the observed dose-dependent responses and the consistency of our findings with prior phthalate reproductive toxicity data.

Limitations, reasons for caution: The tested doses are higher than the expected human exposure levels and animal-to-human extrapolation should be done with caution.

Wider implications of the findings: DiPeP suppresses the expression of steroidogenic genes and induces multinucleated gonocytes in the fetal rat testis. These endpoints seem more sensitive than inhibition of *ex vivo* testosterone production. Although human exposure levels are lower than the doses tested here, DiPeP can potentially act cumulatively with chemicals that share similar mechanisms.

Trial registration number: not applicable

Abstract citation ID: dead093.447
P-082 Estradiol levels in idiopathic male infertility

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Study question: Investigating estradiol in relation with other sperm parameters might reveal new correlations which would help our understanding of male idiopathic infertility

Summary answer: Statistical significance was found in a series of correlations of E2 levels and sperm, hormonal and health parameters

What is known already: Male infertility is a rising major health concern, with sperm counts decreasing by 50-60% in the recent decades. The causes for male infertility are very different (genetic, systemic, neurological, infections, trauma, etc.), but at least 44% of cases are idiopathic. Estradiol (E2) regulates spermatogenesis by binding to classical intracellular estrogen receptors or membrane estrogen receptors to trigger genomic and nongenomic signal transduction pathways. Although E2 doesn't carry any prognostic value by itself, as its levels don't vary significantly between infertile and fertile men, its association with other parameters may lead to a better understanding of male infertility

Study design, size, duration: the design of cross-sectional study comprising 111 subjects- 87 infertile patients (median: 34 years, range 20–55 years) and 24 control (median: 33 years, range 20–45 years). The subjects were included in this study during 3 years (between October 2019- September2022). Inclusion criteria: spermatic parameters according to WHO 2010 Standards Exclusion criteria: Radiotherapy and/or pelvic chemotherapy (over the last 6 months), known genetic aberrations, endocrine diseases, urogenital infections, bilateral orchiectomy, vasectomy and occupational exposure.

Participants/materials, setting, methods: Infertile men were grouped in azoospermia (AZOO), oligoasthenoteratozoospermia (OATS/OATSS) and oligoasthenozoospermia (OAS/OASS). Blood and semen samples were collected and E2 levels were measured in both seminal and blood samples, along with sperm parameters (sperm count, motility, morphology) and other factors (BMI, hormone and vitamin D levels, BsmI polymorphism in the vitamin D receptor gene) were evaluated. E2 levels were assayed using the electrochemiluminescence method with the RocheCobas E601 autoanalyzer.

Main results and the role of chance: E2 varies slightly between the different patients' groups, the highest values being found in the OAT/OATSS group. BMI is higher in the infertile group; significant higher values were found for the OAS/OASS ($p = 0.002$) and OATS/OATS ($p = 0.017$) groups.

Statistical analysis has revealed a significant difference between serum and seminal E2 levels in each group (AZOO $p = 0.0355$; OATS/OATSS $p = 0.0156$; control $p = 0.0009$), but also between OATS/OATSS seminal group and control seminal group ($p = 0.0366$). The E2/Inhibin ratio presented higher values in patients with OATS/OATSS ($p = 0.0031$) and azoospermia ($p = 0.0059$). The lowest values of E2/Vitamin D ratio were detected in the OAS/OASS and OATS/OATSS groups ($p = 0.0254$), A significant increase of E2 serum level was observed in patients from the OATS/OATSS group in the case of homozygous for mutant allele of BsmI polymorphism ($p = 0.0419$). The results bring new evidence regarding the role of E2 in male infertility. The obtained data indicates an interplay between E2 and Inhibin B and vitamin D serum levels, highlighting it's the role in male infertility.

Limitations, reasons for caution: The study could be extended to a larger population sample, in order to further validate the obtained results

Wider implications of the findings: Estradiol activity plays an important role in male fertility and along with the investigated parameters could serve as potential markers in the diagnosis and management of male infertility.

Trial registration number: PN-III-P2-2.1-PED-2019-4402

Abstract citation ID: dead093.448
P-083 Associations of sperm epigenetic aging with semen parameters among men from the U.S. general population

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Study question: Is sperm epigenetic aging (SEA) associated with semen parameters among men in the general population?

Summary answer: While SEA was not associated with general semen parameters, advanced SEA was positively associated with sperm head defects such as length, perimeter, and pyriforms.

What is known already: We have previously shown that advanced SEA was strongly associated with longer time-to-pregnancy among couples in the general population.

Study design, size, duration: A population-based prospective cohort study of couples discontinuing contraception to become pregnant recruited from 16 US counties from 2005 to 2009.

Participants/materials, setting, methods: Sperm DNA methylation from 379 semen samples were assessed via Illumina EPIC Array and SEA was estimated using Super Learner, an ensemble machine learning algorithm. Linear regression models were employed to examine the associations between semen parameters and SEA adjusting for male age and current smoking.

Main results and the role of chance: None of the general semen characteristics such as count, concentration, motility or morphology were associated with SEA. However, several sperm head parameters were positively associated with SEA including length ($\beta = 3.6$, 95% confidence internal (CI): 1.01 - 6.23; $p = 0.007$); perimeter ($\beta = 4.04$, 95% CI: 0.1 – 0.05; $p = 0.045$) and pyriforms ($\beta = 0.29$, 95% CI: 0.1-0.49; $p = 0.003$). SEA was also inversely related to sperm elongation factor ($\beta = -2.9$, 95% CI: -4.8 - -1.1; $p = 0.002$).

Limitations, reasons for caution: This prospective cohort study consisted primarily of Caucasian men and women and thus large diverse cohorts are necessary to confirm the associations between SEA and sperm head defects in other races/ethnicities.

Wider implications of the findings: These data suggest that advanced sperm epigenetic aging may be related to improper sperm head condensation during spermatogenesis.

Trial registration number: N/A

Abstract citation ID: dead093.449
P-084 Oral antioxidant supplementation ameliorates the deleterious effects of plasticizers Bisphenol AF (BPAF) and Dibutyl Phthalate (DBP) on mouse sperm DNA integrity

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Study question: Do common chemicals in plastic, BPAF and DBP, negatively impact sperm parameters and DNA integrity, and can oral antioxidant supplementation mitigate their impact?

Summary answer: Short-term, low-dose exposure to BPAF or DBP did not impact testicular/epididymal histology but caudal epididymal sperm parameters, particularly DNA integrity was significantly compromised.

What is known already: Plasticizers are among the most commonly produced chemicals worldwide with 90% of people showing detectable levels in their urine. As endocrine disruptors (EDCs), they act as estrogen mimetics but can also induce oxidative stress and form DNA adducts. Numerous studies have linked exposure to EDCs to impaired spermatogenesis, sperm DNA damage and male infertility. Limited studies suggest antioxidants may alleviate the reproductive toxicity of EDCs. This study measured the effects of short-term exposures to two common environmental EDCs (BPAF & DBP) on the post-testicular compartment epididymis and investigate the efficacy of an oral antioxidant supplement to safeguard sperm DNA integrity.

Study design, size, duration: Adult male CD1 mice were exposed to low doses (50mg/kg) of BPAF or DBP with or without co-administration of antioxidant supplementation (Fertilix®) for 14 days and evaluated after euthanasia.

Participants/materials, setting, methods: All experiments were performed at the University Clermont Auvergne, Clermont-Ferrand, France. Mice were supplemented with BPAF, DBP or/and Fertilix® in their drinking water. The results of exposure of males were evaluated against controls and included: testicular and epididymal histology, epididymal ROS status via 4-HNE levels in caput protein extracts, general semen parameters: (count, motility, viability, acrosome integrity), sperm nuclear/DNA parameters [decondensation (Toluidine blue stain), fragmentation (TUNEL test), oxidation (8-OHdG residue level)].

Main results and the role of chance: The histology of the testis and epididymis was unaffected by the exposure conditions used. An oxidative stress situation was evident by the EDC exposures, demonstrated by increased level of 4-HNE adducts in the epididymal caput protein extracts. Viability, total and progressive motility, and acrosome integrity of caudal epididymal spermatozoa remained unaltered in exposed animals compared with controls. Exposure to BPAF but not DBP significantly decreased sperm count. Sperm nuclear/DNA integrity assessed by the percentages of sperm with decondensed and oxidized nuclei was significantly increased in EDC-exposed animals. Sperm DNA fragmentation did not significantly increase in any group of exposed animals. Co-administration with Fertilix® significantly corrected the defective sperm parameters (nuclear decondensation and DNA base oxidation) with the exception of sperm count which did not return to control levels in BPAF+ Fertilix® treated animals. These data confirm that even at low doses and short-term exposures, epididymal spermatozoa are susceptible to DNA damage induced by environmental ROS-generating EDCs, confirming its association with male infertility.

Limitations, reasons for caution: The translation of these findings to humans needs to be confirmed, although the nuclear compartment of human sperm is more susceptible to oxidative alterations than that of the mouse.

Wider implications of the findings: These data demonstrate the susceptibility of post-testicular sperm cells to common environmental chemicals. They also support the use of appropriate antioxidant supplementation as an effective therapeutic choice for individuals with multiple exposures to environmental stressors such as ROS-generating EDCs.

Trial registration number: not applicable

Abstract citation ID: dead093.450
P-085 Reactive molecules drive sperm capacitation via protein posttranslational modifications

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Study question: Are S-sulfenylation and S-nitrosylation, two essential post-translational modifications (PTMs), increased during sperm capacitation? Which proteins are subjected to S-sulfenylation?

Summary answer: S-sulfenylation and S-nitrosylation increase during sperm capacitation. S-sulfenylation affects certain proteins, including A-kinase anchor protein 13, Fibrilline-1, and Spermatogenesis-associated protein 7.

What is known already: There is a significant increase in reactive oxygen species (ROS) and reactive nitrogen species (RNS) production within sperm capacitation. In contrast to tyrosine phosphorylation, a well-established mark of sperm capacitation, ROS, and RNS lead to PTMs themselves and modify thiol groups of cysteines. Despite these facts, the dynamics of ROS/RNS-derived PTMs during sperm capacitation remain unknown. Therefore, we consider both S-nitrosylation and S-sulfenylation of sperm proteins as novel modifications associated with the capacitation.

Study design, size, duration: C57BL6 mouse sperm were isolated from the *caudae epididymae* of 12-20-week-old males (n = 5). The study compared non-capacitated and capacitated spermatozoa using proteomic profiling.

Participants/materials, setting, methods: Sperm proteins were extracted into RIPA buffer. The protein lysate was incubated with 5mM dimedone, a hapten that specifically labels S-sulfenylated proteins, and immunogenically detected with an anti-dimedone antibody (Merk, Germany). Alternatively, S-nitrosylated proteins were detected by the anti-nitrosocysteine antibody (Merk, Germany). S-sulfenylated and S-nitrosylated proteins were visualized by western blotting. S-sulfenylated proteins were simultaneously identified by mass spectrometry.

Main results and the role of chance: We demonstrated an increase in S-sulfenylation and S-nitrosylation along with sperm capacitation. In addition, both S-sulfenylation and S-nitrosylation are positively correlated with tyrosine phosphorylation. Using mass spectrometry, we identified S-sulfenylated proteins, mostly belonging to the cytoskeleton: A-kinase anchor protein 13, Fibrilline-1, Spermatogenesis-associated protein 7, and Rab-like protein 2A.

Limitations, reasons for caution: The limitation of this study is the instability of PTMs. ROS/RNS-derived PTMs hypothetically arise and cease during protein sample preparation. The experiment was limited to one animal model. Further experiments should be performed on other mammalian models.

Wider implications of the findings: Studying novel sperm PTMs and their mechanism of action may improve the diagnosis of male infertility. Further, it could bring a novel approach to modulate the fertilizing ability of sperm used for in vitro fertilization.

Trial registration number: 260 536

Abstract citation ID: dead093.451
P-086 Prevalence and clinical factors associated with biochemical hypogonadism in infertile men with non-obstructive azoospermia: a single-center cohort study including 761 patients

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Study question: What is the prevalence, and which factors are associated with hypogonadism in infertile men with non-obstructive azoospermia (NOA)?

Summary answer: The prevalence of biochemical hypogonadism among NOA males is significant. The condition is associated with testicular volume, estradiol levels, paternal age, and testicular histopathology results.

What is known already: NOA is a severe and irreversible condition that accounts for approximately 60% of azoospermia cases, primarily associated with intrinsic testicular deficiencies of various causes. NOA men can present signs of hypogonadism, associated or not with elevated circulating FSH levels; however, the exact prevalence is ill-reported. There is also a lack of data on the clinical factors associated with hypogonadism. We, therefore, estimated the prevalence of biochemical hypogonadism using real-world data and investigated its relationship with clinical factors.

Study design, size, duration: An observational cohort study was conducted, including 767 consecutive NOA patients with primary spermatogenic failure seeking fertility treatment at a University-affiliated tertiary center for

male reproductive health between January 2014 and September 2021. Biochemical hypogonadism was defined as total testosterone (T) levels <350 ng/dL, measured by chemiluminescence immunoassay from a venous sample, collected in the morning, from 8:00 to 10:00 am, and confirmed on a second analysis at least one week apart.

Participants/materials, setting, methods: All included patients (age range: 23-55 years) had complete clinical, hormonal, genetic, seminal, and histopathology data and were naïve concerning previous sperm retrieval attempts. Patients with NOA due to hypogonadotropic hypogonadism and those using hormonal therapy were excluded. Prevalence rates were computed by Goodman's method. The relationship between clinical factors and hypogonadism was assessed by logistic regression analysis. We also assessed the frequency of patients with high and within-range FSH levels in the cohort.

Main results and the role of chance: NOA patients with biochemical hypogonadism represented 80.3% (95% confidence interval [CI] 77.3–43.7) of the studied population. The median (interquartile range [IQR]) of T and FSH levels in hypogonadal men were 276 ng/dL (233.0-303 ng/dL) and 12.3 IU/L (8.9-18.2). Among them, hypergonadotropic (FSH>12 IU/L) and normogonadotropic (within-range FSH levels 1.5-12.0 IU/L) patients were 52.1% (95% CI 48.1-56.0) and 47.8% (43.9-51.8) of studied individuals, respectively. Logistic regression analyses revealed that testicular volume ($p < 0.0001$) and estradiol levels ($p = 0.0005$) had a significant inverse relationship with hypogonadism, whereas paternal age ($p = 0.02$) showed a positive relationship. Moreover, testicular histopathology was a relevant predictor ($p = 0.003$), with an inverse relationship between hypogonadism and germ cell maturation arrest and a positive relationship between hypogonadism and hypospermatogenesis or Sertoli cell-only. Using these variables, a model constructed to predict hypogonadism had an area under the ROC curve of 0.72.

Limitations, reasons for caution: Limitations include possible unmeasured confounding factors and the intra- and inter-assay electrochemiluminescence method variability. Another limitation is that the study center is a referral facility for azoospermic patients; thus, hypogonadism prevalence rates reported herein might not represent that of the general population of NOA males attending general fertility centers.

Wider implications of the findings: Efforts are needed to provide adequate care for this population due to its association with poor quality of life. Moreover, as recent evidence has suggested that hormonal therapy to boost T production might increase sperm retrieval success rates, studies investigating hormonal therapy in such patients may be warranted.

Trial registration number: Not applicable

Abstract citation ID: dead093.452

P-087 Expanding the comparison of an AI automated semen analyses for concentration determinations with manual analyses and a cell counting system

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Study question: Can comparable results be obtained for sperm concentrations determinations in differing clinical settings when using an AI driven analyser, a cytometer, and WHO manual counts?

Summary answer: Outcomes suggest that comparable results can be obtained when utilising the differing counting techniques.

What is known already: Despite robust methods described by WHO, protocol variations exist, creating a lack of reproducible and consistent semen analysis results in standard laboratories, as described by Bjorndahl etal 2016. Human error can influence results where WHO protocols are not utilised in conjunction with rigorous IQA and EQA. A human performing an analysis is considered the gold standard, but it's apparent, as demonstrated by time-lapse embryo evaluation, that human assessments can be enhanced with machine assistance to increase accuracy. To establish the benefit of machine assistance, it is necessary to demonstrate comparable results with the manual operator and reliable machine cell counters.

Study design, size, duration: To assess whether comparable results could be obtained with a well-trained reproductive scientist and a cell counting system, two reputable IVF clinics were selected to perform their standard analyses and perform a repeat test with an automated analyser. A number of semen analyses were performed over a six-month time-period for patients attending IVF clinics for a pre-treatment evaluation.

Participants/materials, setting, methods: 107 samples were collected and analysed between June 2022 to December 2023 in laboratories in Copenhagen and London. Participants were selected on a random basis. The semen analyses were performed in a manner described by the WHO6, with an additional assessment with a NucleoCounter™ chemometec in the Danish laboratory. Simultaneously, a concentration determination was performed on a Mojo AISA ™. (Artificial Intelligence Semen Analysis system).

Main results and the role of chance: Manual concentration assessments, the cytometer data and the AISA concentration results were plotted and examined. Comparable values were obtained when data for the manual assessment v AI machine assessment and the data obtained for fluorescent marked sperm v AI analysis were assessed. A significant Spearman correlation was obtained by the manual analysis team and the team who used the cytometer counting system, values of 0.93 and 0.99, (error average 17.5 and 24.4) respectively.

Limitations, reasons for caution: Increasing the sample frame of this current study, including more laboratories for a multi-centre trial would increase the power of the findings. Inter and intra operator and repeat machine evaluations are planned to establish the human variations during analysis and establish if this can be mitigated by machine assistance.

Wider implications of the findings: The results demonstrate that a machine assisted semen analysis is comparable to a gold standard semen analysis performed by a reputable laboratory staff either when compared to a manual operator and to a cell sorting system where sperm are labelled with a fluorescent probe.

Trial registration number: N/A

Abstract citation ID: dead093.453

P-089 effect of testis tissue retrieval on "Micro Mapping Testicular Extraction" (MMTE): comparison with microscopic testicular sperm extraction (Micro TESE) in non-obstructive azoospermia (NOA)

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Study question: Is a new sperm retrieval method, Micro Mapping Testicular Extraction (MMTE) comparable to that of Microscopic testicular sperm extraction (Micro TESE)?

Summary answer: MMTE is comparable to that of Micro TESE regarding sperm retrieval rate (SRR). It can reduce tissue damage and improve intracytoplasmic sperm injection (ICSI) results.

What is known already: Micro TESE is currently the standard technique for sperm retrieval in NOA. However, the large incision severely damages testicles, complicating repeated Micro TESE, and there is a high risk of testicular atrophy and hypogonadism. New technologies, such as FNA mapping, have also been reported, but these require multiple surgeries, long operation times, and high examination costs. A more accurate and minimally invasive method of sperm retrieval is needed, using a shorter and simpler procedure.

Study design, size, duration: MMTE requires only a small amount of testicular tissue, obtained through multiple needle holes in the tunica albuginea by micro-squeezing under a microscope. Tissue is divided into four testicular areas: ventral-upper, ventral-lower, dorsal-upper, and dorsal-lower, and samples are immediately searched for sperm. If sperm are found, a small incision is made in the area with good sperm and additional tissue is taken under the microscope. If no sperm are found, routine Micro TESE is performed.

Participants/materials, setting, methods: From January to November 2022, 25 NOA cases (35.9+/-4.85 years) with indications for Micro TESE were eligible: 15 idiopathic, 6 gr/gr deletion, 2 Klinefelter's syndrome, 1 b2b4 deletion and 1 other.

MMTE was performed on 45 testes: 5 unilateral (Group A) cases and 20 bilateral (Group B) cases. If sperm were found, they were cryopreserved and later used or will be used for ICSI.

Main results and the role of chance: After MMTE, sperm were found in all 5 cases in Group A. In group B, sperm were found in 6 cases after MMTE. For those sperm positive 11 cases, additional sampling was performed to gain enough sperm for ICSI.

Micro TESE was performed for the rest of group B (14 cases, 28 testes), in which no sperm was found by MMTE. Of 2 cases out of 14 cases, only a few sperm were found by Micro TESE. No sperm was found in the rest of 12 cases.

As a result, the SRR per patient was 52% (13/25). The weight of tissue sampled was 56.2 mg (15.6-96.2 mg) by MMTE and 224.6mg (206.4-433.2 mg) by Micro TESE, a significant difference. ($p < 0.05$).

To date, ICSI has been performed in the MMTE-positive 11 cases with a partner (33.8+/-3.4 years). In those cases, the fertilization rate was 62.7% (79/126). The blastocyst rate was 31.7% (40/126), and the pregnancy rate per transfer was 75% (6/8). The total testosterone levels before and 1 month after surgery were significantly reduced only in the cases in which no sperm were found by MMTE.

Limitations, reasons for caution: Two cases that were sperm-positive only by Micro TESE had severe tissue degeneration and tissue was difficult to retrieve from needle holes, so countermeasures were necessary. It is necessary to consider the number of needle holes depending on the size of the testis. The number of cases is still small.

Wider implications of the findings: MMTE can correct tissue just under the albuginea and can cover areas that are difficult to reach with Micro TESE. The sample can be taken through a small incision, so tissue damage is limited to that of Simple TESE, which improves ICSI results by taking sperm from the best areas.

Trial registration number: non applicable

Abstract citation ID: dead093.454
P-090 The in vitro motility characteristics of human sperm collected by microfluidic device

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Study question: Do the *in vitro* motility characteristics of sperm collected using a microfluidic device (ZyMot) differ from those of sperm collected by density gradient centrifugation (DGC)?

Summary answer: ZyMot-collected sperm had longer-lasting motility than those collected by DGC, but the head beat-cross frequency was inferior due to the absence of washing *in vitro*.

What is known already: ZyMot (DxNow, Inc., USA) can sort highly motile sperm with low reactive oxygen species (ROS), and DNA damage, without centrifugation. In IUI cycles, although the total number of ZyMot collected sperm was lower than that of DGC, it was reported that the pregnancy rate of ZyMot cycles was higher than that of DGC cycles. Washing sperm with a medium is essential for *in vitro* capacitation; however, the protocol for microfluidic devices lacks this procedure. The aim of this study was to clarify the motility characteristics of ZyMot-collected sperm. We also examined how washing ZyMot-collected sperm affected motility.

Study design, size, duration: The specimens were provided by patients who underwent semen analysis between October 2022 and January 2023, with written consent. We used 12 samples with a semen volume of 2.5 ml or more, or a motile sperm concentration of 4 million/ml or more. The same donor samples were divided into three groups (DGC, ZyMot, and ZyMot with wash). Dunnett's test was performed with the DGC group as the control group.

Participants/materials, setting, methods: In the DGC group, 2-layer DGC (400G, 20 min) was performed, followed by washing (400G, 5 min). In the ZyMot group, ZyMot was used according to the manufacturer's protocol. In the ZyMot with wash group, ZyMot-collected sperm was centrifugally washed (300G, 5 min). All samples were cultured at 30 °C until motility analysis. Sperm motility was analyzed using a computer-assisted sperm analysis system (SMAS) after 0, 4, and 24 h of semen preparation.

Main results and the role of chance: There was no significant difference in the motility rate of the two ZyMot groups compared to that of the DGC group at 0 and 4h after sperm preparation. On the other hand, the motility of the ZyMot group tended to be higher (53.3%, $p = 0.0787$), and the motility of the ZyMot with wash group was significantly higher (85.4%, $p < 0.001$) than that of the DGC group (33.5%), after 24h of sperm preparation. The straight-line velocity, curvilinear velocity, and average speed of the ZyMot and ZyMot with wash group did not differ from those of the DGC group at 0 and 4h. All speed parameters of the ZyMot with wash group were significantly higher than those of the DGC group, after 24h of culturing. Regarding sperm head movement, there was no significant difference in the amplitude of lateral head displacement between the two ZyMot groups compared to that of the DGC group at 0 and 4h of culture. In contrast, the sperm head beat-cross frequency of the ZyMot group was lower than that of the DGC group (9.18 ± 0.31 Hz vs 8.71 ± 0.49 Hz, $p = 0.0409$). Both head movement parameters of the ZyMot with wash group were significantly higher than those of the DGC group.

Limitations, reasons for caution: We chose semen samples with a high number of motile sperm because we divided the sperm into three experimental groups. Therefore, it is unclear whether our findings are applicable to patients with poor semen parameters.

Wider implications of the findings: ZyMot may be advantageous for IUI cycles because ZyMot-collected sperm maintains sustainable motility for extended periods. Alternately, ZyMot may be unfavorable compared to DGC in conventional IVF cycles due to inferior sperm head movement, which is a marker of capacitation. However, this disadvantage can be overcome by washing.

Trial registration number: not applicable

Abstract citation ID: dead093.455
P-091 Deep learning-based selection of fertilization-competent human spermatozoa

Abstract withdrawn by the authors

Abstract citation ID: dead093.456
P-092 Interaction of male and female genital microbiota in infertile couples undergoing assisted reproductive technology

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Study question: Is there a crosstalk between the male and female genital microbiota of couples undergoing assisted reproductive technology (ART) and the outcome of this treatment.

Summary answer: Despite the presence of bacteria linked with bacterial vaginosis in male samples, there appears to be very limited impact on the microbiota of female partners.

What is known already: In recent years, thanks to advancements in sequencing technologies, the microbiota has been explored extensively and many of its roles in human physiology were deciphered. Compared to its female counterpart, male genital microbiota has been understudied. Presence of bacteria in sperm was generally associated with a pathological condition, but recent reports indicate that the male genital tract is colonized by bacteria and even good quality semen generally contains bacteria. Previously three main semen microbiota profiles were identified, two of these were composed of bacteria, usually found in the vagina, indicating that bacteria could be shared between partners during sexual intercourse.

Study design, size, duration: Prospective observational study including 65 couples receiving ART between 2018 and 2020 at a single fertility center in Switzerland, to investigate the interaction of male and female reproductive tract microbiota. Samples were collected by swabbing penile skin and seminal fluid as well as vagina and follicular fluid.

Participants/materials, setting, methods: Samples of 64 infertile couples undergoing ART were collected by direct sampling (vagina and penis glans) or by immersion in the biological fluid (follicular fluid and semen). Follicular fluid was obtained during oocyte pick-up. Sampling of vagina and penis glans (self-sampling) as well as immersion of semen was performed on the same day.

16S rRNA profiling (next generation sequencing) was used to characterize bacterial populations in the samples using a previously developed pipeline.

Main results and the role of chance: *Lactobacillus spp.* were highly prevalent in follicular fluid samples and especially in vaginal samples. Generally, penis glans samples were highly colonized by different bacteria and showed the highest alpha diversity. Follicular fluid and semen samples had generally low bacterial biomass. *Lactobacillus iners* was the most prevalent species in female samples. This is also the case for male samples, although the abundance was lower.

For male patients, comparison of beta diversities showed that intra-individual samples were more similar compared to same sample types from different individuals. The opposite was observed for vaginal samples, where inter-individual differences were less marked compared to samples of the same woman. Similar analyses were performed to evaluate relatedness of samples from the same couple, allowing us to estimate the possible impact of microbiota interaction between partners. Despite generally high values, in several cases intra-couple dissimilarities were significantly lower when compared to same sample type of different individuals.

Analysis of differently abundant taxa showed, as expected, that *Lactobacillus* genus was enriched in female samples, along with *Atopobium spp.* A higher number of genera was specifically enriched in male samples, including *Prevotella*, *Campylobacter* and *Mobiluncus* genera among others.

Limitations, reasons for caution: Limitations of this study are, that it is only observational and the number of patients included is relatively small.

Wider implications of the findings: Our results suggest a very limited impact of male microbiota on the female bacterial colonization, despite male samples were enriched with bacterial genera negatively associated with reproduction. Future analysis of the influence of sexual activity on the microbial composition should comprise samples obtained before and after sexual intercourse.

Trial registration number: not applicable

Abstract citation ID: dead093.457

P-093 Single sperm morphokinetic variables during ICSI at the time of sperm aspiration into the microneedle

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Study question: Is it possible to classify sperm morphology as normal or abnormal based on their kinetic characteristics?

Summary answer: Using different classifiers, it was found that balanced dataset the classification scores of individual sperm morphology can be optimized during their motility just before ICSI

What is known already: Sperm selection plays a crucial role during ICSI. This selection by the embryologists is accompanied by two factors: motility parameters of the sperm to be injected, as well as the morphology. Motility affects morphological decision-making which may be subjective due to spermatozoa not being in a narrow vertical space in a PVP droplet, i.e. counting chamber. Machine/Deep Learning models -as classifiers- are gaining popularity in IVF as it could minimize subjectivity in gamete selection. SiD software is an algorithm that from a group of spermatozoa characteristics can support the selection of a single sperm during real time ICSI.

Study design, size, duration: In this prospective study 1699 individual spermatozoa were video-recorded (resolution of 200 X 200 pix.) during sperm selection in a 7%PVP solution. Motility variables (VSL, VCL, LIN, VAP, ALH, WOB, STR, MAD) were obtained from each video using the software SiD. Each sperm was classified as normal or abnormal. Based on motility variables, different classification models were used to make a morphokinetic association of the variables with the classification of each sperm.

Participants/materials, setting, methods: 1699 individual spermatozoa were classified and labeled by three senior embryologists into two categories: normal or anormal. To belong to any category, at least two embryologists had to agree. A normalization was applied to the motility data obtained with SiD. Machine learning classification models were applied. The three classification models with the best results were selected to optimize the hyperparameters and improve their performance.

Main results and the role of chance: A set of motility variables were obtained using the software SiD1 (IVF2.0 Ltd., UK) these were used as features for each of the sperm samples and were tagged either as normal or abnormal having a total of 257 normal samples and 1442 abnormal samples. Different classification algorithms were used to perform sperm classification as normal and abnormal. Then hyperparameter tuning algorithms such as GridSearch were used to compute the optimum values, finding KNN algorithm to achieve the best results to classify normal and abnormal spermatozoa with 80% accuracy. Given the nature of the unbalanced dataset, the F1 score was calculated, achieving 0.77, other algorithms such as Decision-Tree and Random-Forest were used achieving similar results in the F1 score. We will continue growing the dataset until it is balanced and run the same algorithms expecting the performance to improve.

Limitations, reasons for caution: Each clinic's laboratory setup, including the camera used to record the ICSI procedure, is a clinic-specific configuration. Sperm abnormality detection is sensitive to camera resolution, showing the classifiers are resolution-dependent observers. To replicate these results, it is important to consider the quality of the camera.

Wider implications of the findings: Using the selected kinematics features, it is possible to classify individual spermatozoa during motility as having normal or abnormal morphology. Results may improve using standard morphological examination of each population and having more normal sperm samples.

Trial registration number: Not applicable

Abstract citation ID: dead093.458

P-094 Dynamics of progesterone-induced intracellular Ca2+ changes in sperm predicts zygote formation rate by IVF: Preliminary results

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Study question: Can the overall intracellular Ca2+ dynamics evoked by progesterone stimulation predict the fertilizing potential of a non-normozoospermic semen sample under fertility treatment?

Summary answer: Some kinetic parameters of the transient intracellular Ca2+ increase evoked by progesterone stimulation correlate with fertilization rates in conventional IVF but not with ICSI.

What is known already: Stimulation with female hormone progesterone induces the activation of the sperm-specific ion channel CatSper which in turn produces a fast increase in the intracellular Ca2+ concentration ([Ca2+]i). After reaching a maximum [Ca2+]i, different Ca2+ clearance mechanisms become active producing a transient followed by a plateau. We investigated whether the [Ca2+]i dynamics upon progesterone stimulus may be used to assess the fertilizing potential of sperm samples from men undergoing assisted reproduction.

Study design, size, duration: This prospective study employed unidentified semen samples from 39 non-normozoospermic men (age 38 ± 6 years old) undergoing fertility treatment from March 2022 to the present at CITMER Reproductive Medicine, Mexico City. Frozen semen samples and ART procedures with less than 3 oocytes were excluded. Ten normozoospermic semen samples were also analyzed. The progesterone-dependent [Ca2+]i changes were evaluated using time-lapse flow cytometry.

Participants/materials, setting, methods: Semen samples were obtained on the same day as oocyte retrieval. Sperm cells were washed and incubated in capacitating HTF media for further use in conventional IVF or ICSI procedures. When available, the surplus cells were stained with vitality and Ca2+-sensitive fluorescent dyes. Fluorescence changes were evaluated by AccuriC6 flow cytometer. Progesterone-induced [Ca2+]i transient as well as the correlations with the fertilization rates were calculated using custom-made R programming language code.

Main results and the role of chance: The progesterone (1 μM) stimulation evoked a fast and transitory increase in the [Ca2+]i. We then calculated six different kinetics parameters of such responses: basal Ca2+ level, increase and decrease in velocity, maximum Ca2+ level, total response duration, and the area under the curve (AUC). There were no significant differences in any of these parameters between normozoospermic donors (and non-normozoospermic patients. The former group of semen samples were divided into either conventional IVF (n = 17) or ICSI (n = 21). In IVF but not ICSI samples, the duration, and the area under the curve of the progesterone response tended to be higher than in normozoospermic (2.39 ± 0.7 min, p = 0.03, and 113 ± 30 AUF*min, p = 0.18, respectively). Furthermore, both parameters showed a tendency to correlate negatively with the conventional IVF (R= –0.45, p = 0.07 and R= –0. 5, p = 0.04, respectively) but not with ICSI fertilization ratio (number of zygotes/total number of mature oocytes). These results suggest that cells with a better Ca2+ recovery mechanism are well suited for IVF. Interestingly, we observed no difference between the magnitude of the [Ca2+]i increase after progesterone stimulus among donors, IVF, or ICSI samples, as was previously reported. Differences in the concentration of progesterone could explain such results.

Limitations, reasons for caution: The preliminary nature of the results implies a necessity to increase the number of analyzed semen samples (in progress). It is well known that progesterone induces variable responses even among the same donor cells. Our population-based analysis may be underestimating such single cells variations.

Wider implications of the findings: Current protocols for sperm analysis fail to successfully predict the fertilizing capability of semen samples from men seeking reproductive treatment. Further research into the molecular regulation of Ca2+ dynamics could offer promising alternatives for clinicians to appropriately choose between IVF and ICSI for each couple.

Trial registration number: .

Abstract citation ID: dead093.459

P-095 Increased sperm high DNA stainability is associated with a diminished chance of fertilization with IVF cycles: a retrospective study using a propensity score-matching analysis

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Study question: To investigate the association of high DNA stainability (HDS) with fertilization, embryonic development and clinical outcome after IVF treatment.

Summary answer: Increased HDS levels might be associated with decreased fertilization of IVF cycle.

What is known already: The HDS parameter of sperm suggests loosening and weakening of chromatin condensation structure resulting from the lack of full histone-to-protamine exchange. Elevated HDS may negatively correlate with sperm quality, including sperm morphology and concentration.

Study design, size, duration: Couples that underwent IVF cycles from January 2016 to December 2020 were retrospectively studied, including a total of 2604 target cycles after IVF treatment consisting of 676 cycles in the HDS > 15% group and 1928 cycles in the HDS ≤ 15% group. In addition, 548 cycles undergoing fresh IVF-embryo transplantation treatment consisting of 151 cycles in the HDS > 15% group and 397 in the HDS ≤ 15% group were included.

Participants/materials, setting, methods: HDS was assessed by sperm chromatin structure assay method. Couples were classified into HDS > 15% group and HDS ≤ 15% group based on the HDS thresholds recommended by the manufacturer's instructions. After controlling the bias between groups by the propensity score-matching method, the Mann-Whitney U test or Chi-squared test was used to evaluate the differences between groups. Regression analysis was used to assess the effect of HDS levels on fertilization, embryo quality, and clinical outcome of IVF cycles

Main results and the role of chance: No significant differences were observed between the HDS > 15% group and HDS ≤ 15% group regarding the number of fertilized oocytes (10.00 [6.00, 15.00] *vs.* 10.00 [6.00, 15.00], $P = 0.230$), the number of 2 pronuclei zygotes (8.00 [4.00, 12.00] *vs.* 8.00 [5.00, 12.00], $P = 0.311$), the number of cleaved zygotes (10.00 [5.00, 15.00] *vs.* 10.00 [6.00, 15.00], $P = 0.301$), and the number of high-quality embryos on day 3 (5.00 [2.00, 9.00] *vs.* 5.00 [3.00, 9.00], $P = 0.729$). However, linear regression analysis showed that HDS levels negatively impacted fertilization (adjusted B value = −0.015, 95% CI: −0.028 ~ −0.002, $P = 0.022$) and 2 pronuclei formation (adjusted B value = −0.015, 95% CI: −0.029 ~ 0.000, $P = 0.046$). The implantation rate (47.1% *vs.* 50.2%, $P = 0.418$), clinical pregnancy rate (60.9% *vs.* 64.5%, $P = 0.440$), ongoing pregnancy rate (83.7% *vs.* 86.7%, $P = 0.475$), early miscarriage rate (16.3% *vs.* 13.3%, $P = 0.475$), late miscarriage rate (1.1% *vs.* 3.5%, $P = 0.232$), and live birth rate (82.6% *vs.* 83.2%, $P = 0.896$) were not significantly different between groups. Linear regression analysis showed that HDS did not affect the implantation rate. Binary logistic regression analysis also showed that HDS did not affect clinical pregnancy, ongoing pregnancy, early miscarriage, late miscarriage, and live birth.

Limitations, reasons for caution: The main weakness of this study was the nature of the retrospective design, and additionally, patients were not randomized.

Wider implications of the findings: Although our results are not entirely new, it can be considered as reliable because 2604 IVF cycles and 548 fresh IVF-ET cycles were included and subsequently controlled the bias between groups This study suggested that HDS might be an indicator in predicting the fertilization ability of sperm in IVF treatment.

Trial registration number: not applicable

Abstract citation ID: dead093.460

P-096 Prediction of fertilization success by voltage-dependent intracellular Ca2+ dynamics in non-normozoospermic human semen samples undergoing in vitro fertilization: preliminary results

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Study question: Does the change in intracellular calcium levels evoked by artificial membrane depolarizations predict fertilization success in a non-normozoospermic semen sample undergoing assisted reproductive techniques?

Summary answer: The increase in the intracellular calcium after artificial plasma membrane depolarization correlates negatively with fertilization rates in conventional IVF but not with ICSI

What is known already: Sperm capacitation-related features, like the regulation of the intracellular calcium concentration ([Ca2+]i) and the hyperpolarization of the plasma membrane potential (Vm), have been proposed as predictors of success during artificial reproduction techniques (ART). Intriguingly, upon Vm depolarization, sperm exhibit a rapid and transitory increase in the [Ca2+]i controlled, likely, by the activation of the sperm-specific calcium channel CatSper. The regulation of CatSper activity is essential for sperm function and is crucial for fertilization. Therefore we wondered if the Vm depolarization-dependent rise in the [Ca2+]i can be used to predict fertilization success rates in non-normozoospermic semen samples of men undergoing ART.

Study design, size, duration: This prospective study employed unidentified semen samples from 29 non-normozoospermic men (37.8 ± 5.6 years old) undergoing fertility treatment from March 2022 to the present at CITMER Reproductive Medicine, Mexico City. Frozen semen samples and procedures with less than 3 oocytes were excluded. Ten normozoospermic semen samples were analyzed as a control. The depolarization-dependent [Ca2+]i changes were evaluated by flow cytometry under four different Vm conditions: resting (variable among samples), –64, –46, and –32 mV.

Participants/materials, setting, methods: Semen samples were obtained on the same day as oocyte retrieval. Sperm cells were washed and incubated in capacitating HTF media for further use in conventional IVF or ICSI procedures. When available, the surplus cells were stained with vitality, Ca2+, and Vm-sensitive fluorescent dyes. Fluorescence changes were evaluated by AccuriC6 flow cytometer. The kinetical analysis of the transitory [Ca2+]i increase as well as the statistical comparisons were performed using custom-made R programming language code.

Main results and the role of chance: Analysis of resting Vm showed that normozoospermic semen samples presented more depolarized Vm than non-normozoospermic controls (–75.7 ± 6.9 vs –67.3 ± 10.5 mV, respectively, $p = 0.02$). The former group of semen samples was either used for conventional IVF ($n = 11$) or ICSI ($n = 14$). The semen samples in these last categories showed a tendency to present a more depolarized Vm than non-normozoospermic samples (–69.9 ± 8.8 and –66.6 ± 11.3 mV, respectively). No differences were seen in the resting Ca2+ levels. We then induced a Vm hyperpolarization by adding the K+ ionophore, valinomycin followed by increasing concentrations of extracellular K+. Each depolarizing stimulus evoked a fast and transitory increase in the [Ca2+]i. We then calculated seven different kinetic parameters of such responses: basal Ca2+ level, increase and decrease velocity, maximum Ca2+ reached, response durability, and area under the curve (AUC). The basal Ca2+ level reached after depolarizing stimuli correlated negatively ($R = -0.64$, $p = 0.03$) with the conventional IVF fertilization ratio (number of zygotes/total number of mature oocytes) when the Vm was clamped either to –64 and –46 mV. A negative and significant correlation ($R = -0.76$, $p = 0.006$) was seen for the AUC analysis at –64 mV. No relevant correlations were found in the ICSI samples.

Limitations, reasons for caution: This is an in vitro study, and caution must be taken when extrapolating these results in vivo. The preliminary nature of the results implies a necessity to increase the number of analyzed semen samples (in progress).

Wider implications of the findings: Further research into the molecular regulation of the CatSper channel could offer promising alternatives for clinicians to appropriately choose between IVF and ICSI for each couple. This protocol is fast and simple to perform in a laboratory equipped with a flow cytometer or any fluorescence-based reader.

Trial registration number: NA

Abstract citation ID: dead093.461

P-097 Embryological and Clinical Outcomes of Microdissection testicular sperm extraction combined with Intracytoplasmic sperm injection (microTESE-ICSI) in patients with and without Klinefelter syndrome

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Study question: This retrospective study aims to identify the embryo development and clinical outcomes in non-mosaic Klinefelter syndrome patients treated with microTESE-ICSI

Summary answer: Non-mosaic Klinefelter patients can retrieve their sperm using microTESE and can have their biological offsprings by combining microTESE with ICSI

What is known already: Klinefelter syndrome patients are classified into two main groups, namely non-mosaic Klinefelter (47, XXY) and mosaic Klinefelter (46, XY/47, XXY). To retrieve sperm from non-mosaic Klinefelter, microTESE is a crucial treatment that yields a successful rate of approximately 50%. Recent research reports that microTESE-ICSI is a treatment regime that brings a higher *in-vitro* fertilization (IVF) success rate for those with non-mosaic Klinefelter syndrome. However, most of the previous studies on this group focuses on sperm retrieval rate and male factors. Few studies have reported embryo development and clinical outcomes, especially, under the adjustment for female partner's age and oocyte quantity

Study design, size, duration: This retrospective study was conducted to evaluate spermatozoa retrieval, embryo development, and clinical outcomes in 931 patients with microTESE treatment at Andrology and Fertility Hospital of Hanoi from 6/2019 to 9/2022. Patients were divided into two groups including 118 patients with non-mosaic Klinefelter syndrome (KS) and 813 patients without Klinefelter syndrome (non-KS).

Participants/materials, setting, methods: In 931 patients, 63 KS and 417 non-KS had spermatozoa retrieved from microTESE were carried out ICSI. In ICSI group, 59 KS and 319 non-KS patients whose partners were under 35 years old were selected to compare embryological and clinical outcomes. Outcomes included fertilization rate, day 3 usable embryo rate, usable blastocyst rate, pregnancy rate (positive beta-hCG test), biochemical pregnancy rate, ongoing pregnancy, and live-birth rate (calculated on the total number of IVF cycles).

Main results and the role of chance: Spermatozoa retrieval rate by microTESE in the KS group was 53.4% (63/118), while this rate in the non-KS group was 51.2% (417/813) ($p > 0.05$). The mean age of female partners was significantly lower in the KS group than in non-KS group (26.6 ± 4.0 vs. 28.0 ± 3.5; $p = 0.006$). The mean of mature oocytes between these two groups was similar (15.7 ± 8.1 vs 14.5 ± 7.2; $p > 0.05$). Fertilization rates in KS and non-KS groups were 62.7% and 65.3%, respectively. Day 3 usable embryo rate (embryo with over 6 blastomeres at day 3) in two groups was not significantly different (69% vs 69.5%, $p > 0.05$). Usable blastocyst rate (embryo quality is over 2BB, Gardner grading system) in the KS group (19.9%,

n = 54) was significantly lower than in non-KS group (41.1%, n = 281) ($p < 0.001$).

Pregnancy rate and biochemical pregnancy rate were 75.9% and 6.9% in KS group (n = 58), respectively. Those rates were 81.5% and 3.2% in non-KS group (n = 313), respectively. There was no significant difference in ongoing pregnancy rate between these two groups (61.1% vs 74.5%; $p > 0.05$). Live-birth rate in the KS group was 43.2% (16/37), while this rate in the non-KS group was 46.5% (67/145).

Limitations, reasons for caution: This research had limitations of a retrospective study. Male hormonal factors and baby health could not be thoroughly measured because of difficulties in contact with patients at the time of the study. Besides, day 3 top-quality embryos were directed to freeze, so usable blastocyst rate was low in this study.

Wider implications of the findings: These data confirmed that microTESE provides opportunities for non-mosaic Klinefelter syndrome patients to retrieve their own sperm. Combination of microTESE and ICSI is an effective treatment regimen that helps these patients to have their own usable embryos and babies.

Trial registration number: not applicable

Abstract citation ID: dead093.462

P-098 Cryopreservation process deregulates the expression of pathways important for fertility in human spermatozoa

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Study question: Does cryopreservation impact on transcriptome of conventional frozen human spermatozoa?

Summary answer: The cryopreservation of human sperm alters pathways important for fertility, although the upregulation of some genes can compensate for the harmful effects of freezing.

What is known already: Sperm cryopreservation is a widely used procedure for storing gametes for later use, for preserving fertility in patients before gonadotoxic treatments or surgery, and for sperm donation programs. The cryopreservation technique can cause damage to sperm through induction of DNA alterations, fragmentation and oxidation, and altered motility, mitochondrial activity and morphology. Transcriptomic alterations have been reported in cryopreserved sperm from various animal species, but no experimental data are available on the effect of cryopreservation on the transcriptome-wide profile of human sperm.

Study design, size, duration: Semen samples were obtained from 20 normospermic men from April to May 2022. Each sample was divided in two aliquots. From one aliquot total RNA was immediately extracted. The second aliquot was slowly cryopreserved and after a week of storage in liquid nitrogen total RNA was extracted. A total of 13 paired RNA samples passed quality control and were analyzed after randomization into 4 pools, each of 6 patients.

Participants/materials, setting, methods: The men who participated in this study had a median age of 35.0 years (range: 29.0-46.0). RNA was extracted by miRNeasy Micro Kit (Qiagen), analyzed by High Sensitivity RNA on 2200 Tape Station system (Agilent), amplified by Ovation Pico WTA SystemV2 (Nugen), labeled and hybridized on GE 4x44K v2 microarrays (Agilent). The paired Significance Analysis of Microarray (SAM) was performed using the limma hgug4112a.db and samr packages in R/BioConductor.

Main results and the role of chance: The expression profiles of cryopreserved sperm significantly differed from those of fresh sperm in 219 down-regulated and 28 up-regulated unique transcripts. The gene ontology analysis disclosed that cryopreservation downregulates genes involved in sperm motility by the mitochondria function (*CABS1*, *SPATA19*) and the correct organization of the sperm midpiece and flagellum cytoskeleton (*ACTL7A*, *AKAP4*, *C9ORF24*, *CAPZB*, *CCIN*, *IQCG*, *ODF2*, *SPATA6*, *SPATA6L*, *TBC1D21*), in fertilization (*ACTL7A*, *AKAP4*, *PLCZ1*, *PRSS37*, *TUBGCP3*), early embryo development (*AGT*, *CLMN*, *DCC*, *DHRS3*, *EGFLAM*, *FAM20A*, *FREM1*, *GPI*, *HEMGN*, *KRTDAP*, *IFT172*, *MICAL2*, *MLF1*, *NF2*, *PGRMC2*, *PHACTR1*, *POPDC3*, *RO60*, *ROBO1*, *RORA*, *SGCA*, *SPRR2D*, *TCF4*, *TNC*, *TNNI3*), oxidant detoxification (*TXNDC29*) and DNA repair (*BRIP1*, *ERCC6*, *TRIP12*), calcium ion binding and homeostasis (*AMY1C*, *ANO1*, *ANO2*, *ASGR1*, *CABS1*, *CPNE9*, *FREM1*, *KCNIP2*, *ITPR3*, *PKD2L1*, *PLCZ1*, *SELENOK*, *SYN3*, *TNNI3*). Upregulated genes were enriched in pathways related to the negative regulation of DNA damage response (*ALOX15B*, *CD74*, *RPL10*, *SNAI1*, *THBS1*), cellular response to calcium ion (*ALOX15B*, *FOSB*, *HLA-DQB1*, *S100A4*, *THBS1*), regulation of transcription (*CD74*, *FOSB*, *HLA-DRB1*, *MAMLD1*, *RASD1*, *RPL10*, *SNAI1*, *TCF15*, *TLE1*), protein stabilization (*CD74*), protein ubiquitination (*BAG1*, *DCAF6*, *ERCC6*, *HERPUD2*, *KLHL7*, *MARCHF8*, *NF2*, *PSMA6*, *RNF133*, *SERGEF*, *TRIP12*, *UBE2DNL*, *UBL3*, *UBQLN3*, *ZNRF3*) and ubiquitin protein ligase binding (*GPI*, *HSPA1L*, *PRKAR2A*, *STX8*).

Limitations, reasons for caution: Data validation in a larger sample cohort and by quantitative PCR would be useful, although we have applied stringent criteria for gene selection (FDR = 0). Further research should be undertaken to experimentally test whether the length of cryostorage can have any effect on the gene expression profile of sperm.

Wider implications of the findings: Specific pathways are deregulated by cryopreservation, in accordance with altered sperm morphology, motility and molecular integrity reported in literature. Functional enrichment analyses also revealed gene expression changes to compensate for the sperm cryoinjury. This finding is noteworthy for safety issue of sperm banking i.e., fertility preservation, gamete donation.

Trial registration number: not applicable

Abstract citation ID: dead093.463

P-099 Alterations in microRNA expression profiles may be implicated in increased prevalence of cancer in Sertoli cell-only syndrome cases: A systematic review and in-silico analysis

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Study question: Could microRNA dysregulation be an underlying molecular mechanism leading to the observed increased prevalence of cancer in patients with Sertoli-cell only syndrome (SCOS)?

Summary answer: Patients with SCOS are characterized by altered microRNA profiles and dysregulated gene pathways involved in SCOS pathophysiology and in cell-cycle control and therefore in carcinogenesis.

What is known already: Sertoli cell-only syndrome constitutes a histopathological subtype of non-obstructive azoospermia, affecting 26.3–57.8% of azoospermia patients. It is characterized by partial or complete absence of active spermatogenesis due to germ cell aplasia. Except from infertility, SCOS is associated with increased risk of testicular nodules and cancer, rendering research on the topic essential. Despite advances, the underlying molecular mechanisms connecting SCOS with cancer remain unknown. Data

demonstrates that microRNAs could play crucial roles in both SCOS pathophysiology and carcinogenesis. Identifying relevant microRNAs and conducting in-silico analysis on affected pathways may lead to mapping the way forward.

Study design, size, duration: A systematic review was performed in PubMed/Medline and Embase up to April 2022. Only full-length original studies in humans were included. Strict inclusion-exclusion criteria were applied aiming to select studies comparing microRNA profiling between SCOS cases versus men with normal spermatogenesis or men with proven fertility. Following study selection, data on altered microRNA expression patterns were analyzed to underline differences between the abovementioned groups. Subsequently, in-silico functional analysis was performed to compare affected gene pathways.

Participants/materials, setting, methods: The studied population consisted of SCOS cases. Men with normal spermatogenesis or proven fertility served as the control group. Predicted microRNA–target pairs were retrieved from microT-CDS, while a 0.8 cutoff threshold was applied. The GTEx repository was used to identify microRNA-targeted genes in the testis. Annotations derived from Ensembl and miRbase. Gene-set enrichment analysis was performed employing the KEGG-database. Fisher's exact test was performed in R package limma, setting a 0.01 p-value threshold.

Main results and the role of chance: Four studies reported altered microRNA expression profiles in SCOS (n = 45) versus normal spermatogenesis cases or men of proven fertility (n = 16). Functional analysis revealed that six microRNAs, which were downregulated in the SCOS cases, namely hsa-miR-34b-5p, hsa-miR-202-3p, hsa-miR-34c-5p, hsa-miR-449a, hsa-miR-141-3p, and hsa-miR-34b-3p, affected 66 statistically significant gene-targets in the testis. Two pathways were reported to be statistically significantly dysregulated from these microRNAs, namely the ''microRNAs in cancer'' pathway (40 affected genes, p-value = 0.004), and the ''TGF-beta signaling'' pathway (26 affected genes, p-value = 0.01). Furthermore, four microRNAs, namely hsa-miR-10b-5p, hsa-miR-4270, hsa-miR-181c-5p, and hsa-miR-605-3p, reported to be upregulated in the SCOS group. These microRNAs had 108 statistically significant gene-targets in the testis. Three pathways were statistically significantly dysregulated from these microRNAs, namely the ''Herpes simplex virus 1 infection'' pathway (61 affected genes, p-value = 0.01), the ''microRNAs in cancer'' pathway (28 affected genes, p-value = 0.01), and the ''longevity regulating'' pathway (19 affected genes, p-value = 0.01). The molecular role of the affected gene pathways in proper cell-cycle regulation and germ cell differentiation is herein underlined as critical. In the disrupted testicular microenvironment of the SCOS cases these disrupted genes may act as inducers of carcinogenic mechanisms.

Limitations, reasons for caution: The main limitation is the small number of the included studies and the small number of participants investigated per study, especially with regards to the control group. Moreover, the observed heterogeneity among the studies regarding the molecular tools employed for the microRNA profiling is another reason for caution.

Wider implications of the findings: Our data suggest that the dysregulation of microRNAs affecting several gene pathways that control cell-cycle and differentiation may lead to increased cancer risk in SCOS cases. Further studies employing our findings as a starting point will indicate whether microRNA profiling can serve as an effective evaluation tool for cancer predisposition.

Trial registration number: Not applicable

Abstract citation ID: dead093.464

P-100 The influence of vasectomy on seminal microbiome

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Study question: Does male sterilisation with vasectomy induce changes in the seminal microbiome composition?

Summary answer: Human semen harbours an enriched microbial community and the seminal microbiome composition significantly differed in semen samples from the same men before and after vasectomy.

What is known already: The semen harbours a polymicrobial community, where *Lactobacillus*, *Corynebacterium*, *Staphylococcus*, *Prevotella*, and *Finegoldia* are common. The origin of the seminal microbiome, however, has not yet been established. One-third of the seminal microbes originate from the urethra, whereas a considerable part could originate from the upper genital tract. Similarly, male reproductive organs, such as prostate, seminal vesicles, and testicles contain its own microbiome. Recent pioneering studies analysing a total of 18 men indicates that vasectomy procedure alters the seminal microbiome, suggesting a testicular or epididymal microbial origin.

Study design, size, duration: This cohort study included 36 men (age = 40.4 ± 5.2 years, BMI = 26.2 ± 3.9 kg/m^2) who were planning to undergo vasectomy at the University Hospital from February to June 2021 and volunteered to donate semen samples before vasectomy and 3 months after the procedure. The study was approved by the Ethics Committee of Investigación Biomédica de Andalucia.

Participants/materials, setting, methods: Samples were collected after washing the penis with soap and water, and after urinating. Semen was obtained by masturbation (3-5 days of abstinence) before the vasectomy and 3 months post-vasectomy. The seminal microbiome was analysed by sequencing the V4 hypervariable region of the 16S rRNA gene (Illumina MiSeq). Raw sequences were pre-processed and taxonomy assigned using *Quantitative Insights Into Microbial Ecology version 2* (QIIME2). Bacterial diversity and abundance analyses were performed in R software.

Main results and the role of chance: Fifty-six bacterial genera were detected in the semen samples, where *Campylobacter*, *Finegoldia*, *Ezakiella*, *Streptococcus*, *Staphylococcus*, *Corynebacterium*, *Peptoniphilus*, and *Anaerococcus* prevailed (each genus with a relative abundance ≥5%). Post-vasectomy semen samples had significantly higher alpha diversity (observed richness diversity Wilcoxon signed-rank p-value = 0.035) and markedly increased abundance of genera *Porphyromonas*, *Finegoldia*, and *Anaerococcus*, while *Acetobacter* genus was reduced when compared with semen samples collected before the vasectomy procedure (FDR p-value<0.05). Beta diversity analysis indicated a significant microbial dissimilarity between seminal samples collected before and after vasectomy (weighted and unweighted PERMANOVA p-value = 0.001).

Limitations, reasons for caution: This study did not include urine samples, which do share around 30% of the microorganisms with semen. Therefore, in the next step we aim to integrate urine samples into the analysis.

Wider implications of the findings: Male sterilisation with vasectomy gave rise to fluctuations in the seminal microbiome composition and abundance. Our study findings provide new insight into the origin of seminal microbes, indicating that some accompanying bacteria could originate already from the testicular environment and their changes could entail microbial unbalance in semen.

Trial registration number: NA

Abstract citation ID: dead093.465
P-101 A comprehensive profile of cell-free RNAs in seminal plasma reveals their noninvasive potential in male infertility

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Study question: Could the cell-free RNAs (cfRNAs) in seminal plasma be associated with male infertility and used as clinical biomarkers?

Summary answer: CfRNAs in seminal plasma show different patterns in men with infertility and could serve as diagnostic and predictive factors in male infertility.

What is known already: Seminal plasma is a biological fluid containing large amounts of tissue-specific components secreted by organs of the male reproductive system. Thus, seminal plasma has great potential as a clinical sample for biomarker discovery and noninvasive diagnostics of male reproductive disorders. Several metabolites, metal ions, proteins and nucleotides are associated with semen quality and could be predictive factors of azoospermia forms and subtypes. However, the comprehensive transcriptome of cfRNAs in seminal plasma and their clinical value is not clear.

Study design, size, duration: We applied a previously developed library method of polyadenylation ligation-mediated sequencing (PALM-Seq) to capture and sequence the cfRNAs of seminal plasma from adult men, including 129 patients with asthenozoospermia, 101 patients with oligozoospermia, 102 patients with azoospermia (obstructive and nonobstructive azoospermia) and 107 healthy individuals.

Participants/materials, setting, methods: We performed PALM-Seq on the seminal plasma of recruited individuals and comprehensively profiled cfRNAs including miRNA, tRNA, piRNA, lncRNA and mRNA. In addition, we followed up the clinical outcome of assisted reproductive technology (ART) during reproductive therapy.

Main results and the role of chance: Hundreds of miRNA and tRNA could be detected in all samples. Unlike other body biofluids, we defined a large amount of piRNA in seminal plasma since piRNA is highly expressed in germ cells. Besides, the piRNA showed a group-specific pattern in men with infertility, especially in patients with azoospermia. Differentially abundant mRNA in patients with asthenozoospermia, oligozoospermia and azoospermia were mainly involved in regulating mononuclear cell migration, thermogenesis and germ cell development, respectively. The abundance of seminal plasma cfRNAs was associated with parameters of semen quality, as well as sperm DNA fragmentation. At last, using the collected omics data and machine learning models, we could accurately classify the subtypes of azoospermia and successfully predict the ART outcome before treatment.

Limitations, reasons for caution: We only recruited a limited number of individuals in this research and more studies focused on the ART outcome are needed.

Wider implications of the findings: We comprehensively characterized kinds of cfRNAs in seminal plasma for the first time and revealed changes in men with infertility. Our results contribute a new insight into understanding the mechanism of male infertility. Our findings could provide noninvasive diagnostics before invasive biopsies and help to improve the treatment of infertility.

Trial registration number: Not applocable

Abstract citation ID: dead093.466
P-102 Inhibition of RANKL increases the number of motile sperm in a subgroup of infertile men

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Study question: Does inhibition of RANKL increase semen quality in infertile men?

Summary answer: Overall, inhibition of RANKL (Denosumab) did not affect semen quality. However, in a subgroup of men with high serum AMH sperm production was significantly increased.

What is known already: Currently, there is no treatment available for male infertility, but studies in animal and preclinical human data have suggested a role for RANKL in male reproduction.

Study design, size, duration: A single-center, double-blinded, randomized clinical trial in 100 infertile men randomly assigned (1:1) to s.c. injection with 60 mg/mL Denosumab *or* s.c. saline injection 1 mL (0.9%) (placebo). The primary endpoint was changes in semen production, though the study was designed as an explorative study, with the aim of investigating the profile of which infertile men who would have a beneficial treatment outcome of Denosumab.

Participants/materials, setting, methods: All participants were infertile men referred to our andrological clinic due to impaired semen quality. Blood and semen samples were collected at baseline and after one and two cycles of spermatogenesis on day 80 and day 160, respectively.

Main results and the role of chance: There were no differences in semen quality or reproductive hormones at day 80 or day 160 between men treated with Denosumab or placebo. However, men with a serum AMH $\geq$ 40 pmol/L had a significant increase in total motile sperm (143% (SD 116%); $p = 0.011$), progressive motile sperm (133% (SD 100%); $p = 0.039$) and a borderline increase in sperm concentration (64% (SD 95%); $p = 0.056$) at day 80 compared to baseline. Further stratifying men according to both serum AMH ($\geq$ 40 pmol/L) and serum RANKL (highest 50%), showed a significantly higher increase in sperm concentration (107% (SD 111%) vs. -8% (SD 68%); $p = 0.009$), total motile sperm (256% (SD 270%) vs. 31% (SD 125%); $p = 0.039$) and progressive motile sperm (191% (SD 194%) vs. 18% (SD 134%) $p = 0.013$) at day 80 in men treated with Denosumab compared to placebo treated men. Furthermore, serum FSH (-28% (SD 33%) vs. 0%, (SD 22%); $p = 0.041$) and serum LH (-29% (SD 28%) vs. 20% (SD 51%), $p = 0.048$) were significantly lower and testosterone/LH ratio (50% (SD 51%) vs. -7% (SD 43%); $p = 0.024$) significantly higher at day 80 in men treated with Denosumab compared to placebo treatment.

Limitations, reasons for caution: This is a report from a randomized clinical trial, with an explorative design. Randomized clinical trials in a predefined group of infertile men with high serum AMH, are needed to explore if inhibition of RANKL improves semen quality and pregnancy rate in infertile couples with male factor infertility.

Wider implications of the findings: The increase in sperm production after Denosumab treatment in infertile men with preserved Sertoli cell function needs to be validated, in a trial selecting men based on serum AMH. Ultimately, Denosumab may be a potential breakthrough in treating male infertility at least in some infertile men with high serum AMH.

Trial registration number: NCT03030196

Abstract citation ID: dead093.467
P-103 Three-dimensional oviductal organoid model to study spermatozoa-oviduct interaction in humans

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Study question: Is it possible to establish an *in vitro* model that recapitulating human oviduct to investigate sperm-oviduct interaction

Summary answer: Three-dimensional (3D) oviductal organoid model was established from either primary human oviductal tissues or immortalized human OE-E6/67 cell line, based on our protocol.

What is known already: The sperm reservoir in oviduct is formed to maintain the fertilizing capacity of spermatozoa until ovulation by the binding of sperm to the epithelial lining of the oviduct, which is also significant to select

sperm with normal chromatin structure and superior fertilization ability. The regulation and mechanism of sperm reservoir formation in human is still unknown, mainly due to the ethical issue and technical difficulty in obtaining enough human oviduct. We previously demonstrated that sperm fucosyltransferase-5 (sFUT5), a carbohydrate-binding protein on spermatozoa, is responsible for spermatozoa-oviduct interaction in humans.

Study design, size, duration: Human oviductal epithelial cell line immortalized by HPV 16 E6/E7 open reading frame by retroviral expression system was employed to establish the co-culture model. Fresh human samples (human spermatozoa,N = 30; 1-2 cm primary oviductal tissue,N = 15) were collected in hospital with signed consent. Fluorescent pre-labelled sperm were co-cultured with organoids for 2 hours. At least three replicates were conducted for each assay.

Participants/materials, setting, methods: Morphological characterization and markers expressions of the organoid were determined by immunohistochemistry and confocal microscopy analysis. The sperm binding capacity of the oviductal organoid was studied by co-culture system. Sperm chromatin structure of the organoid-bound sperm was assessed by flow cytometry-based TUNEL assay. Affinity chromatography followed by nano-liquid chromatography–mass spectrometry was used to identify the sFUT5-binding proteins on ovidutal epithelial cells (OECs) and their expressions on OECs were validated by immunostaining/flow cytometry.

Main results and the role of chance: The organoid exhibited a hollow structure surrounded by a single layer of OECs. Immunohistochemistry analysis revealed the high expressions of epithelial marker E-cadherin (basolateral membrane), secretory marker pair-box 8 (PAX8) and proliferative marker Ki67. Expression of ciliated cell marker, acetylated tubulin (AcTUB) can also be detected. The modification of culture environment successfully reversed the OEC organoids polarity as demonstrated by immunostaining using antibodies against ZO-1 (apical marker) and E-cadherin (basolateral marker), making the organoid accessible during the co-culture. These apical-out OEC organoids have significantly higher sperm binding capacities than their basal-out counterparts. Furthermore, OEC organoid-bound spermatozoa possessed more intact DNA than the unbound spermatozoa. The results indicate the relevance of using OEC organoid-spermatozoa co-culture model as a tool to assess spermatozoa-oviduct interaction in humans. By using immuno-affinity chromatography and mass spectrometry analysis, cell adhesion molecule 4 (CADM4) was then identified as a potential sFUT5-interacting protein on OECs. This result was further supported by flow cytometry and immunofluorescent staining. 3-D oviductal organoid model demonstrates profound potential for *in vitro* investigation of human oviduct physiology.

Limitations, reasons for caution: Our 3D model may not fully represent the entire cellular complexity and tubular architecture of the oviductal epithelium. Further investigation on the sperm morphology and DNA integrity and analysis on organoid transcriptome will enhance our understanding on oviduct in human reproduction.

Wider implications of the findings: The results from co-culture may give a clue to future study on whether oviductal organoid can be applied clinically for functional sperm selection in *in vitro* fertilization. Our apical-out organoid model may also be applied to study tubal ectopic pregnency.

Trial registration number: not applicable

Abstract citation ID: dead093.468
P-104 Impact of Semen Microbiota on the Embryo Quality

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Study question: Is there a relationship between the microbiota composition of semen samples used for assisted reproductive technologies (ART) and quality of embryos?

Summary answer: The obligate anaerobes prevalence in semen microbiota was associated with reduced embryo quality in patients with astenozoospermia, but no difference was found for normozoospermic samples.

What is known already: The success of the ART depends on a number of factors, including embryo quality. The viability of IVF embryo could be affected by various environmental factors, including contamination with semen microbiota. Semen is not sterile, and even processed it may contain some bacteria, which may colonize culture dishes with oocytes and embryos. It was shown that the presence of bacteria might alter the sperm quality and the contamination of embryo culture dishes with bacteria was associated with poor quality of the developing embryos.

Study design, size, duration: 126 infertile couples attending the "Garmonia" Medical Center (Yekaterinburg, Russia) for IVF in 2020-2021, were included in the study. Depending on the spermiogram results, they were divided into two groups Group 1 (n = 51) — asthenozoospermia, Group 2 (n = 75) — normospermia.

Participants/materials, setting, methods: Semen microbiota was analyzed using RT-PCR kit Androflor (DNA-Technology, Russia). Cluster analysis was performed for 78 samples with the total bacterial load (TBL) of at least 10^3 GE/ml (asthenozoospermia = 31, normozoospermia = 47). Cluster analysis was conducted using the k-means++ algorithm, scikit-learn. The blastocyst development rate (BDR), as the proportion of 2PN zygotes, was determined on day 5 after injection. BDR $\geq$ 40% was considered satisfactory, BDR < 40% - unsatisfactory.

Main results and the role of chance: When analyzing semen microbiota, 8 stable clusters were distinguished. Cluster 1 was lactobacilli-dominated, Cluster 2 and 3 - Gram-positive facultative anaerobes dominated with prevalence of *Corynebacterium spp.* and *Streptococcus spp.* respectively; Cluster 4 – *Enterobacteriaceae/Enterococcus spp*-dominated,; Cluster 5, 6 - obligate anaerobes dominated with prevalence of *Bacteroides spp./Porphyromonas spp./Prevotella spp.* or *Peptostreptococcus spp./Parvimonas spp.*, respectively. Cluster 7 was formed by mixed obligate anaerobes (MOA); Cluster 8 - by *Mycoplasmae*.

There was no difference in detection rate of individual clusters in samples with normospermia and asthenozoospermia.

Depending on their BDR each group were divided into subgroups: Subgroup 1A (n = 25) with BDR $\geq$ 40%, Subgroup 1B (n = 26) with BDR < 40%; Subgroup 2A (n = 38) with BDR $\geq$ 40%, Subgroup 2B (n = 37) with BDR < 40%.

The detection rate of different microbiota clusters in Subgroups 2A and 2B (normospermia samples) was almost similar.

In asthenozoospermia samples the lower embryo quality (Subgroup 1B) was associated with higher prevalence of Cluster 7 (53.8%) comparing to those of Subgroup 1A (16.0%), $p < 0.001$. Cluster 1 was detected more often in Subgroup 1A (20.0%) than in Subgroup 1B (7.8%), but $p > 0.05$. TBL > 10^3 GE/ml was present in 52% of Subgroup 1A samples and in 69.2% of Subgroup 1B samples.

Limitations, reasons for caution: Cluster analysis was not conducted for the samples with TBL lower than 10^3 GE/ml, since their results were incompatible with the data received for the negative control samples.

Wider implications of the findings: Further research could determine the significance of the described bacterial clusters in semen with other pathologies. Establishing the relationship between the characteristics of semen microbiota and poor embryo quality might allow the development of new algorithms for treating patients with reproductive disorders, depending on the composition of semen microbiota.

Trial registration number: not applicable

Abstract citation ID: dead093.469

P-105 The Reliability of Sperm Chromatin Dispersion (SCD) Test to Predict Assisted Reproductive Technology (ART) Treatments Outcomes -A Systematic Review and Meta-Analysis

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Study question: Is the sperm chromatin dispersion (SCD) test reliable for predicting reproductive technology (ART) treatment outcomes?

Summary answer: Assessment of DNA fragmentation using the SCD test failed to predict pregnancy or live births rates.

What is known already: The detrimental effect of high DNA fragmentation on semen quality, fertility, and poor ART outcomes has been well-studied in recent years. Still, it remains contentious. Several techniques can detect sperm DNA fragmentation, such as TUNEL, comet assay, and SCSA. Although these techniques have been implemented in many andrology laboratories, they remain complex, time-consuming, and expensive. On the other hand, the SCD test indirectly estimates the DNA fragmentation level by manually quantifying the number of halos representing nuclear dispersion after sperm lysis and acid denaturation. While the SCD test is commonly used, it remains subjected to bias and high variability.

Study design, size, duration: Systematic review and meta-analysis.

A PRISMA-guided literature search in the English language was performed in January 2021 using PubMed/MEDLINE, Scopus, and Google scholar.

We looked for rates of fertilization, cleavage rate embryos, implantation, clinical pregnancy, miscarriage, and live birth.

MedCalc software using the random effects model was used, Sensitivity analysis was conducted to examine heterogeneity and the robustness of the results.

Participants/materials, setting, methods: After duplicate removal, 179 abstracts were assessed for eligibility, and 55 full-text articles were screened. Forty-one articles were included in the qualitative syntheses, and 16 were included in the quantitative synthesis, representing 6989 participants.

Main results and the role of chance: No significant difference was identified between the group with high SDF vs. low SDF regarding fertilization (SMD −0.334, 95% CI −0.695, 0.027, $p = 0.07$), clinical pregnancy (OR 0.901, 95% CI 0.719, 1.130, $p = 0.367$), miscarriage (OR 0.901, 95% CI 0.719, 1.130, $p = 0.367$) and live birth (OR 1.105, 95% CI 0.627, 1.946, $p = 0.730$) rates.

The rates of cleavage stage embryos (SMD -1.153, 95% CI -2.113, −0.194, $p = 0.019$) and implantation (OR 0.472, 95% CI 0.303, 0.737, $p = 0.001$) were significantly lower in the group with high SDF vs. low SDF.

Limitations, reasons for caution: A high index of heterogeneity and publication bias was reported regarding fertilization (I2 = 93.23%, Egger's test: intercept -5.29866, 95% CI -10.41, −0.19, $p = 0.02$) and cleavage-stage embryos(I2 = 98.49%, Egger's test: intercept -14.02395, 95% CI -27.47, −0.57, $p = 0.02$)

Wider implications of the findings: Assessment of DNA fragmentation using the SCD test failed to predict pregnancy or live births. As the goal of ART is live birth, clinicians should consider the limited yield of this assay to predict the likelihood of live birth.

Trial registration number: NA

Abstract citation ID: dead093.470

P-106 The inhibition of sperm SIRT1 enhances fertilization and modifies subsequent embryonic epigenetic quality

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Study question: Is SIRT1 present in sperm and thus involved in fertilization and/or sperm epigenetic code? Do the SIRT1-driven epigenetic marks be transmitted to the progeny?

Summary answer: SIRT1 localized at the sperm head and influences fertilization and sperm epigenetic code via its deacetylation activity. SIRT1-modulated epigenome is transmitted to zygotes and blastocysts.

What is known already: The addition or removal of acetyl group from lysine residua (i.e. acetylation) post-translationally modulates proteins through the action of acetylases and deacetylases, respectively. SIRT1 (also Sirtuin 1) is capable to deacetylate a number of proteins, including histones, with roles in male reproduction. Testicular SIRT1 deficit leads to subfertility and increases number of abnormal and immotile spermatozoa that fail to fertilize. However, neither sperm SIRT1 presence nor its function has been investigated despite the significance of acetylation in the events around fertilization. Moreover, the epigenetic mode of action of sperm SIRT1 highlights its relevance for the success of the pre-implantation embryo.

Study design, size, duration: Mouse sperm of 14-18-weeks-old males of CD-1 x C57Bl6 hybrid strain were isolated from the epididymis. Sperm were incubated under capacitating conditions in presence of sirtinol (5 μM), a selective SIRT1 inhibitor, or the vehicle (0.1% v/v DMSO) for 1 h in 37 °C and 5% CO_2.

Participants/materials, setting, methods: Motility and acrosome reaction was evaluated as previously stated (Ritagliati et al., 2018). Moreover, sperm proteins were extracted into RIPA-buffer and acetylated proteins were depicted by western blot. Finally, *in vitro* fertilization and embryo culture were performed. Above parameters defining IVF outcomes (i.e., fertilization, cleavage, and blastocyst rate), immunocytochemistry of acetylated histone 4 in the 16th residua of lysine (H4K16) in zygotes and blastocyst was quantified.

Main results and the role of chance: SIRT1 was localized in the sperm head, apparently at the perinuclear theca and perforatorium. Inhibition of SIRT1 leads to the increase of lysine acetylation (2.28 *vs* 1, fold change values) and hyperactivation (16.55 ± 0.79 *vs* 10.84 ± 0.79 %sperm), the enhanced motility that facilitates sperm to penetrate the oocyte, as well as the acrosome reaction (28.69 ± 0.41 *vs* 17.98 ± 0.41 %sperm). Subsequently, these spermatozoa were able to better fertilize oocytes *in vitro* (90.5 ± 7.21 *vs* 66.25 ± 7.21, %). Interestingly, zygotes derived from sirtinol-pre-treated spermatozoa developed to 2-cell embryos at a higher rate than those fertilized by untreated spermatozoa (79.33 ± 7.21 *vs* 67.00 ± 7.21, %). Moreover, we observed significant hyperacetylation of H4K16 in the male pronuclei of zygotes and embryos derived from sirtinol-treated sperm (8.19 ± 1.02 *vs* 6.10 ± 2.09 and 45.55 ± 13.96 *vs* 27.62 ± 13.96, integrated density of the signals, respectively).

Limitations, reasons for caution: Although acetylation of H4K16 was accumulated in male pronuclei, we do not know which subset of genes was affected. Therefore, we only can assure that SIRT1-driven epigenetic marks are transmitted to the offspring, but their consequences should be further elucidated. For knowledge transfer to humans, a verification study is required.

Wider implications of the findings: the reduction in testicular SIRT1 expression is an age-related phenomenon. Therefore, our results support SIRT1 as the basis for new interventions against age-related male infertility. Moreover, due to the high reactivity of SIRT1 to its inactivation, SIRT1

becomes a target to pharmacologically enhance sperm fertilization ability in assisted reproductive techniques.

Trial registration number: Animal procedures were conducted in accordance with Act No. 246/1992 Coll. on the Protection of Animals Against Cruelty under the supervision of the Animal Welfare Committee of the Ministry of Education, Youth and Sports of the Czech Republic, approval ID MSMT-11925/2016-3.

Abstract citation ID: dead093.471
P-107 MICROFLUIDIC SPERM SEXING: An XLD (X-linked Disorder) model for preventing genetic disorders in newborns

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Study question: Can sperm sexing based on unique surface membrane markers – enable genotypic sperm sorting by allosomal content to prevent certain X-linked disorders in newborns?

Summary answer: In an X-Linked-Disorder prevention model, we utilized a microfluidic device functionalized with H-Y monoclonal antibodies attached to magnetic beads to enrich Y-chromosome bearing sperm sorts.

What is known already: About 4% of newborns are at risk of being born with an X-linked disease such as Fragile X Syndrome. The current clinically approved mitigation plan for preventing such genetic disorders is to consider Preimplantation Genetic Testing for Monogenetic diseases (PGT-M) which involves an invasive microsurgical procedure on fragile embryos. To minimize this invasiveness, we proposed a less invasive microsurgical exclusion approach to prevent such genetic disorders by genotypically sorting sperm cells that can be used for IUI/IVF/ICSI – with microfluidics – using their allosomal characteristics.

Study design, size, duration: Semen samples purchased from FairFax Cryobank from anonymous donors were used in immuno-magnetic microfluidic sorting protocols with the use of phycoerythrin (PE) binding Tetrameric Antibody Complexes (TAC) coupled with magnetic beads to tag H-Y monoclonal antibody (mab) binding activities on the sperm surface membrane.

Participants/materials, setting, methods: Immuno-magnetic sorting was conducted with specially fabricated microfluidic-sperm-sorting (MSS) chips. Sort yield and purities of positively selected sperm populations were assessed by flow-cytometry, immunofluorescence, fluorescent-in-situ-hybridization (FISH) and by quantitative-PCR (qPCR – Cq & Tm). Quantitative experiments were run in triplicates and the results were expressed as absolute means for comparison between paired variables. Statistical analyses were performed using Student's t-test and a p-value of less than 0.05 was considered to be statistically significant.

Main results and the role of chance: For the assessment of H-Y sperm sorts, our preliminary data showed that flow cytometry was able to confirm the positive sperm selection (which were mostly Y-chromosome bearing sperm cells) in a sperm genotyping protocol – initially utilizing immuno-magnetic sort tubes prior to flowing through microfluidic chips. Both immuno-magnetic sort tubes and the microfluidic chips were able to optimally confirm from qPCR-Cq means – the negative selection – being the X-chromosome bearing sperm that may have been largely unselected by the H-Y-mab. Values for Cq means compared under H-Y-qPCR data sets were statistically significant with a p value of 0.000023 (p values is significant at < 0.05).

Our study was able to elucidate a potential less invasive clinical application of genotypically sorting sperm cells with mab-functionalized microfluidic chips for an XLD-model. Molecular down stream applications like flow cytometry may therefore be utilized to experimentally confirm sort purities of positively selected sperm populations (with H-Y markers) – based on the utility of the SMCX and SMCY gene in the X and Y sperm chromosome bearing sperm cells respectively.

Limitations, reasons for caution: Physiological effect of magnetic fields of varying tesla or gauss strengths on sperm selected with immuno-magnetic beads may warrant further safety studies prior to actual clinical applications. Optimal selection results may be achieved with the use of freshly collected normospermic rather than frozen-thawed samples.

Wider implications of the findings: Although we had de-emphasized the clinical application of the microfluidic sperm sexing protocol for legitimate use in sex selection and gender balancing, this study design was solely focused on adapting a less invasive method of preventing X-linked genetic disorders with the potential exclusion of microsurgical procedures leading to PGT-M.

Trial registration number: NA

Abstract citation ID: dead093.472
P-108 Does DNA Fragmentation.level significantly impact clinical outcomes in patients undertaking Assisted Reproduction with using donor oocytes?

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Study question: Does an abnormal Sperm DNA Fragmentation Index significantly impact clinical outcomes in patients undergoing Assisted Reproduction with ICSI using donor oocytes?

Summary answer: No statistical difference was seen in pregnancy or miscarriage rates between high or low DNA fragmentation indexes with oocyte donation cycles using intracytoplasmic sperm injection.

What is known already: High levels of sperm DNA fragmentation are proposed to have an adverse impact on reproduction. While subject to debate, generally accepted ranges of $> 30\%$ considered high and associated with poorer outcomes, with levels $< 15\%$ considered normal. Impact in the intermediate range if 15-30% is less understood. Potential interventions for elevated DFI include ICSI, lifestyle changes, antioxidant therapy or more controversially TESE. It is thought that high quality oocytes have the potential to repair sperm DNA damage- thus the use of younger donors with higher fertility in oocyte donation cycles may improve outcome and overcome a high DFI.

Study design, size, duration: A retrospective cohort analysis was performed on our database of donor oocyte FET treatment between January 2016 and December 2020, with 1370 cycles identified Screening to identify those couples where pre- treatment sperm DFI was performed as part of the treatment workup, and DFI was analysed in 9% of these cycle ($n = 124$). Clinical outcomes were recorded including positive HCG, clinical pregnancy rate, miscarriage rate and ongoing pregnancy rate at 12/40 gestation, and compared between groups.

Participants/materials, setting, methods: Oocyte donation cycles with baseline Sperm DFI measurements in a tertiary academic centre were identified. Donor treatments were chosen to reduce the impact of female factor variation on results. 49 cases had normal DFI($< 15\%$), and were compared to 21 patients in the high group (> 30). 54 patients were on the intermediate range (15-30%) and were excluded from the analysis. Pregnancy and miscarriage rates were compared between high and low groups to assess impact of DFI.

Main results and the role of chance: Elevated DFI has been proposed to have an abnormal impact on assisted reproduction outcomes. Other studies have suggested that ICSI may compensate for this, or that healthy oocytes may be able to negate any detrimental impact from the sperm. With autologous gamete treatments, large variations in the expected prognosis are due to female characteristics such as age, ovarian reserve or gynaecological disorders such as endometriosis or PCOS. This use of younger egg donors with normal AMH and the exclusion of any identifiable pelvic abnormality allows for some standardisation of the female cohort, therefore allowing a more direct assessment of the impact of any male factor abnormality on the outcome.

Positive HCG rates per embryo transfer were the same between DFI groups.

Normal 53.1% (26/49) vs High 47.65% (10/21) $p = 0.68$

Similarly there was no difference in Clinical Pregnancy Rates based on DFI level

Normal 44.9% (22/49) vs High 47.7% (10/21) $p = 0.83$

Miscarriage rates per positive HCG test were again not impacted by male sperm DFI:

Normal 30.7% (8/26) vs High 33.3 (3/10) $p = 0.72$

The study data demonstrates that in treatment cycles using a combination of ICSI and good quality oocytes, the clinical outcomes do not appear to be impacted by raised DFI

Limitations, reasons for caution: We previously demonstrated that fertilisation and blastulation rates were unaffected by DFI. The study was limited by a relatively small sample size, as DFI analysis was not routinely measured before treatment. Larger multi-centre studies to increase the study population and statistical power would be useful for confirmation of the findings.

Wider implications of the findings: Our data suggests that biochemical, clinical and ongoing pregnancy rates, and also miscarriage rate, were not adversely impacted by high DFI in donor oocyte cycles.This could be attributed to the ability of a high quality oocyte to repair sperm DNA damage when combined with ICSI for sperm selection and fertilisation.

Trial registration number: Not applicable

POSTER VIEWING
EMBRYOLOGY

Abstract citation ID: dead093.473
P-109 Letrozole versus hormone replacement therapy for frozen embryo transfer: A randomized clinical trial

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Study question: Do the outcomes of frozen embryo transfer cycles differ according to endometrial preparation (EP) with letrozole versus hormone replacement therapy (HRT)?

Summary answer: Letrozole seems to be as effective as HRT preparation in frozen cycles in terms of treatment outcomes.

What is known already: Vitrification improved the survival of vitrified-warmed embryos and increased the number of FET cycles. A common EP strategy for FET includes augmentation of the endometrium using estrogen followed by progesterone supplements. Retrospective studies have shown that using aromatase inhibitors (letrozole) in FET cycles was associated with higher clinical pregnancy and live birth rates, with a lower miscarriage rate, as compared to HRT cycles.

Letrozole maintains regular feedback mechanisms, facilitating normal follicular growth, and promotes single follicle development and ovulation. Moreover, it does not have any negative effects on the endometrium and can improve endometrial receptivity.

Study design, size, duration: This randomized controlled trial was performed in two university-affiliated medical centers.

A total of 170 patients undergoing frozen embryo transfer cycles from June 2018 to June 2021 were eligible.

Participants/materials, setting, methods: Patients were randomized to either letrozole (n = 69) or HRT protocol (n = 101) for EP, using a blocked randomization method. Inclusion criteria were single blastocyst transfer with normal uterine cavity. Exclusion criteria were patients older than 40 years, oocyte donation cycles, or more than 3 previous transfers without a pregnancy. The primary study outcome was percentage of clinical pregnancies, defined as clinical pregnancies/embryo transfers, excluding canceled treatments.

Main results and the role of chance: The characteristics of the study groups were comparable in age, BMI, gravidity, parity, previous treatment history and characteristics of the relevant fresh cycle in which the embryo was frozen. After excluding 5 cancelled treatments in the letrozole group and 2 in the HRT group, the percentage of clinical pregnancies between the letrozole and HRT arms were similar (24 (38.0%) versus 34 (34.6%), respectively, p = 0.67). There was no difference in the percentages of live births (22 (34.9%) versus 28 (28.6%), respectively, p = 0.4) or missed abortions (1 (4.1%) versus 5 (14.7%), p = 0.19). For patients who did not achieve pregnancy in the randomized treatment cycle, we retrospectively examined the outcome of the next treatment cycle with the other EP protocol, when possible. Achievement of a clinical pregnancy was the same for change in treatment for both study groups (7(50%) in the letrozole group and 9(50%) in the HRT group).

Limitations, reasons for caution: Limitations of this study included the heterogeneous study groups. Even though class II ovulation disorders were similar in prevalence in both groups, they included different indications for ART. Pregnancy complications were not evaluated and should be further investigated in a study with a larger sample size.

Wider implications of the findings: Letrozole seems to be as effective as HRT for EP in frozen cycles, with possible advantages of fewer missed abortions and pregnancy complications. Letrozole could be a suitable option for EP, as it is effective and, enables more convenient timing, versus natural FET.

Trial registration number: NCT03540979

Abstract citation ID: dead093.474
P-110 Effects of embryo culture under atmospheric oxygen concentration on cumulative live birth rate and birth weight

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Study question: The purpose of this study is to compare cumulative live birth rate and perinatal outcomes of embryos cultured in 20% oxygen and 5% oxygen.

Summary answer: Atmospheric oxygen concentration not only affects the quality of embryos, but also affects the cumulative live birth rate and birth weight after embryo transfer.

What is known already: Whether 5% oxygen culture can effectively improve the outcome of embryonic pregnancy is still controversial. There is a lack of scientific data support from a large sample, especially regarding the cumulative live birth outcome as the primary outcome.

Study design, size, duration: This retrospective study included patients who received in vitro fertilization (IVF) treatment with their own first oocyte retrieval cycles from 1 January 2016 through 31 November 2019, with transfer dates limited to 31 November 2020. Our final analytic cohort included 31,566 women.

Participants/materials, setting, methods: Embryos were cultured at 5% or 20% oxygen concentration until the third day for transplantation or freezing. If prolonged culture was required, embryos of both groups were transferred to the incubator with 5% oxygen concentration to the blastocyst stage until transplantation or freezing. Other laboratory and clinical procedures were consistent between the two groups.

Main results and the role of chance: The rate of high-quality embryos in the 5% oxygen group was significantly higher than that in the 20% oxygen group (0.51 ± 0.33 vs 0.49 ± 0.33; β = −0.03; 95%CI, −0.03–−0.02; P < 0.001), while the rate of slow-developing embryos was significantly lower (0.52 ± 0.31 vs 0.56 ± 0.31; β = 0.04; 95%CI, 0.03–0.05; P < 0.001). The cumulative live birth rate was significantly higher in the 5% oxygen group than in the 20% oxygen group (62.4% vs 58.6%; adjusted OR = 0.85; 95%CI, 0.81-0.90). The birth weight and Z score (birthweight corrected for gestational age at birth, gender and parity were significantly lower in the 5% oxygen group than in the 20% oxygen group (birth weight: 3.28 ± 0.48 vs 3.30 ± 0.50, adjusted OR = 0.022, 95%CI, 0.004-0.04; Z score: 0.22 ± 1.01 vs 0.26 ± 1.04, adjusted OR = 0.037, 95%CI, 0.001–0.074). No matter what age group, the cumulative live birth rate, pregnancy rate and good birth outcome of the 5% oxygen concentration group were higher than those of the 20% group, while the proportion of no transferable embryos was lower than those of the 20% group.

Limitations, reasons for caution: Limitations include that oocytes were cultured at 20% oxygen concentration on the fertilization day (D0), and randomly distributed to incubators with different oxygen concentrations until day 1. Because it was a retrospective study, the grouping was not randomized.

Wider implications of the findings: We counted the largest sample data at present, took the cumulative live birth rate as the main outcome indicator, and tracked the perinatal outcome, using reliable data to prove that atmospheric oxygen concentration has serious and long-term irreversible effects on embryonic development.

Trial registration number: not applicable

Abstract citation ID: dead093.475

P-111 External validation of a model for prioritizing day 3 embryos for transfer based on deep learning and time-lapse images

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Study question: Could an objective embryo assessment by iDAScore version 2.0 perform as well as a conventional morphological assessment according to the Istanbul consensus report?

Summary answer: iDAScore version 2.0 performed as well or even better than conventional embryo assessment in fresh day 3 embryo transfer cycles.

What is known already: The pregnancy prediction performance of iDAScore version 1.0 has been confirmed in a large cohort study with vitrified-warmed blastocyst transfer, the validation results showed that it performs as well or better than conventional embryo evaluation. iDAScore version 2.0 was developed for transfers on both day 2, day 3 and day 5 transfers, however, the performance of iDAScore version 2.0 has not been verified with independent datasets.

Study design, size, duration: A single-center large cohort retrospective study from an independent validation test was carried out, including 4328 cleavage embryo transfers cycles performed between January 2020 and June 2022.

Participants/materials, setting, methods: The selection of embryos for transfer was based on conventional morphological assessment model according to Istanbul consensus report, and the transferred embryos were retrospectively evaluated by the iDAScore version 2.0 model. The pregnancy prediction performances of the two day 3 embryo assessment models were compared using the area under curve (AUC) for predicting the FHB for all cycles and subgroup analysis(SET and DET; female age <35 and $\geq$ 35 year; number of blastomeres <8c and ≥ 8c).

Main results and the role of chance: The FHB rate in the iDAScore (2.0-3.9) group was lower than in all iDAScore group, iDAScore (4.0-5.9), and (6.0-8.0) groups within the poor, fair and good group. Additionally, for fair and good group, the FHB rate in iDAScore (6.0-8.0) group was higher than that in other iDAScore groups. The AUCs of iDAScore were significantly greater than the conventional morphology assessment, for all cycles (0.62 vs. 0.59, $P < 0.05$), SET cycles (0.63 vs. 0.59, $P < 0.05$) and DET cycles (0.61 vs. 0.59, $P < 0.05$). For the sub-groups of age, the AUC of iDAScore was significantly greater than the conventional morphology assessment in <35 group (0.62 vs. 0.59, $P < 0.05$). However, there was no significant difference between the two assessment model in ≥ 35 group (0.60 vs. 0.58, $P > 0.05$). For the sub-groups of the number of blastomeres, a significantly greater AUC was observed for iDAScore than for conventional morphology assessment, for both the <8c group (0.67 vs. 0.56, $P < 0.05$) and the $\geq$ 8c group (0.58 vs. 0.55, $P < 0.05$).

Limitations, reasons for caution: the limitation of the findings is that all analyzed embryos were selected previously by morphology. Although the conventional morphological assessment is a widely used morphological grading system, it is subjective and susceptible to inter-observer variation.

Wider implications of the findings: iDAScore version 2.0 may be a promising tool for selecting day 3 embryos with the highest likelihood of implantation. A fully automated evaluation system also significantly reduced the time used for embryo selection and eliminates subjectivity.

Trial registration number: none

Abstract citation ID: dead093.476

P-112 Outcome of laser collapsing blastocyst on clinical pregnancy rate

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Study question: To determine clinical pregnancy rate after laser-induced collapse of blastocyst before cryopreservation

Summary answer: Laser collapse of the blastocyst before cryopreservation increases clinical pregnancy rate regardless of assigned embryo quality group compared to the blastocysts vitrified without laser collapsing

What is known already: Vitrification has been associated with the certain risks to the structure of an embryo due to intracellular ice crystal formation and impaired shrinkage rate. Blastocyst is a large fluid filled cavity requiring additional preparation to reduce the potential risks of damage. Collapsing a blastocyst has been shown to improve survival rate of an embryo following thawing and improve in vitro fertilization (IVF) outcomes including clinical pregnancy and live birth rates

Study design, size, duration: . It is a single-centre retrospective study conducted at a private clinic in Kazakhstan. We analyzed the outcome of IVF treatment following cryopreservation and subsequent frozen embryo transfer of 2959 blastocysts from 1642 women during January 2019-December 2021

Participants/materials, setting, methods: The data on blastocyst cryopreservation technique and IVF treatment outcome was collected from the clinic's electronic database. The blastocysts were divided into two groups based on performed laser collapsing prior to vitrification and further stratified by embryo quality group including excellent, good and fair groups. The primary outcome was clinical pregnancy rate. Laser-assisted collapsing procedure was initiated in August 2020 at the clinic. Thus, all previously vitrified blastocysts served as control group for the study

Main results and the role of chance: 1556 blastocysts from 777 women vitrified following laser collapsing and 1403 blastocysts from 865 women without prior laser collapsing were analyzed in the study. Stratification of blastocysts by the embryo quality were comparable between two groups with more than half of embryos defined as excellent embryo quality group, 54% in collapsed group and 52% in non-collapsed group ($p > 0.05$). Most blastocysts were from women under the age of 35 years old in both study groups. 1 in 4 collapsed blastocysts of the excellent embryo quality group were from women older than 35 years old, whereas non-collapsed group of blastocysts was evenly distributed between embryo quality groups ($p < 0.05$). Clinical pregnancy rate was the highest in the excellent embryo quality group with prior laser collapsing compared to the group without collapsing, 53% vs 43% respectively ($p < 0.001$). Overall, collapsed blastocysts showed higher rate of clinical pregnancy rate in all three embryo quality groups. Despite of changes associated with the COVID-19 pandemic IVF outcomes had not been affected but improved with the use of laser collapsing prior to embryo vitrification

Limitations, reasons for caution: Retrospective design of the study did not provide full baseline information on potential confounders including paternal characteristics and concurrent conditions. Moreover, the study period included the COVID-19 pandemic start which could have affected treatment outcomes

Wider implications of the findings: Collapsing the blastocyst embryo before vitrification should become a routine procedure to improve treatment outcomes of frozen embryo transfer irrespective of woman's age and embryo's quality

Trial registration number: not applicable

Abstract citation ID: dead093.477

P-113 The performance of pregnancy prediction is improved in an updated deep-learning based embryo selection model: A retrospective validation study using 3960 single blastocyst transfer cycles

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Study question: Does increasing the size of a training dataset improve the performance of pregnancy (fetal heartbeat) prediction in a deep-learning model?

Summary answer: Yes. Increasing the training data for a deep-learning model for embryo selection improved prediction of the likelihood of implantation after single vitrified-warmed blastocyst transfer (SVBT).

What is known already: Recently, artificial intelligence (AI) for implantation prediction after blastocyst transfer has been extensively studied. AI can address the issue of subjective assessment for the selection of blastocyst transfer. Generally, the performance of deep learning models is said to improve by increasing the training dataset. However, to the best of our knowledge, there exists no study on the effect of increasing training data on the performance of pregnancy prediction on the same deep-learning model.

Study design, size, duration: A total of 3,960 SVBT cycles (1 patient,1 cycle) were retrospectively analyzed. Embryos were stratified according to SART age groups. The quality and scoring of embryos were assessed by iDAScore v1.0 (iDAV1; Vitrolife, Sweden), v2.0 (iDAV2; Vitrolife, Sweden), and Gardner grading. The discriminative performance of the pregnancy prediction for each embryo scoring model was compared using the area under the curve (AUC) of the receiver operating characteristic curve for each maternal age group.

Participants/materials, setting, methods: Embryos were cultured in the EmbryoScope+ and/or EmbryoScope Flex (Vitrolife). iDAV2 and iDAV1 were based on an identical deep-learning architecture, but the training data for iDAV2 has been increased with 15% more data. ICM and TE were annotated according to the Gardner grading system. The degree of blastocyst expansion was Grade 4 due to our freezing policy. Furthermore, Gardner's grading (GG) was stratified into four grades (A: AA, B: AB BA, C: BB, D: others).

Main results and the role of chance: The AUCs of the < 35 years age group (n = 757) for pregnancy prediction were 0.718 for iDAV1, 0.733 for iDAV2, and 0.694 for GG. The AUC of iDAV2 was significantly higher compared to GG ($P < 0.05$).

For the 35–37 years age group (n = 821) the AUCs were 0.696, 0.712, and 0.695 for iDAV1, iDAV2, and GS, respectively, and were significantly different between iDAV1 and iDAV2 ($P < 0.05$). The AUCs of the 38–40 years age group (n = 1,007) were 0.698 for iDASV1, 0.706 for iDAV2, and 0.700 for GG and there was no significant difference. The AUCs of the 41–42 years age group (n = 715) were 0.734, 0.745, and 0.696 for iDAV1, iDAV2, and GS, respectively, and the AUC of iDAV2 was not significantly different compared to iDAV1 ($P = 0.174$) but significantly different compared to GG ($P < 0.05$). For the > 42 years age group (n = 660) AUCs were 0.685 for iDAV1, 0.698 for iDAV2, and 0.682 for GS and were not significantly different among groups. The AUCs of iDAV1, iDAV2, and GG in all ages were 0.736, 0.720, and 0.702, respectively. iDAV2 were significantly higher than iDAV1 and GG ($p < 0.05$).

Limitations, reasons for caution: This study was based on minimal stimulation and natural cycle IVF treatment, and a freeze-all strategy whereby all transferred blastocysts had previously been vitrified. Therefore, we had only a few cycles with elective blastocyst transfer. In addition, this study was retrospective in nature.

Wider implications of the findings: For all age groups, iDAV2 had a higher AUC than iDAV1, although a significant difference was only observed for the young age group. This result suggests that the increased dataset used for the development of iDAV2 improved the performance of pregnancy prediction for SVBT in the deep-learning model.

Trial registration number: not applicable

Abstract citation ID: dead093.478

P-114 Abdominal obesity in Polycystic ovary syndrome patients can increase endometrial stromal cells decidualization

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Study question: Whether abdominal obesity can change the decidualization induction potential in PCOS patients?

Summary answer: We hypothesized that considering the inflammatory origin of abdominal obesity, it may affect decidualization potential of endometrial stromal cells (ESCs).

What is known already: Polycystic ovary syndrome (PCOS) as a chronic inflammatory disease is the most common hormonal disorder in women of reproductive age, which is often associated with subfertility or infertility due to ovulation disorders and subsequently low pregnancy rate, live birth rate and a high miscarriage rate. Inflammatory Abdominal obesity (AO) is the most common phenotype in PCOS patients, which has a high association with PCOS pathogenesis. Embryo-endometrium cross-talk has a key role in successful embryo implantation, which contains inflammatory phase (decidualization induction). Followed by inflammatory phase for pregnancy support.

Study design, size, duration: Our study population were consisting of 53 infertile women including PCOS diagnosed according to Rotterdam criteria (n = 25) and normal oogenesis women (male factor infertility) (n = 28) with in vitro fertilization (IVF) / intracytoplasmic sperm injection (ICSI) and frozen embryo transfer (FET) indication. All patients were aged 25-35 referred to Royan Institute between 1st May until 1st December in 2021 for infertility treatments.

Participants/materials, setting, methods: The embryos were subdivided into four groups: PCOS with AO, PCOS without AO, nonPCOS with AO, and nonPCOS without AO patients. The embryos from each group were single cultured up to the blastocyst stage. Their embryo condition media (ECM) were pooled and added to the culture media of healthy ESCs monolayer to investigate their effects on decidualization potential via gene and protein expression analysis and ESCs migration assay.

Main results and the role of chance: Results showed that ECM generally can improved decidualization capacity in ESCs. Morphological analysis, migration assay, protein and gene expressional analysis showed PCOS with AO had the highest decidualization potential and revealed by higher expression of decidualization markers ($P \leq 0.05$). NonPCOS individuals without AO had the lowest level of both gene and protein decidualization markers ($P \leq 0.05$).

Limitations, reasons for caution: Due to small sample population and the ECM volume limitation, the present study remained focused on the some main decidualization markers rather than extended study.

Wider implications of the findings: Inflammation may increase endometrial receptivity, allowing more suboptimal embryos to implant; this could explain the high rate of abortion in these cases. Controlling the inflammation in PCOS patients with AO may correct the decidualization profile of these patients.

Trial registration number: Not applicable

Abstract citation ID: dead093.479
P-115 Clinical prognosis of abnormally cleaved embryos: time-lapse evidence of self-correction before blastulation

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Study question: What is the clinical prognosis of embryos displaying abnormal cleavage (ABNCL) observed via time-lapse videography (TLV) and the role of blastulation in embryo self-correction?

Summary answer: ABNCL leads to reduced blastulation rate, but not live birth rates and neonatal outcomes once blastulation has been achieved.

What is known already: It is widely accepted that ABNCL is associated with reduced viability in cleavage stage human embryos. However, evidence is scarce in literature reporting birth outcomes from blastocysts arising from such embryos, mostly because they are ranked low priority for transfer. Knowledge is also limited regarding the potential self-correction mechanism in those leading to live births. In this study, using a large dataset of blastocysts with known live birth outcomes, we aimed to explore the role of blastulation in the self-correction process of ABNCL embryos, and their subsequent live birth rates as well as neonatal outcomes following intrauterine transfer.

Study design, size, duration: This retrospective study involved 1562 consecutive autologous *in vitro* fertilization cycles (maternal age 35.1 ± 4.7 years) performed at Fertility North between January 2017 and June 2022. Fresh transfers were routinely performed on Day 3 with remaining embryos cultured up to Day 6. A total of 9451 embryos were subject to blastocyst culture, with a subset of 664 resulting blastocysts (following single frozen transfers up to August 2021) included for live birth and neonatal outcome analysis.

Participants/materials, setting, methods: A total of 6019 out of 9451 embryos were annotated for ABNCL up to Day 3, including direct cleavage (DC), reverse cleavage (RC) and <4 intercellular contact points at 4-cell stage (<4ICCP). The remaining 3432 embryos were excluded from TLV annotation due to poor quality as per the 2011 Istanbul consensus. For DC and RC, the number of affected blastomeres was also recorded for the 1st (1-cell), 2nd (2-3-cell), and 3rd cleavage cycles (4-8-cell), respectively.

Main results and the role of chance: Embryos showing DC (19.5%), RC (41.7%), <6ICCP (58.8%), and Mixed (affected by 2 or 3 ABNCL types, 26.4%) had lower blastulation rates than those without ABNCL (67.2%, $P < 0.01$ respectively). Subgroup analysis showed increasing blastulation rates along cleavage cycles of DC/RC occurrence, from 1st through 2nd to 3rd cleavage cycles (DC 6.3%, 20.7% and 41.0%, $P < 0.001$; and RC 10.0%, 32.3% and 45.8%, $P < 0.05$). Negative correlation ($P < 0.05$ respectively) was also identified between the number of blastomeres affected by DC/RC and subsequent blastulation. However, once blastulation was achieved, clinical results were comparable between blastocysts with DC, RC, <6ICCP and No ABNCL; including live birth rates (25.9%, 33.0%, 36.0% vs 30.8%, $P > 0.05$ respectively), gestational age (38.7 ± 1.6, 38.5 ± 1.2, 38.3 ± 3.5 vs 38.5 ± 1.8 weeks, $P > 0.05$ respectively) and birthweight (3343.0 ± 649.1, 3378.2 ± 538.4, 3352.6 ± 841.3 vs 3313.9 ± 509.6 grams, respectively). Above findings held true following multivariate regression (logistic or linear as appropriate); adjusting for maternal age and BMI, vitrification day, blastocyst expansion stage, ICM/TE grades, insemination methods and sperm types. Also, no difference was detected using either Z-score as endpoint combining birthweight, baby sex and gestational age; or preterm delivery (<37 weeks) or low birthweight (<2500 grams) incidences as endpoints. Live-birth embryo TLV indicated self-correction mechanisms by excluding/extruding DC/RC cells before blastulation.

Limitations, reasons for caution: Our study is limited by its retrospective nature, making it impossible to control all known/unknown confounders. Embryos in our dataset, being surplus after selection for those with no or minimum ABNCL for fresh transfer on Day 3, may not represent the full embryo cohort.

Wider implications of the findings: Our findings further support the embryo self-correction mechanism where DC/RC affected blastomere is excluding/extruded at blastulation, which is evidenced by TLV of live-birth embryos. The assuring live birth rates and neonatal outcomes in ABNCL blastocysts provide valuable guidance to clinical practice and patient counselling.

Trial registration number: not applicable

Abstract citation ID: dead093.480
P-116 How does the short insemination method affect clinical outcomes and laboratory management?

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Study question: Does the short insemination method using a time-lapse monitoring system improve clinical outcomes and laboratory management?

Summary answer: The method increased the normal fertilization rate of Conventional IVF and also increased the 3PN rate. The working environment of the laboratory staff was improved.

What is known already: Short insemination using a time-lapse monitoring system has been reported to reduce fertilization confirmation failures due to loss of pronuclei, but many reports indicate that the method was implemented to perform so-called 'rescue ICSI' for unfertilized oocytes on the day of egg retrieval and there are not many reports of clinical outcomes in conventional IVF without rescue ICSI. There are also few reports on the working environment and working hours in the laboratory.

Study design, size, duration: This was a retrospective study of 3,714 oocytes from 715 cycles in 595 patients who underwent Conventional IVF with egg retrieval in 2016-2022.

Long insemination (1,577 oocytes) was performed in cycles up to May 2019 and short insemination (2,137 oocytes) in later cycles.

In IVF, 40,000 motile sperm were placed in 1 ml of culture medium per oocyte.

Participants/materials, setting, methods: Oocytes of the long insemination group (group L) were inseminated 5 hours after egg retrieval and denuded 19 hours later to observe their pronuclei.

Oocytes from the short insemination group (group S) were inseminated 2 hours after egg retrieval, denuded 4 hours later, and cultured in EmbryoScope (Vitrolife, Sweden) to observe the pronuclei.

Since the working hours set at our clinic are 8:00 a.m.-5:00 p.m., the time schedule for both groups followed this schedule.

Main results and the role of chance: The 2PN rate was 60.9% in group L and 64.7% in group S. The 3PN rates were 7.5% and 10.2% respectively, both rates being significantly higher in group S ($P < 0.05$). The non-fertilization rates were significantly higher in group L (25.3% and 21.7% respectively); the 1PN rate was not significantly different (3.6% vs. 3.4%).

The rate of missed pronuclei was 2.8% in group L and 0% in group S.

Early embryo transfer pregnancy rate (25.4% vs 27.8%), good blastocyst development rate (52.2% vs 63.6%) and blastocyst transfer pregnancy rate (41.1% vs 31.7%) in groups L and S respectively.

The good blastocyst incidence was significantly higher in group S.

In the case of the Conventional IVF cycle in which egg retrieval was performed at 8:00 a.m., the embryologists in group L were to observe the pronuclei at 8:00 a.m. the next day, but in reality the embryologists had to come to work earlier because of denudation, and even then pronuclear loss occurred.

In contrast, the group S did not require an earlier attendance because denudation was not performed the next morning, and the time-lapse images did not fail to confirm the pronuclei at all.

Limitations, reasons for caution: Since the short insemination method was introduced at our clinic in June 2019, this study compared clinical outcomes before and after that date.

Wider implications of the findings: It has been reported in the past that early insemination time increases both 2PN and 3PN rates. The short insemination method in this study also used a faster insemination time, which may have led to similar insemination results.

Trial registration number: not applicable

Abstract citation ID: dead093.481

P-117 The reason why direct cleavage and rapid cleavage should be differentiated

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Study question: Direct cleavage (DC) and Rapid cleavage (RaC) both appear to be 1 cell divided into 3 (or more) cells, should they be distinguished?

Summary answer: Since blastomeres by RaC had higher developmental potential than blastomeres by DC, and since many RaC embryos had "normal" blastomere, the two should be distinguished.

What is known already: DC, in which one cell divides into three (or more) cells without the two-cell stage, and RaC, in which one or two cells divide rapidly after the short two-cell stage (The former lead to segregation of chromosomes into three cells, while the latter leads to inadequate DNA replication), are both often recorded as "DC", but when classified in detail, there are reports that the blastocyst development rate is higher in RaC embryos. However, the reason for this has not been clarified.

Study design, size, duration: This was a retrospective study of 643 embryos collected and cultured for at least 5 days in 2020 at our clinic. The embryos were time-lapse monitored by EmbryoScope (Vitrolife, Sweden) and classified into three groups according to the style of first division.

Participants/materials, setting, methods: The embryos were classified as follows: DC group: embryos that have divided into three (or more) cells without a two-cell stage; RaC group: embryos that rapidly (<5 hours) divided one (or both) cells after the 2-cell stage; Normal cleavage (NC) group: embryos that had a 2-cell stage for more than 5 hours. The subsequent development of blastomeres of all embryos was observed and whether they participated in blastocysts or not was recorded.

Main results and the role of chance: Of the embryos, 63 were in the DC group and 199 were in the RaC group (173 of which had one rapidly dividing blastomere and 26 of which had two). The blastocyst participation rate of blastomeres was 4.4% in the DC group, 19.5% of rapidly dividing blastomeres and 61.6% of normal blastomeres in the RaC group, with significant differences between all groups ($p < 0.01$). The good blastocyst (4BC or higher in Gardner grade) development rate was 4.8% (3/63) in the DC group, 40.5% (70/173) in the RaC group with one rapidly dividing blastomere (RaC1 group), 19.2% (5/26) in the RaC group with two such blastomeres (RaC2 group), showing a significant difference between the DC and RaC1 groups ($P < 0.01$). The live birth rate for single blastocyst transfer was 50% (1/2) in the DC group, 27.8% (5/18) in the RaC1 group, and 100% (1/1) in the RaC2 group. (Note that there were 381 in the NC group, and the above rates were 76.5%, 63.3% (241/381), and 29.3% (22/75), respectively)

Limitations, reasons for caution: This study examined only the style of first division and did not consider second and subsequent divisions. In addition, as of 2020, the clinical use of PGT-A is not approved in principle in Japan, and the subject embryos have not undergone chromosome analysis.

Wider implications of the findings: The reason for the higher blastocyst development rate in RaC embryos was indicated by the difference in blastocyst participation rates of the respective blastomeres and the presence of normal blastomeres, but since live births were obtained in both embryos, so these irregular divisions would not completely compromise the blastocyst euploidy.

Trial registration number: not applicable

Abstract citation ID: dead093.482

P-118 Are cumulus cells required during in vitro maturation of human oocytes at the germinal vesicle stage? a systematic review

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Study question: Does the presence of cumulus cells affect the *in vitro* maturation (IVM) rate of human oocytes at the germinal vesicle (GV) stage obtained in vivo?

Summary answer: The oocyte maturation and fertilization rates are higher in the presence of cumulus cells (CC) comparing with their absence in the culture system.

What is known already: According to the ESHRE guidelines IVM is currently not considered experimental, but nevertheless it is not very often used in reproductive medicine clinics. Accumulated data of more than 30 years of existence of IVM method can present to us how effective is the level of oocytes maturation *in vitro* obtained by aspiration follicles *in vivo*. Some studies reported that oocytes were matured after denudation, and in others they are cultured in cumulus oocyte complexes or in the presence of CC. Therefore, a comparison of oocyte maturation at the GV stage with and without CC was performed in this study.

Study design, size, duration: We searched Medline and Embase up to January 10, 2023. Full texts of classical articles, clinical studies, clinical trials, clinical trial protocols, comparative studies, controlled clinical trials, multicentre studies, randomized controlled trials were included

Participants/materials, setting, methods: Participants were women undergoing infertility treatment with IVM. All oocytes from the included studies were obtained by follicular aspiration *in vivo*.

Main results and the role of chance: A preliminary screening of the word combination "human oocyte in vitro maturation" yielded 355 results, from which 42 were original publications with full-text. Selected papers included a full description of the stimulation protocol, maturation medium, and the group of immature oocytes consisted of gametes only at the GV stage. A total of 24,649 GV oocytes were examined for IVM rate. The average of oocyte maturity was 61.5 ± 10.7%. However, the distribution of oocyte maturation data depending on the presence or absents of CC in the culture media. The maturation rate was higher in oocytes with cumulus cells than in denuded oocytes without cumulus (64.7% *vs* 53.5%, respectively, $p<0.05$). The fertilization rate had the same tendency (69.2% vs. 59.5%, $p<0.05$). However, there was no significant difference between the groups in embryo development up to the cleavage stage (74.9% vs 76.5%, $p > 0.05$). Unfortunately, there are very only a few studies that provide data on the more advanced stages of embryo development as a blastocyst, which does not allow a large sample to be made for any IVM method.

Limitations, reasons for caution: This study does not take into account the influence of stimulation protocols and the composition of the maturation medium, which may in turn also affect the results of oocyte IVM.

Wider implications of the findings: The obtained data can be used for comparison with new maturation protocols that will be aimed at increasing the maturation rates, in addition, these data are important for comparing the efficacy of maturation obtained from ovaries outside the body for the purpose of preserving fertility.

Trial registration number: not applicable

Abstract citation ID: dead093.483

P-119 Relation of graphical follicle models to the cumulative live birth rate in GnRH-antagonist stimulation treatment cycles

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Study question: Is it possible to create a model that combines follicle number and size to help physicians visualize the trigger requirements to predict oocyte utilization?

Summary answer: Findings support graphical follicle model as an innovative, simple, and practiced parameter for predicting clinical results in ovary stimulation treatment and facilitating personalized protocol adjustment.

What is known already: Previous studies have observed the number and size of follicles are two independent indicators of whether the oocyte is

adequate and mature and are used to predict the trigger time in routine clinical practice. However, due to individualized differences, it currently relies mainly on physician experience.

Study design, size, duration: This retrospective study included 8405 patients started their first in vitro fertilization cycle with a GnRH-ant protocol including fresh and subsequent frozen-thawed cycles during 2016–2020.

Participants/materials, setting, methods: We classified follicles recorded on the HCG day by size. We then produced graphical models, and classified into Inverted-trapezoid (large follicles in dominant proportion), Polygon (moderate follicles in dominant proportion), Trapezoid (small follicles in dominant proportion), Rectangle (equivalent proportions of the three size categories). The Cochran–Mantel–Haenszel and Generalized Linear Model(GLM) were used to evaluate the difference among models in relation to maturity, fertilization, and number of viable embryos, as well as cumulative live birth rate.

Main results and the role of chance: In GLM analysis, after adjusting the confounders, there are differences between models of CLBR. The CLBR of the different models was higher in the Polygon and Inverted-trapezoid model than Trapezoid and Rectangle model (42.75%, 39.56%, vs. 37.38%, 28.57%, respectively; all $P<0.05$). For oocytes derived from very large follicles (>20 mm), the CLBR was lower than that of patients with ≤ 20 mm follicles [26.10% vs. 42.10%, OR = 1.74 (95% confidence interval 1.52–2.00), $P<0.01$] in Inverted-trapezoid model, but there was no difference between models.

And the risk of ovarian hyperstimulation syndrome (OHSS) rate of patients with ≤ 20 mm follicles was lower than that of patients with >20 mm follicles [8.64% vs. 17.89%, OR = 0.57 (95%CI: 0.49−0.65), $P<0.01$] in Inverted-trapezoid model. Patients who received an adjusted Gn dose(whether Decreased or Increased-dose protocol) showed no difference in CLBR among models (Polygon vs Inverted-trapezoid vs Trapezoid vs Rectangle model: 47.07% vs. 49.21% vs. 47.69% vs. 42.42%, $P>0.05$); but when patients continued with the same starting dose in Fixed-dose protocol, the CLBR of the Polygon model was higher than that of other models (40.43% vs. 34.32, 31.13% vs. 26.46%, respectively; all P value <0.05).

Limitations, reasons for caution: This study is retrospective. Due to analysis of real-world data, the follicular corresponding outcome following measurement was not obtained. Besides, the majority of patients received 2 cleavage stage embryos transfer, this approach may impact the external validity of follicle model.

Wider implications of the findings: The follicle models can demonstrate ovarian response of patients. It is better for patients to reach the Inverted-trapezoid model with dominate follicles >18 mm and <20 mm. Adjusting the protocol is critical to the outcome. But how to improve follicle models by regulating stimulation remains to be further studied.

Trial registration number: not applicable

Abstract citation ID: dead093.484

P-120 Seasonal effects at the time of oocyte collection on the success of frozen embryo transfers

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Study question: Does season and duration of daylight hours at the time of oocyte collection impact on live birth rates following frozen embryo transfer?

Summary answer: Frozen embryo transfer following oocyte collection in summer had 30% increased odds of live birth compared to oocytes collected in autumn.

What is known already: Season and conditions at the time of fresh or frozen embryo transfer do not appear to impact live birth rates. Recent data from the northwest USA suggest increased live birth rates following frozen embryo transfer from oocytes collected in summer, independent of the season at the time of transfer.

Study design, size, duration: Retrospective cohort study of all frozen embryo transfers at a single centre in Western Australia from 2013 - 2021 for which oocyte collection also occurred between these dates. This study included 3,659 frozen embryo transfers with embryos generated from 2,155 IVF cycles in 1,835 patients. Data were analysed by season, temperature and recorded sunshine hours at the time of oocyte collection and embryo transfer.

Participants/materials, setting, methods: All transfers during the study period were included; demographics, IVF cycle characteristics, embryological data and clinical outcomes were collected from the clinic database. Weather data were recorded by the Australian Bureau of Meteorology. Statistical adjustment was performed for factors known to affect live birth rates, and we corrected for multiple cycles in the same patient and conditions at embryo transfer.

Main results and the role of chance: Compared to frozen embryo transfer (FET) with oocyte retrieval dates in autumn, FET with oocyte retrieval dates in summer had 30% increased odds of live birth (OR: 1.30, 95% CI: 1.04-1.62). Temperature at the time of oocyte retrieval did not affect LBR; there was a 28% increase in odds of live birth when the sunshine hours were in the highest tertile compared to the lowest on the day of oocyte retrieval (OR: 1.28, 95% CI: 1.06-1.53). These findings remained consistent when adjusted for season or sunshine hours on the day of FET respectively.

Odds of livebirth were decreased by 18% when the minimum temperature on the day of transfer was high compared with low (OR: 0.82, 95% CI: 0.69−0.99).

The duration of bright sunshine on the day of oocyte retrieval appeared to drive the seasonal variations, whereas ambient temperature was not associated with clinical outcomes.

Limitations, reasons for caution: This was a retrospective study, however study populations across seasons, temperature tertiles and sunshine tertiles were similar. We did not analyse subgroups of low prognosis patients. We also did not analyse environmental pollutants in this study, nor did we assess impacts on sperm quality. These may all affect the results.

Wider implications of the findings: Optimal conditions for livebirth appear to be associated with summer and increased sunshine hours on the day of oocyte retrieval. Consideration could be given to timing oocyte retrieval to optimise outcomes. The mechanisms remain to be clarified, though may involve melatonin and its impact on oocyte quality.

Trial registration number: 2022/ET000980

Abstract citation ID: dead093.485

P-121 Identification of differentially expressed genes common to the developmental potential, maternal age, and morphology in human blastocysts

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Study question: Is it possible to apply RNA-sequencing to assisted reproductive technology (ART) to predict blastocyst quality?

Summary answer: We identified 14 commonly identified differentially expressed genes by grouping blastocysts according to three distinct parameters such as developmental potential, maternal age, and Gardner score

What is known already: In ART, while the selection of a suitable embryo for transfer is critical for a successful pregnancy, neither preimplantation genetic testing nor morphological and chronological evaluation of the embryo can fully guarantee a successful pregnancy. Recently, transcriptional events in early human embryonic development have been analyzed using RNA-sequencing (RNA-seq) and researchers are attempting to apply this information to ART. We have reported that 96 differentially expressed genes (DEGs) were identified using RNA-seq of each inner cell mass (ICM) and trophectoderm (TE) in blastocysts classified according to the developmental potential which correlates with pregnancy rate at the ESHRE 37th annual meeting.

Study design, size, duration: After retrospectively analyzing 1,890 cases undergoing freeze-thaw blastocyst transfer from March 2018 to December 2020 to examine the correlation between blastocyst developmental potential and pregnancy rate, a total 13 blastocysts cryopreserved between February 2011 and September 2018, then scheduled for disposal and with consented, were subjected to RNA-seq to identify genes associated with pregnancy expectation. RNA-seq data were then examined whether common DEGs could be found when classified by maternal age and Gardner score, respectively.

Participants/materials, setting, methods: Blastocysts were donated by infertile couples undergoing c-IVF or ICSI cycles at the Yamashita Shonan Yume Clinic with informed consent under ethical approval. TE cells and ICM cells were collected from blastocysts classified by developmental potential and subjected to RNA-seq to identify DEGs. In addition, RNA-seq data were regrouped by maternal age and Gardner score to find common DEGs. DEGs (q-value < 0.01) were identified using the R package "DESeq2" (version 1.32.0).

Main results and the role of chance: When the RNA-seq data obtained from blastocysts classified according to pregnancy expectation were re-grouped by maternal age and re-analyzed, we identified 7 and 17 genes that were down- and up-regulated in the elder group, respectively, in ICM. In TE, 2 and 12 genes were down- and up-regulated in the elder group, respectively. On the other hand, when re-grouped by Gardner score (<BB versus <BB), we identified 26 and 29 genes that were down- and up-regulated in the poor group, respectively, in ICM. In TE, 64 and 20 genes were down- and up-regulated in the poor group, respectively. A comparison of these genes and DEGs identified by the pregnancy expectation found 14 common genes. By comparison with the elder group, we identified one gene (*UCHL1*) that was commonly up-regulated in TE. By comparison with the poor Gardner score group, one gene (*CMTM7*) was commonly down-regulated and three genes (*LINC00458*, *DYNLRB1*, and *UBL4A*) were commonly up-regulated in ICM, and 5 genes (*LRIG3*, *SLC28A3*, *CXADR*, *LOC727896*, and *ATP1B1*) were commonly down-regulated and 5 genes (*UCHL1*, *BZW2*, *NABP1*, *HEXIM1*, and *PLEKHA6*) were commonly up-regulated in TE.

Limitations, reasons for caution: Although we established an expected pregnancy rate concerning the degree of blastocyst development from retrospective clinical outcomes and used it as a surrogate marker for assigning biopsied blastocysts to different analysis groups, it remains unknown whether the gene expression profiles accurately reflect the pregnancy outcomes.

Wider implications of the findings: *UCHL1* expression was commonly increased with lower blastocyst developmental potential, higher maternal age, and lower Gardner score. Since *UCHL1* has been reported to be essential for blastocyst development in mice, our results suggest that *UCHL1* may also be a marker of blastocyst quality in humans.

Trial registration number: not applicable

Abstract citation ID: dead093.486
P-122 Evaluation of an updated artificial intelligence embryo viability model on implantation and miscarriage

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Study question: Is the implantation and miscarriage prediction performance of the iDAScore model v2.0 increased as compared to the original v1.0 model?

Summary answer: The predictive performance is better for iDAScore v2.0 than for v1.0 on both implantation and miscarriage, although the differences did not reach statistical significance.

What is known already: In recent years, several artificial intelligence (AI) models for the prediction of embryo implantation based on image or time-lapse data have been published and validated in various ways. Some of these models are commercial products while others are more of an academic nature. The long-term benefit of AI models in clinical practice relies on their use in clinical routine and that the models are continuously monitored and updated. The iDAScore time-lapse model has been available from Vitrolife since 2021 and several external validations of iDAScore v1.0 have been published. An updated version (iDAScore v2.0) has recently been released.

Study design, size, duration: This was an observational retrospective study with data collected during the years 2019 to 2022 from one Danish clinic from fresh single embryo transfers performed on day 5. Blastocysts were selected for transfer by experienced embryologists based on morphology (Gardner score). Within the study period, we included all 849 embryos for which we had information on hCG test results, implantation (fetal heartbeat (FH) detected by ultrasound) and live birth (LB).

Participants/materials, setting, methods: Embryos were cultured in an EmbryoScope+ incubator (Vitrolife A/S). Time-lapse images were automatically captured throughout the incubation period. We used the original time-lapse images to calculate scores for iDAScore v1.0 and v2.0 retrospectively. For each version, the predictive performance for miscarriage (early pregnancy loss and clinical miscarriage), FH and LB prediction was expressed in terms of area under the ROC curve (AUC). Differences in performance between versions were assessed in paired comparisons of the AUCs.

Main results and the role of chance: The median age of the transfer recipients was 34 years. One third of the blastocysts was derived from ICSI and the remaining from standard IVF cycles. Among all transferred blastocysts, the hCG rate was 58.0% (492/849), the FH rate was 46.5% (395/849), the LB rate was 42.4% (360/849), and the overall miscarriage rate from hCG to live birth was 26.8% (132/492).The overall AUC for FH prediction was 0.652 (0.616 to 0.689) for iDAScore v2.0 compared to 0.632 (0.595 to 0.669) for v1.0. Similarly, the AUC for LB prediction was 0.644 (95% CI: 0.607 to 0.681) for iDAScore v2.0 compared to 0.626 (95% CI: 0.588 to 0.663) for iDAScore v1.0. However, these differences were not statistically significant (P-values: 0.12 for FH and 0.17 for LB) in paired comparisons.For miscarriage, we found an increase in AUC from v1.0 (0.574; 95% CI: 0.514 to 0.633) to v2.0 (0.592; 95% CI: 0.534 to 0.649), P-value: 0.40.

Limitations, reasons for caution: This study only includes data from a single clinic. Patient characteristics and procedures followed might not be representative for other clinics. The data is limited to blastocysts that were chosen for transfer by morphology from trained embryologists. Furthermore, the limited sample size does not provide statistically significant differences in performance.

Wider implications of the findings: It is important that predictive models are constantly updated and improved. Updated versions should take into account a larger database as well as potential new methodologies that evolve in the rapidly developing AI field. This study shows that the iDAScore model update seems to represent an actual performance increase.

Trial registration number: 1-16-02-277-22

Abstract citation ID: dead093.487
P-123 Early irregular division of human embryos changes in style between the first and second divisions

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Study question: Is there a difference in irregular division kinetics between first and second division of human embryos?

Summary answer: The incidence of rapid cleavage was reduced in the second division, but the developmental potential of the blastomeres by this dynamics was unexpectedly low.

What is known already: The styles of irregular division of early human embryos are known: direct cleavage (DC), in which one cell becomes three (or more) cells without a two-cell phase, and rapid cleavage (RaC), in which one (or both) cells dividing rapidly after two-cell phase, both of which reduces the embryonic developmental potential. However, there are no reports that have examined in detail whether these division styles occur similarly in the first and second divisions, and how these dynamics affect the potential.

Study design, size, duration: This is a retrospective study of 635 embryos collected in 2020 at our clinic and cultured for at least 5 days. The embryos were time-lapse monitored by EmbryoScope (Vitrolife, Sweden) to observe the first and second divisions, and the blastomeres of each embryo were classified according to their style of division.

Participants/materials, setting, methods: It was observed whether there was a DC (division into 3 cells without a 2-cell phase) or RaC (One or two blastomeres divide within 5 hours after the 2-cell phase) at the first division. First dividing normal embryos were observed for the presence of a DC or RaC at the second division. Blastomeres by DC or RaC were observed for subsequent development and whether they participated in blastocysts or not was recorded.

Main results and the role of chance: Among the subject embryos, 63 embryos had DC and 199 embryos had RaC in the first division, and their blastomeres were classified into the DC1 and RaC1 groups, respectively. Of the 373 first division normal embryos, 39 had DC and 23 had RaC (there were 4 embryos with both DC and RaC) in the second division, and their blastomeres were classified into DC2 and RaC2 groups. The blastomeres blastocyst participation rate was 4.4% in the DC1 group and 19.5% in the RaC1 group, respectively, the former was significantly lower ($P < 0.01$), however, in the DC2 and RaC2 groups, 23.3% and 4.2%, respectively, the latter was significantly lower ($P < 0.01$). The incidence of DC was similar in the first (9.9%) and second (10.5%) divisions, but the incidence of RaC was 31.3% in the first division and 6.2% in the second division, the latter was significantly lower ($P < 0.01$). The development rate of good blastocysts (≥4BC in Gardner grade) was 4.8% in embryos with DC1 and 19.5% in embryos with RaC1, with the former significantly lower ($P < 0.01$), 37.0% in embryos with DC2, and 26.3% in embryos with RaC2, with no significant difference.

Limitations, reasons for caution: As of 2020, the clinical use of PGT-A is not approved in principle in Japan, and the subject embryos have not been chromosomally analyzed.

Wider implications of the findings: RaC would lead to inadequate DNA proliferation, but the incidence of this and the prognosis of the blastomeres were different in the first and second divisions. In human embryos, the mechanisms that control blastomeres from dividing may change as development progresses, but further studies are needed to elucidate this.

Trial registration number: not applicable

Abstract citation ID: dead093.488

P-124 Behavior of the second polar body and its relationship to subsequent human embryonic development

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Study question: Is the behavior of the second polar body (PBII) associated with subsequent human embryonic development?

Summary answer: Human zygotes with morphologically static PBII showed significantly higher rates of good quality embryos and utilization than zygotes with PBII showing various behaviors.

What is known already: In recent years, the time-lapse incubators have become widely available, enabling continuous observation of human embryos. With the time-lapse imaging, zygotes with second polar bodies that exhibit a variety of behaviors can be observed from the time of PBII extrusion to the first cleavage. Although the ploidy and morphology of the first polar body have been variously reported to reflect the quality of embryos after fertilization, there are few studies on the second polar body, which is thought to directly reflect the state of oocyte after sperm entry, and its relationship to the embryonic development.

Study design, size, duration: This is a retrospective study involving the time-lapse imaging of 285 normally fertilized ICSI zygotes between January and August 2019. Of those, 262 showing suitable images of PBII from the extrusion to the first cleavage were examined. Zygotes with morphologically static PBII during the observation were classified into Group 1 (n = 68), and zygotes with moving-like-amoeba, shrinking, fragmenting, and/or ruffling PBII were classified into Group 2 (n = 194).

Participants/materials, setting, methods: This study included 64 cycles of ART treatment. Gonadotrophin-releasing hormone analogues (GnRHa) and gonadotrophins were used for ovarian stimulation prior to the oocyte retrieval. Time-lapse imaging was performed in EmbryoScope®. The rates of irregular divisions at the first cleavage, good quality embryos at the early cleavage stage based on the modified Veeck's criteria, good quality blastocysts (≥4BB) based on the Gardner grading system and utilization for cryopreservation and embryo transfer were compared between groups.

Main results and the role of chance: The proportion of Group 1 (morphologically static PBII) and Group 2 (PBII with various behaviors) was 26.0% (n = 68/262) and 74.0% (n = 194/262). The mean maternal age (years) was 34.8 ± 5.4 in Group 1 and 35.0 ± 4.8 in Group 2. The incidence of irregular (direct/reverse) divisions at the first cleavage was 22.1% in Group 1 and 28.9% in Group 2. Good quality embryos at the early cleavage stage were cryopreserved/transferred on Day-2/3 according to our clinic policy, and only those that were not cryopreserved or freshly transferred on Day-2/3 were extendedly cultured up to Day-7. Of those, the rate of good quality blastocysts was 30.6% (n = 11/36) in Group 1 and 23.3% (n = 30/129) in Group 2. Thus, there were no significant differences between Group 1 and 2 in the mean maternal age and the rates of irregular divisions and good quality blastocysts. Meanwhile, the rate of good quality embryos at the early cleavage stage was significantly higher in Group 1 than in Group 2 [$P<0.01$; 57.4% (n = 39/68) in Group 1, 37.1% (n = 72/194) in Group 2]. The utilization rate for cryopreservation and embryo transfer was also significantly higher in Group 1 than in Group 2 [$P<0.05$; 64.7% (n = 44/68) in Group 1, 49.5% (n = 96/194) in Group 2].

Limitations, reasons for caution: This is an observational study using only ICSI zygotes. Since good quality embryos were cryopreserved or freshly transferred on Day-2/3 according to our clinic policy, the number of embryos cultured up to the blastocyst stage was limited to those which were fair or poor quality at the early cleavage stage.

Wider implications of the findings: This study revealed that the rates of good quality embryos and the utilization for cryopreservation or embryo transfer were significantly higher in zygotes with morphologically static PBII than in those with PBII showing various behaviors. This suggests that the behavior of PBII may be a new indicator for embryo evaluation.

Trial registration number: not applicable

Abstract citation ID: dead093.489

P-125 Day 5 endometrium, Is the pregnancy rate following day 5 or day 6 vitrified/thawed blastocysts transfer affected?

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Study question: Are ongoing pregnancy rates of embryos vitrified on day 5 and day 6 when transferred into a day 5 endometrium comparable?

Summary answer: Day 5 blastocysts implant better in day 5.5 endometrium than day 6, whether they were vitrified on day 6 or 5 and cultured 24 hours.

What is known already: Preparation of the endometrium for transfer of cryopreserved embryos is usually done so that transfer takes place on endometrial day 5, although embryos may be at day 5 or 6 of development, so there may be asynchrony between the endometrium and the embryo if a day 6 embryo is transferred. Fresh transfer of day 5 blastocysts has been reported to have better results than day 6 blastocysts, predictably due to this endometrial asynchrony. However, data from frozen blastocyst transfers are less

clear, possibly due to heterogeneity of the patient population and/or embryo quality.

Study design, size, duration: In this study we aim to analyse whether ongoing pregnancy after vitrified/thawed embryo transfers is affected if day 5 or day 6 embryos are transferred into day 5 endometrium. For this purpose, we examined 2266 cryotransfers performed in three Next Fertility clinics in Spain between 2016 and 2022. Day 5 (n = 1583) and 6 (n = 519) blastocyst transfers and day 5 embryos that devitrified one day before transfer (n = 164) were retrospectively compared.

Participants/materials, setting, methods: In this work blastocyst transfers were performed from donor or autologous oocytes, with or without PGT-A. Embryos were vitrified on day 5 or 6. Endometria were prepared with hormone replacement therapy or in natural cycle. All FET were performed after 5.5 days of progesterone exposure, regardless of embryo stage (day 5 or 6). The results were analysed considering all the above groups.

Main results and the role of chance: Ongoing pregnancy are superior with day 5 blastocysts versus day 6 and day 5 cultured for 24 hours prior to transfer. However, day 6 blastocysts were similar to cultured ones.

Own oocytes: Day 5 44.98% (n = 538) vs Day 6 35.75% (n = 207) ($p < 0.03$); Day 5 44.98% vs Day 5 cultured 32.58% (n = 89) ($p < 0.03$); Day 6 35.75% vs Day 5 cultured 32.58% ($p > 0.05$).

Donated Oocytes: Day 5 45.86% (n = 726) vs Day 6 28.51% (n = 221) ($p < 0.00001$); Day 5 44.98% vs Day 5 cultured 39.34% (n = 61) ($p > 0.05$); Day 6 28.51% vs Day 5 cultured 39.34% ($p > 0.05$).

PGT-A Own oocytes: Day 5 51.72% (n = 203) vs Day 6 25.39% (n = 63) ($p < 0.003$); Day 5 51.72% vs Day 5 cultured 40.00% (n = 10) ($p > 0.05$); Day 6 25.39% vs Day 5 cultured 40.00% ($p > 0.05$).

PGT-A Donated Oocytes: Day 5 42.24% (n = 116) vs Day 6 35.71% (n = 28) ($p > 0,05$); Day 5 42.24% vs Day 5 cultured 40.00% (n = 4) ($p > 0.05$); Day 6 35.71% vs Day 5 cultured 50.00% ($p > 0.05$).

Miscarriage rates were similar across all groups.

Limitations, reasons for caution: Not all cohorts in the study were equally large, so some results have less statistical power, especially in abortion rates.

Wider implications of the findings: Day 5 blastocysts should always be transferred into a 5.5-day endometrium on the day they are devitrified, as this is when they are most synchronous with the endometrium. Further studies are needed to assess whether day 6 embryos implant better in a 6.5-day endometrium than in a 5.5-day endometrium.

Trial registration number: not applicable

Abstract citation ID: dead093.490

P-126 The KAT-Score is a useful indicator for predicting ongoing pregnancy in mosaic risk assessment in PGT-A: a prospective study with single vitrified-warmed blastocyst transfer

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Study question: Does the mosaic risk score assessed by the KAT-Score correlate with clinical pregnancy and ongoing pregnancy?

Summary answer: A lower mosaic risk score assessed by the KAT-Score correlated with a higher ongoing pregnancy rate, but not with the clinical pregnancy rate.

What is known already: As the result of preimplantation genetic testing for aneuploidy (PGT-A), the result of analysis for mosaicism is obtained in addition to those for euploidy and aneuploidy. Viotti, *et al.* retrospectively analyzed 1000 mosaic embryo transfers, and showed that the rates of pregnancy and live birth differentiated stepwise depend on the type and level of mosaicism. Although it is possible to determine to the priority of embryo transfer according to the type of mosaicism, no prospective studies have been reported in which embryo transfer was performed using this method of selection.

Study design, size, duration: This is a prospective study conducted in a single IVF center between January 2020 and August 2022. A total of 104 single vitrified-warmed blastocyst transfer cycles were analyzed. The scoring of PGT-A embryos was assessed using the knowledge-based aneuploidy theoretical score (KAT-Score: Varinos Inc., Japan) in the range of 0 to 10. This study was approved by the Ethics Committee of the Institutional Review Board (IRB) of Kinutani Women's Clinic, Hiroshima, Japan.

Participants/materials, setting, methods: The results of 104 vitrified-warmed single blastocyst transfers were classified into pregnancy (54 cycles) and non-pregnancy (50 cycles) groups. They were also classified into ongoing pregnancy (47 cycles) and non-ongoing pregnancy (57 cycles) groups. The correlations between KAT-Score and clinical pregnancy/ongoing pregnancy rates, were analyzed using the Wilcoxon signed-rank test and logistic regression analysis.

Main results and the role of chance: The KAT-Scores for 104 blastocysts ranged from 0 to 9. The distribution of scores is (KAT-Score 0: n = 43; 1-2: n = 21; 3-4: n = 4; 4-5: n = 16; 5-6: n = 7; 6-7: n = 5; 7-8: n = 6; 9: n = 2). First, mean values were compared between two different groups by use of the Wilcoxon signed-rank test. No significant difference in KAT-Score was observed between the pregnancy group and the non-pregnancy group (1.81 ± 2.42 vs. 2.78 ± 2.87; N.S.). On the other hand, the KAT-Score in the ongoing pregnancy group, was significantly lower than that in the non-ongoing pregnancy group (1.34 ± 1.98 vs. 3.04 ± 2.94; $P < 0.01$). Second, binary logistic regression analysis was used to calculate the area under the curve (AUC) as a predictive measure of clinical pregnancy or ongoing pregnancy. The KAT-Score assessment of PGT-A embryos was a predictive indicator of ongoing pregnancy (AUC = 0.67: $P < 0.01$), but not of clinical pregnancy (AUC = 0.59: N.S.). Multivariate logistic regression analysis, which included maternal age and morphological grade as confounding factors, showed that lower KAT-Scores correlated significantly with higher ongoing pregnancy rates (adjusted odds ratio: 0.77, 95% CI: 0.64–0.93, $P < 0.01$). In addition, the ongoing pregnancy rate from embryo transfer with euploid or mosaic embryos was not affected by maternal age or morphological grade.

Limitations, reasons for caution: The main limitation of this study is small sample size. Further research is required to gain a more complete understanding of whether the KAT-Score is associated with pregnancy and ongoing pregnancy.

Wider implications of the findings: This study suggests that the KAT-Score may be used as one of the indicators to predict ongoing pregnancy regardless of maternal age and morphological grade. The scoring of 104 embryo transfers by KAT-Score provides statistically valid evidence for ranking mosaic embryos in the infertility clinic.

Trial registration number: not applicable

Abstract citation ID: dead093.491

P-127 Micro-Magnetic Resonance Spectroscopy of individual mammalian embryos: a safe and non-invasive diagnostic tool for embryo screening in assisted reproduction

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Study question: Is micro-magnetic resonance (micro-MRS) a safe tool to non-invasively unravel the metabolic fingerprint of single mammalian embryos?

Summary answer: We successfully tested the safety of micro-MRS in a mouse model. No long or short-term adverse effect was found in vitro and in vivo.

What is known already: Non-invasive selection of the best embryo to transfer is one of the most significant challenges for ART professionals. The chemical sensitivity, resolving power, and, more importantly, the non-invasive nature of MRS makes it an excellent candidate to investigate the building blocks of complex organisms. Although MRS is a well-established technique for the biochemical profiling of large organisms, handling small samples like embryos and 3D cell cultures and spheroids alongside sensitivity issues has prevented its adoption for clinical and research applications. Our group has

overcome these limitations with microchip-based sensors to leverage non-invasive MRS technology down to the embryo scale.

Study design, size, duration: This safety study was divided into two main phases in order to test all the components needed to operate the micro-MRS analysis. In phase 1 we used >800 2-cell embryos to test a) radiofrequency exposure, b) device activation, and c) static magnetic field exposure. In phase 2 we confirmed in-vivo that MF exposure was not affecting live animals (n267) over 3 generations of mice by assessing different IVF outcomes, natural mating, live parameters and histopathology.

Participants/materials, setting, methods: Cryopreserved 2-cell B6D2F1 mouse embryos were purchased cryopreserved from JanvierLabs. In phase1 embryos were thawed and used for testing micro-MRS main components' safety. Mouse embryo assay (MEA) was used to first assess micro-MRS adverse effects in-vitro. In phase 2 embryos were exposed to magnetic field in a 9.4T Magnet for 1h at 37 °C, then surgically transferred to surrogate mothers. Both mothers and progeny were tracked up to F3. Statistical significance was set at $p < 0.05$.

Main results and the role of chance: This is the first study ever conducted to assess the safety and efficacy of micro-MRS on pre-implantation mammalian embryos. Our results show, with statistical significance, that micro-MRS is safe for use in mammalians. In particular, we have de-risked the three main aspects/components that could affect the embryos: 1) Materials to which the embryos are exposed when loaded into the device, 2) Radiofrequency energy required to activate the device and perform a measurement, and 3) Magnetic field required to analyze the embryos biochemistry in a non-invasive way. Overall no adverse effect was observed when the primary IVF outcomes were assessed, such as implantation rate and live birth rate. In addition, no visible alteration of the overall appearance of ED14.5 foetuses, F1, F2 and F3 mice was observed when comparing the mice obtained from treated or control embryos. The surrogate mothers did not display any significant alteration or visible health issues after embryo transfer. F1 and F2 mice were able to naturally mate and conceive new progeny suggesting that the reproductive potential of the animals is not affected by the magnetic field exposure. Furthermore, histopathological analysis of 5 representative organs did not reveal adverse effects on both surrogate mothers or F1 pups.

Limitations, reasons for caution: A vital step towards establishing MRS as a clinical and research tool is the reliable detection of a wide range of signals from cellular components. Our method is ready for R&D studies, while its clinical application requires further safety studies and protocol optimization.

Wider implications of the findings: A non-invasive quick assay would provide the means to reveal the role of embryonic lipids throughout development. Micro-MRS can further develop into a safe embryo assay for selection before embryo transfer. This would apply to both human and animal ART, whose success rate is relatively low.

Trial registration number: NA

Abstract citation ID: dead093.492
P-128 Morphokinetics and blastocyst biomarkers of twin and triplet monozygotic pregnancy using artificial intelligence

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Study question: *Can AI* reduce the rate of twin and triplet monozygotic pregnancies following elective single embryo transfer (eSET)?

Summary answer: AI can automatically annotate morphokinetic developments and other biomarkers. Embryos leading multiple monozygotic pregnancies have slower cell divisions and larger ICM than monoamniotic embryos.

What is known already: Monozygotic twin (MZT) and monozygotic triplet (MTP) pregnancies are a rare phenomenon in spontaneous pregnancies, but the incidence increases significantly when pregnancies are achieved by assisted reproductive technology (ART); 0.4% vs. 1.56% MZTs, and 0.004% vs. 0.048% MTPs. It is unclear what mechanisms cause an embryo to split into two or three, although several have been proposed such as culture to blastocyst, decompacting ICMs, frozen-warmed embryo transfers, assisted hatching and even ICSI. In order to study this split phenomenon, time-lapse imaging has been used to discover any signs of embryo division.

Study design, size, duration: This is a retrospective assessment of a total of 8 embryos from 2018 to 2022 that led to single pregnancy (n = 4), twin pregnancy (n = 3) and triple pregnancy (n = 1) following a fresh single embryo transfer. All embryos were inseminated using the ICSI technique and assisted hatching was performed before fresh transfer.

Participants/materials, setting, methods: Using CHLOE (Fairtility, Tel Aviv) we automatically assessed the morphokinetics, blastocyst biomarkers and scores. Singletons and multiple pregnancies were compared in terms of morphokinetics (t-test) and surface area of ICM, ICM diameter, ICM area/embryo area ratio, ICM shape and CHLOE embryo quality score were compared using the ANOVA test.

Main results and the role of chance: Embryos that led to multiple pregnancies had slower embryo development than embryos that led to singletons (single vs multiple: t2: 22.1+-2 vs 25.2+-2, p = 0.04; t3: 32.56+-2.65 vs 36.98 +-1.48, p = 0.01; t5: 44.09+-4.84 vs 50.37+−0.69, p = 0.02; t6: 47.26+-3.87 vs 53.59+-2.40, p = 0.01; t7: 48.76+-4.04 vs 55.15+-3, p = 0.02; t8: 50.18+-4.27 vs 57.55+-1.5, p = 0.008; t9: 63.27+-3.76 vs 73.04+-5.33, p = 0.03). Embryos leading to triplets were slower than twins which were, in turn, slower than singletons (single vs twins vs triplets: t4: 33.86+-3.41 vs 37.69+-1.61 vs 48.95, p = 0.007; t8: 50.18+-4.27 vs 58.07+-1.29 vs 55.96, p = 0.04; t9: 63.27+-3.76 vs 71.23+-4.81 vs 78.44, p = 0.01).

Embryos that led to multiple pregnancies had a larger ICM to embryo surface area ratio (single vs multiple: 0.14+−0.08 vs 0.27+−0.06, p = 0.04) and smaller embryo diameter (single vs multiple: 177.2+-16.5 vs 138.15+-8.25, p = 0.003).

We didn't find statistically significant differences between the groups in CHLOE EQ score (single vs multiple: 0.97 vs 0.76, p = 0.36), Blast score (single vs multiple: 0.87+−0.06 vs 0.78+−0.13, p = 0.26), CHLOE Rank, Trophectoderm quality, ICM Area (single vs multiple: 3629+-1361 vs 4151 +- 569, p = 0.48) and ICM shape (single vs multiple: 1.64+−0.45 vs 1.27+−0.12, p = 0.15).

Limitations, reasons for caution: The main limitation of this study is the number of cases included in the study, as we studied one triplet, 3 twins and 4 single pregnancies.

Wider implications of the findings: This might be the first time that AI has been used to analyse the behaviour of embryos resulting in multiple pregnancies. The transfer of a slowly dividing embryo and/or with a large ICM could result in a multiple pregnancy.

Trial registration number: not applicable

Abstract citation ID: dead093.493
P-129 Morphokinetic embryo behavior in oocyte presenting with dimorphisms: an analysis of 1158 injected oocytes

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Study question: Are morphokinetic embryo behavior and embryo quality as per Known Implantation Diagnosis Score (KIDScore) assessment related to oocyte dimorphism in intracytoplasmic sperm injection (ICSI) cycles?

Summary answer: Morphokinetic embryo behavior and embryo quality as per KIDScore assessment are positively related with oocyte dimorphisms, which was demonstrated by delayed cell cleavage and blastulation.

What is known already: As a routine procedure in intracytoplasmic sperm injection (ICSI) cycles, denudation of retrieved oocytes allows the determination of maturation status and the assessment of morphological features of the cytoplasm, perivitelline space (PVS) and zona pellucida. Oocytes presenting extra and intracytoplasmic dimorphisms have been correlated to impaired embryo developmental potential and implantation. Time-lapse imaging (TLI) systems allow for the mapping of morphological changes or events with the exact time-point of occurrence. The aim of this study was to investigate the relationship between oocyte dimorphisms an embryo morphokinetic events.

Study design, size, duration: This cohort study, performed in a private university-affiliated IVF center from Mar/2019-Dec/2021, included 156 ICSI cycles and 1158 injected oocytes cultured in a TLI incubator (EmbryoScope^{+}). The presences of intracytoplasmic dimorphisms (granulation clusters, smooth endoplasmic reticulum (SER), dark cytoplasm and vacuoles), and extracytoplasmic dimorphisms (large perivitelline space (PVS), PVS granulation, polar body (PB) fragmentation and abnormal zona pellucida) were recorded, and their potential association with embryo morphokinetic events were investigated considering clustering of data.

Participants/materials, setting, methods: Evaluated kinetic markers were timing to pronuclei appearance (tPNa) and fading (tPNf), to two, three, four, five, six, seven, and eight cells (t2 - t8), to morulation (tM), start of blastulation (tSB) and blastulation (tB). Durations of the second (t3-t2) and third (t5-t3) cell cycles (cc2 and cc3) and timing to complete synchronous divisions s1 (t2-tPNf), s2 (t4-t3), and s3 (t8-t5) were calculated. The KIDScore ranking was recorded. The post hoc power was $>$ 95.0%.

Main results and the role of chance: Morphologically normal oocytes (n = 747) reached tPNa (6.0 ± 0.1h vs. 6.4 ± 0.1h), t3 (36.5 ± 0.3h vs. 37.5 ± 0.2h), t6 (50.9 ± 0.5h vs. 52.1 ± 0.3h), tM (88.3 ± 0.8h vs. 91.6 ± 1.2h), tSB (97.4 ± 0.8h vs. 108.8 ± 3.6h), tB (104.7 ± 0.8h vs. 109.3 ± 0.5h) and cc2 (10.0 ± 0.3h vs 10.7 ± 0.2h) significantly faster than oocytes showing intracytoplasmic dimorphisms (n = 411). Significantly faster t5 (48.4 ± 0.3h vs. 50.5 ± 0.8h), t6 (51.5 ± 0.3h vs. 53.0 ± 0.7h), t7 (54.0 ± 0.3h vs. 57.1 ± 0.8h), t8 (57.9 ± 0.4h vs. 60.5 ± 0.9h), tB (107.9 ± 0.5h vs. 113.2 ± 1.1h) and cc3 (11.6 ± 0.2h vs. 13.5 ± 0.5h) were observed in normal oocytes compared to oocyte showing extracytoplasmic dimorphisms (n = 162). The incidence of extracytoplasmic dimorphisms per cohort was associated with decreased implantation (B: −23.9, CI: −37.8 – −10.1) and odds of pregnancy (OR: 0.360, CI: 0.163 – 0.796). KIDScore ranked significantly different between normal oocytes and those presenting ZP (3.8 ± 0.8 vs. 2.7 ± 0.8) and shape dimorphisms (4.1 ± 0.7 vs. 2.4 ± 1.0), and non-resistant membranes (4.4 ± 0.1 vs. 2.8 ± 0.1).

Limitations, reasons for caution: Despite the use of a nested statistical analysis that accounted for the fact that embryos from the same patient share comparable developmental behavior, this study has limitations, such as the retrospective design and small sample size, though an adequate power has been achieved.

Wider implications of the findings: This study adds to knowledge of oocyte quality role in embryo development. Dimorphic oocytes may have inefficient biological machinery. Significant differences, that could not have been noticed in a conventional incubator, were observed in embryo morphokinetic development when normal and abnormal oocytes were compared.

Trial registration number: N/A

Abstract citation ID: dead093.494

P-130 Progesterone-primed cycles result in slower embryos without compromising the implantation potential, with the advantage of oral administration and potential cost reduction: A time-lapse imaging study

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Study question: Could exogenous progestin replace the use of an antagonist of GnRH (GNRH-antagonist) without any effects on embryo morphokinetics and implantation rate in freeze-all cycles?

Summary answer: Exogenous progestin leads to slower embryo development and increased cyclés cancellation rate, with increased implantation rate when compared to cycles using an GnRH-antagonist.

What is known already: Improvements in cryopreservation techniques associated with the expansion of elective-single embryo transfer have steadily increased the use of deferred embryo transfers. This gives the opportunity to break away from the standard sequence of stimulation–retrieval–transfer, and to consider new strategies for pharmacological control of follicle growth. Usual ovarian stimulation regimens use an analog of the GnRH to prevent the LH surge and premature ovulation. Since progesterone is able to block the LH surge question remains on whether exogenous progesterone may replace the use of an GnRH analogue without compromising embryo development, in cycles followed by embryo cryopreservation.

Study design, size, duration: This cohort study, performed in a private university-affiliated IVF center, from Mar/2019 - Mar/2021, included 288 freeze-all ICSI cycles and its 2,768 respective embryos. Patients were age matched, according to the age, into groups depending on the protocol used to prevent the LH surge: Progestin-Primed-Group (n = 144 cycles and 1,360 embryos) and GnRH-Antagonist-Group (n = 144 cycles and 1,408 embryos). Embryos were cultured in a time-lapse imaging incubation system (TLI) and embryo morphokinetics were compared among the groups

Participants/materials, setting, methods: Evaluated kinetic markers were timing to pronuclei appearance (tPNa) and fading (tPNf), to two, three, four, five, six, seven, and eight cells (t2 - t8), to morulation (tM), start of blastulation (tSB) and blastulation (tB). Durations of the second (t3-t2) and third (t5-t3) cell cycles (cc2 and cc3) and timing to complete synchronous divisions s1 (t2-tPNf), s2 (t4-t3), and s3 (t8-t5) were also calculated. The KID-Score ranking, laboratorial and clinical outcomes were also evaluated.

Main results and the role of chance: Mean maternal and paternal ages were 37.0 ± 3.8 and 39.0 ± 6.4 years old. Slower tPNa (6.2 ± 0.2 vs. 7.0 ± 0.2, p = 0.008), t2 (27.2 ± 0.3 vs. 26.2 ± 0.3, p = 0.045), t7 (56.4 ± vs. 54.7 ± 0.5, p = 0.046), tM (89.3 ± 0.8 vs. 87.1 ± 0.6, p = 0.045), tSB (101.5 ± 0.8 vs. 110.8 ± 0.1, p = 0.012), tB (111.0 ± 0.8 vs. 108.5 ± 0.7, p = 0.034), were observed among embryos derived from Progestin-Primed cycles. Similar tPNf, t3, t4, t5, t6, t8, timing to cc2, cc3, s1, s2, and s3 were observed among the groups. No significant differences were noted in the Kid-score D5, number of aspirated follicles, retrieved oocytes, oocyte yield, mature oocytes, and rates of mature oocytes, fertilization, and blastocyst formation. The cyclés cancelation (15.7% vs. 2.0%, p = 0.004) and implantation rates (63.1% ± 6.1 vs. 44.4% ± 6.3, p = 0.004) were increased in the Progestin-Primed-Group, however no significant differences were noted in the cumulative pregnancy (64.4% vs. 49.0%, p = 0.104) and miscarriage rate (2.6% vs. 8.6%, p = 0.554) for Progestin-Primed and GnRH-Antagonist groups, respectively. The expense for the ovulation prevention using the GnRH-Antagonist was U$318.18, while by using progestins U$ 11.05 was sufficient to inhibit the premature LH surge.

Limitations, reasons for caution: This study has limitations, such as the retrospective design and small sample size.

Wider implications of the findings: Despite the increased implantation rate and potential cost reduction, the high cancellation rate and slower embryo development in the progestin-protocol cannot be ignored. Delayed embryo transfer due to the freeze-only approach may also be inconvenient. Before considering any protocols for LH surge prevention, pros and cons must be carefully evaluated.

Trial registration number: N/A

Abstract citation ID: dead093.495

P-131 Predicting Embryo Utilisation Rate on Day 5 using an Artificial Neural Network: A Multicentre Retrospective Study

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Study question: Can artificial intelligence (AI) identify which patient and cycle specific variables are most important for predicting blastocyst utilisation rate (BUR) on day 5?

Summary answer: Number of mature oocytes (MII) injected is the most significant variable for predicting BUR. Highest BUR association with MII is found when six MII injected.

What is known already: The day 5 usable blastocyst rate originating from the accumulation of embryos being transferred and cryopreserved is one of the most informative key performance indicators (KPI) in an IVF laboratory. As per the Vienna consensus, the blastocyst development rate competency value is set as $\geq$ 40% with the benchmark value set as $\geq$ 60%. Artificial intelligence (AI) has been integrated into clinical settings as a technological advancement aimed to improve clinical success rates. Identifying the predictors that can result in an increased BUR on day 5 can be an additional tool towards improved cycle outcomes.

Study design, size, duration: In this retrospective, multicentre study, we evaluated six variables for predicting BUR using an artificial neural network (ANN). Study was performed in two fertility centres, between March 2021 and August 2022. A total of 865 cycles were included. Cycle exclusion criteria: Preimplantation genetic testing (PGT), advanced maternal age (AMA) $>$38, infertility aetiology of endometrial origin, severe oligospermia and epididymal/testicular sperm extraction. Primary outcome measure was the BUR on day 5 for the different variables.

Participants/materials, setting, methods: A total of 865 cycles were analysed using the ANN model with six variables: Number of MII oocytes injected (ICSI), use of autologous/donated gametes, maternal age at oocyte pickup (OPU), sperm concentration, progressive sperm motility rate, and fertilisation rate. Cycles were divided into training and test set through stratified random sampling: 73.2% (633) training and 26.8% (232) test. BUR on day 5 was grouped into $<$60% and $\geq$60% as per Vienna consensus benchmark values.

Main results and the role of chance: Number of MII oocytes was found to be the most important variable (100%) followed by the type of gametes used (54.1%), sperm concentration (32.9%), age at OPU (24.7%), progressive sperm rate (23.4%) and fertilisation rate (17.1%).The effect of MII injected on BUR was then investigated and results indicated an inverse correlation, with increasing MII injected resulting in decreased BUR (correlation coefficient of $r=0.344$,$p<0.001$). According to the performed model, 6 injected MII produces the higher rate of utilisation (62.9%).

Performance of ANN model was assessed by positive predicted value (PPV), negative predicted value (NPV), false positive rate (FPR), false negative rate (FNR), overall accuracy (OA), sensitivity and specificity. PPV for the training and test sets was 62.2% (194) for the $<$60% EUR on day 5 group and 79.8% (256) for $\geq$60% EUR on day 5 group and 64.8% (83) for the $<$ 60% EUR on day 5 and 81.7% (85) for $\geq$60% EUR on day 5 group, respectively. OA obtained from training and test sets were 71.1% and 72.4% for the $<$60 and $\geq$60% EUR on day 5 groups, respectively. The area under the curve (AOC) to predict the UR on day 5 group was found to be 77.6%.

Limitations, reasons for caution: The results represent the experience gained from current practice and not of a prospective controlled study. Clinical outcomes using this approach were not explored. Several other patient and cycle variables can also be investigated.

Wider implications of the findings: The number of oocytes retrieved is associated with live birth rate with 15 being the ideal maximum. Increasing oocyte numbers however, can bear greater risks. Predicting embryo utilisation rate on Day 5 can guide the way towards personalised treatment and safety.

Trial registration number: Not applicable

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P-132 Does the automated blastocyst assessment system, iDAScore®, provide valuable data for predicting the results of preimplantation genetic testing for aneuploidy (PGT-A)?

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Study question: What iDAScore® could indicate the presence of euploidy in PGT-A? Is it a valid predictor of the result of PGT-A?

Summary answer: PGT-A results with an iDAScore® of 8.3 or higher can indicate euploidy. However, patient age and trophectoderm (TE) grade can be effective predictors as well.

What is known already: PGT-A was approved as a general test in Japan in 2022. Currently, only patients who meet the criteria of Japan Society of Obstetrics and Gynecology can undergo the test. It is expected that the test will improve the live birth rate and reduce the miscarriage rate. The iDAScore® uses artificial intelligence and deep learning to score blastocysts. It is anticipated to be more objective than is morphological evaluation, which varies among individuals. Unlike PGT-A, the iDAScore® can assess embryos without cellular invasion. However, only a few reports have investigated the association between PGT-A and iDAScore® results.

Study design, size, duration: The study was performed retrospectively on 3,781 cycles of *in vitro* fertilization (IVF) conducted at our clinic between August 2020 and October 2022. Of the total of 5,684 embryos derived from conventional IVF or intracytoplasmic sperm injection that were cultured in a time lapse incubator, 293 blastocysts were used in the study.

Participants/materials, setting, methods: On the basis of their PGT-A results, the blastocysts were divided into euploidy and aneuploidy groups. The mean age of patients in each group, the methods of insemination, and the median iDAScore® were evaluated. The area under the curve (AUC) and the cut-off value were calculated from the PGT-A and iDAScore® results. A logistic regression analysis of the predictive parameters of PGT-A results, including the iDAScore®, was also performed.

Main results and the role of chance: Patients' mean ages in the euploidy and aneuploidy groups were 38.5 and 41.5 years, respectively. This was significantly higher in the aneuploidy group ($P<0.001$). There was no significant difference in the stage of maturity at oocyte retrieval ($P=0.302$). Similarly, no significant differences were associated with method of insemination ($P=0.961$). The median iDAScore® for the euploidy group was 8.7; for the aneuploidy group, it was 7.8. This result was significantly higher in the euploidy group ($P<0.001$). The AUC, calculated from PGT-A results and the iDAScore® with receiver operating characteristic curves, was 0.685 (95% CI [0.617−0.754]). The cut-off value was 8.3. The logistic regression analysis of age, stage of maturity, insemination method, timing of blastocyst freezing, diameter before blastocyst freezing, inner cell mass, TE grade in the Gardner classification, and iDAScore® as parameters for the PGT-A results indicated significant differences for age and TE grade (age: $P<0.001$, odds ratio [95%CI]: 1.320 [1.19-1.45]; TE grade 2: $P=0.03$, odds ratio [95%CI]: 2.480 [1.11-5.57]; TE grade 3: $P=0.02$, odds ratio [95%CI]: 3.740 [1.27-11.00]), while no significant differences were associated with the iDAScore®.

Limitations, reasons for caution: The iDAScore® is likely to depend on the timing of blastocyst freezing. Furthermore, the iDAScore® utilised in this study was the score of blastocysts that fulfilled our freezing criteria; the blastocyst that reaches a size of at least 150-160 μm in diameter on Day 5,6, or 7 is frozen.

Wider implications of the findings: An iDAScore® of 8.3 or above could indicate a euploidy PGT-A result. However, the logistic regression analysis indicated that age and TE grade were more significant predictors than the iDAScore® was. Therefore, it is difficult to predict PGT-A results from the iDAScore® itself.

Trial registration number: Not applicable

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P-133 A study of the relationship between in vitro embryo development and maternal hypothyroidism

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Study question: Are there an association between the maternal thyroid condition and in vitro fertilization (IVF) outcomes?

Summary answer: Maternal hypothyroidism was found to be related to oocyte maturation and blastocyst development.

What is known already: Previous studies have shown that thyroid-stimulating hormone (TSH) levels affect ovarian stimulation in assisted reproductive technology (ART). In addition, changes in serum TSH levels have been linked to a sub-optimal environment for the implantation and development of the embryo. Thyroid-related receptors are present in granulosa cells, ovaries, and the endometrium. In addition, those receptors play a role in ovulation and folliculogenesis. This study aims to determine the association between maternal hypothyroidism and adverse implications for IVF outcomes in IVF/ICSI treated women.

Study design, size, duration: This retrospective study performed 333 cycles of 208 patients with TSH levels ranging from 0.043-16.627 μIU/mL, treated at the RMC National IVF Center between 2019 and 2022. The study population consisted of 1171 oocytes, which were divided into two groups based on their maternal basal TSH levels: normal TSH $\leq$2.5 μIU/mL (control group, n = 865, euthyroid) and higher TSH $>$2.5 μIU/mL (case group, n = 306, hypothyroid).

Participants/materials, setting, methods: Maternal basal serum TSH levels were measured using Fluorescence Enzyme Immunoassay on the third day of the menstrual cycle. IVF outcome expressed oocyte maturation, fertilization, cleavage, and blastocyst formation rate. Oocytes were divided into mature (metaphase II) and immature (metaphase I and prophase I) groups. Blastocysts are graded by the Kato Ladies Clinic classification system and divided into high potential (A and B grades) and low potential (C, D, and E grades) groups.

Main results and the role of chance: In our study, there was no statistically significant difference in age and partner's semen criteria between the two groups ($p > 0.05$). As regards oocyte maturation, MII oocytes retrieved significantly higher in the control group (82.2% vs. 75.8%). Logistic regression analysis found that TSH levels $>$2.5 μIU/mL were related to statistically significantly increased risks of oocyte maturation stages like MI or GV (Age affect adjusted model: B = 0.456, p = 0.005; OR = 1.578, 95% CI = 1.146-2.175). The fertilization, cleavage, and blast formation rates have no differences between the groups. However, 91% (n = 324/356) of higher potential blastocysts in the control group were significantly higher than the case group (79.7%, n = 106/133). While, by the logistic regression analysis found that higher TSH levels ($>$2.5 μIU/mL) were related to statistically significantly increased risks of lower potential for implantation grades (B = 0.947, p = 0.001; OR = 2.579, 95%CI = 1.477-4.502).

Limitations, reasons for caution: The study relied on TSH as the primary indicator of thyroid function and excluded participants with abnormal prolactin levels. Other markers, such as FT4 and TSHR, should also be considered for a thorough evaluation of thyroid function.

Wider implications of the findings: Thyroid dysfunction can negatively impact IVF success, and screening and managing thyroid function in women undergoing IVF are essential. More research is needed to understand the mechanisms and develop strategies to improve oocyte and blastocyst quality.

Trial registration number: not applicable

Abstract citation ID: dead093.498

P-134 An artificial intelligence method based on time-lapse images and deep learning may predict if a day2/3 embryo will form a utilizable blastocyst

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Study question: Can iDAScore v2.0 predict likelihood of development into a utilizable blastocyst from time-lapse images captured during the first two or three days of embryo development?

Summary answer: iDAScore v2.0 predicts utilizable blastocyst formation from time-lapse images of early embryo development. Specific score thresholds can be established to ensure high specificity of prediction.

What is known already: Treatment efficacy would benefit from a reliable method to predict the potential of early embryos to develop into utilizable blastocysts and select fresh IVF treatment cycles suitable for blastocyst culture. Reports indicate that time-lapse images captured during the first few days of development and analyzed by deep learning methods may be an accurate approach. A fully automated AI model, iDAScore, is reported to be more accurate than routine morphology and morphokinetics for prediction of implantation. However, the applicability of iDAScore calculated on day 2 and 3 as a tool to predict blastocyst development has yet to be tested.

Study design, size, duration: This is a retrospective multicenter cohort study including time-lapse videos of 9111 embryos cultured to blastocyst stage, from 1283 fresh IVF treatments between 2016-2021 at two ART clinics together performing $>$2000 OPU per year. Images captured during the first days of culture were used to calculate iDAScores (at 48 and 66 hours post insemination) and determine the ability to discriminate embryos likely to develop into utilizable blastocysts according to Gardner score of 3BB or more.

Participants/materials, setting, methods: Patients were required to be undergoing IVF treatment using their own (autologous) oocytes whereby all zygotes were cultured up to the blastocyst stage in GTL® culture medium using the Embryoscope+® time-lapse system (Vitrolife, Gothenburg, Sweden). At least two experienced embryologists graded blastocysts on day 5/6, selecting utilizable blastocysts for transfer/vitrification. To evaluate the performance of iDAScore to predict development into utilizable blastocysts, sensitivity, specificity, and area under ROC curve (AUC) values were calculated.

Main results and the role of chance: The blastocyst utilization rate was 43.8% (3990 utilizable blastocysts of 9111 day 2/3 embryos developing from 2PN zygotes). Prediction of utilizable blastocysts obtained AUCs of 0.768 (0.759 - 0.778) and 0.782 (0.773 - 0.791) for day 2 and day 3, respectively. These results suggest IDAScore prediction performed slightly better when calculated on day 3 than on day 2. The ability to discriminate between utilizable and non-utilizable blastocysts at different thresholds of iDAScore on day 2 and on day 3, suggests that embryos should have a minimum iDAscore of 5 on day 2 to obtain a high specificity of 89%, and a threshold of 6 on day 3 to obtain a high specificity of 88%. Lower sensitivities were observed for these potential thresholds, 35% on day 2 and 41% on day 3.

Limitations, reasons for caution: This is a retrospective analysis where most cycles have at least five zygotes that supported subsequent blastocyst development, limiting relevance for patients with fewer zygotes. IDAScore is developed to predict implantation potential, not utilizable blastocyst, thus could be an underestimation of the potential performance of a model for utilization prediction.

Wider implications of the findings: The iDAScore v2.0 may provide valuable information for embryologists and be useful as a prognostic tool to select patients with high chance to obtain blastocysts based on day2/3 time-lapse images and thereby may achieve similar live birth rate per transfer with no need for extended culture.

Trial registration number: Not applicable

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P-135 No difference in morphokinetic patterns between male and female preimplantation embryos

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Study question: Do morphokinetic parameters vary between male and female preimplantation embryos?

Summary answer: We observed no significant differences in morphokinetic characteristics between male and female preimplantation embryos

What is known already: Studies across various mammalian species, including humans, suggest that male and female embryos differ in their gene expression and metabolic phenotypes. These variations may inherently affect the timing and duration of key preimplantation events. In the context of ART, such developmental differences may ultimately bias embryo selection, leading to an unbalanced sex ratio in the resulting offspring. To date, only a few studies have explored the relationship between morphokinetic patterns and human embryo sex. While some suggest subtle sex-specific variations in human embryo growth dynamics, results have been conflicting and currently no clear consensus has been reached.

Study design, size, duration: Retrospective study of morphokinetic data obtained from 175 preimplantation embryos. The study included 175 cycles performed at two IVF centers, between March 2018 and June 2021. Only fresh oocyte donation cycles using intracytoplasmic sperm injection (ICSI) and fresh single embryo transfer (SET) were assessed. Sex of the embryos was obtained from live birth data. Overall, our analysis included 92 female and 83 male embryos.

Participants/materials, setting, methods: All embryos were cultured in a timelapse incubator in standardized conditions. The morphokinetic parameters assessed included: time to pronuclear fading (tPNf), times to 2–5 cells (t2, t3, t4, t5), time to 8 cells (t8), time to start of blastulation (tSB) and time to full blastocyst stage (tB). All parameters were measured in hours post insemination (hpi). A two-tailed Student's *t*-test was used to compare morphokinetics between embryo sexes. A $p<0.05$ was considered statistically significant.

Main results and the role of chance: We observed no significant differences in morphokinetic parameters when comparing cycles resulting in female vs. male live births: tPNf (21.8 ± 3.3 vs. 22.3 ± 3.4 hpi; $p>0.39$); t2 (24.6 ± 2.5 vs. 25.0 ± 2.5 hpi; $p>0.34$); t3 (35.3 ± 3.3 vs. 35.8 ± 3.1 hpi; $p>0.28$); t4 (36.3 ± 3.4 vs. 36.9 ± 3.7 hpi; $p>0.20$); t5 (47.9 ± 4.7 vs. 48.0 ± 4.8 hpi; $p>0.88$); t8 (54.0 ± 6.5 vs. 54.1 ± 6.5 hpi; $p>0.91$); tSB (86.3 ± 14.6 vs 85.7 ± 15.5 hpi; $p>0.78$) and tB (93.0 ± 16.9 vs. 93.2 ± 17.2 hpi; $p>0.94$). These findings suggest that the timing of key developmental events does not vary between male and female ICSI-derived preimplantation embryos.

Limitations, reasons for caution: The main limitation of this study is its retrospective nature and sample size. We analyzed the data of embryos leading to a live birth; therefore, caution is warranted when generalizing results to non-implanting embryos.

Wider implications of the findings: Male and female human embryos display similar morphokinetic patterns throughout preimplantation development in our study. Accordingly, embryo selection in a timelapse setting based on developmental characteristics does not bias sex ratios in resulting live births.

Trial registration number: not applicable

Abstract citation ID: dead093.500

P-136 An embryo evaluation algorithm based on deep learning correlates strongly with cell number and degree of fragmentation on day 2 and 3

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Study question: Does the embryo evaluation model iDAScore v2.0 correlate with manually assessed cell numbers and fragmentation grade in early-stage embryos?

Summary answer: iDAScore v2.0 significantly correlated with morphological assessment of preimplantation embryos on days 2 and 3. Correlation between cell numbers and iDAScore decreased with increasing fragmentation.

What is known already: Time-lapse technology, including continuous documentation of embryo development, has enabled the implementation of more automated scoring systems, based on images and video sequences. iDAScore (ranging from 1-9.9) is a fully automated annotation-free scoring system based on deep learning and has been shown to discriminate between implanting and non-implanting embryos to a similar extent as KIDScore, which required manual annotations. iDAScore v2.0 also demonstrated a good agreement between predicted probabilities and observed implantation rates. However, not much is known regarding the correlation between specific morphological day 2 and day 3 variables and iDAScore v2.0.

Study design, size, duration: Retrospective observational study from two ART centers, together performing >2000 OPU per year. The study includes all normally fertilized embryos (n = 5378) from 1792 treatments between 2016 and 2021 where fresh single embryo transfer was performed on day 2 or day 3 post fertilization.

The association of iDAScore v2.0 with manual assessment of cell numbers and grade of fragmentation on day 2 (treatments = 762, embryos = 2170) and day 3 (treatments = 1030, embryos = 3208) was analysed using video sequences.

Participants/materials, setting, methods: Inclusion criteria were treatment cycles with own (autologous) oocytes and embryo transfer on day 2 or 3. Exclusion criteria were cycles with double embryo transfers or PGT cycles.

Clinical and laboratory processes were performed according to standard procedures. Time-lapse culture and embryo annotations were performed using the Embryoscope+® with GTL® culture medium (Vitrolife, Gothenburg, Sweden). Wilcoxon rank sum test was used to compare iDAScores between groups.

Main results and the role of chance: Day 2: For the number of cells, iDAScore differed between embryos having <= 3 cells compared to >= 4 cells (medians: 2.3 vs. 4.0, $p<0.001$). The median iDAScores for fragmentation grade 0%; <20%; <50%; >50% were 4.7; 3.9; 2.6 and 2.1, respectively. Furthermore, 0% was significantly different from each of the other groups ($p<0.001$). When cell numbers and fragmentation grade were combined, it was seen that having <= 3 cells versus >= 4 was significantly correlated to iDAScore when the fragmentation grade was scored as 0%, but this association decreased as the fragmentation grade increased.

Day 3: A similar pattern was seen for day 3 morphology analyses. It was found that having <= 5 cells vs. >= 6 cells correlated to a significant difference in iDAScore (medians: 2.5 vs. 4.4, $p<0.001$). The median iDAScores for fragmentation grade 0%; <20%; <50%; >50% were 5.6; 3.8; 2.6 and 2.3, respectively. Furthermore, 0% was significantly different from each of the other groups ($p<0.001$). When combining cell numbers and fragmentation grade in the same analysis, the association of cell numbers to iDAScore decreased as fragmentation increased.

Limitations, reasons for caution: The present study is of retrospective design, with analysis of data from two clinics, and the results may not apply in a wider context. In addition, only two morphological variables, cell numbers and fragmentation, were included.

Wider implications of the findings: Our results show that there is a strong correlation between iDAScore v2.0 and the number of cells and the fragmentation grade of the embryo, both for each individual variable and when combined. This indicates that iDAScore v2.0 identifies some of the well-established morphological evaluations for cleavage stage transfers.

Trial registration number: Not applicable

Abstract citation ID: dead093.501
P-137 pronuclear characteristics and chromosomal constitutions of mono-pronuclear zygotes

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Study question: Limited studies have explored the correlation between the pronuclear characteristics, developmental potential, and genetic constitution of mono-pronuclear (1PN) zygotes.

Summary answer: Those 1PN zygotes developed into blastocysts have unique pronuclear characteristics and significantly higher rate of diploidy euploidy in comparison with the arrested embryos.

What is known already: It has been reported that 1PN blastocysts have similar morphokinetic features to two-pronuclear (2PN) embryos. Cytogenetic analyses reveal that some of the 1PN embryos have a normal chromosomal constitution.

Study design, size, duration: This retrospective cohort study included the 1PN zygotes from December 2021 to September 2022 (n = 388).

Participants/materials, setting, methods: Patients who underwent IVF or intracytoplasmic sperm injection (ICSI) in the Women and Children's Hospital of Chongqing Medical University were recruited. Only 1PN zygotes cultured in the time-lapse incubators from the oocyte retrieval day were included. The pronuclear characteristics, including the area and diameter of the pronuclear, the number of nuclei, and the distance between the pronuclear and the near-polar body (DPNP), and the genetic constitutions were investigated.

Main results and the role of chance: The overall blastocyst formation and good-quality blastocyst rates in 1PN zygotes were 22.94% and 16.24%, significantly lower than that of 2PN zygotes (63.25% and 50.23%, respectively, $P = 0.000$). Compared to arrested embryos, 1PN zygotes that developed into blastocysts showed significantly larger area (752.57 ± 131.53 vs. 653.65 ± 116.58, $P = 0.000$), longer diameter of pronuclear (30.10 ± 2.90 vs. 27.28 ± 2.83, $P = 0.000$), a greater number of nuclei (11.92 ± 4.06 vs. 7.72 ± 3.07, $P = 0.000$), and shorter DPNP (13.73 ± 8.65 vs. 24.20 ± 13.51, $P = 0.000$). Of the tested embryos, the diploidy euploidy rate was significantly higher in good-quality blastocysts in comparison with the arrested embryos (66.67% vs. 11.76%, $P = 0.000$), which was also significantly higher in IVF-1PN blastocysts than in ICSI-1PN blastocysts (75.44% vs. 25.00%, $P = 0.001$).

Limitations, reasons for caution: The correlation between pronuclear characteristics and genetic constitution was investigated only in limited embryos. The small sample size might weaken the conclusions. In addition, part of the 1PN blastocysts were tested by NGS, which has inherent limitations because it could not find the uniparental diploidy.

Wider implications of the findings: The 1PN blastocysts, especially from IVF-1PN zygotes, have high diploidy euploidy rates. The utility of 1PN blastocysts is potentially beneficial for patients without other viable embryos.

Trial registration number: Chongqing Science and Technology Committee (grant number: CSTB2022NSCQ-MSX0253), Chongqing Health Committee (grant number: 2021MSXM108), Yuzhong District Science and Technology Committee (grant number: 20190143), and Women and Children's Hospital of Chongqing Medical University (grant number: 2021YJQN07).

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P-138 Protein concentration of 5 mg/mL in recovery medium for thawed blastocysts is equivalent to 10 mg/mL in post-thaw survival and pregnancy rates

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Study question: Are comparable survival and pregnancy rates obtained when recovery media are supplemented with 5mg/mL protein versus customary 10mg/mL protein in Frozen Embryo Transfer cycles?

Summary answer: Recovery medium containing 5mg/mL protein compared to 10mg/mL yielded similar outcomes in blastocyst survival and pregnancy rates.

What is known already: Accepted practice has been to culture thawed blastocysts in medium with the same protein concentration as the vitrification media (20% protein) to minimize osmotic stress and optimize survival and implantation rates. The potential to acquire similarly positive outcomes with supplementation of only 5 mg protein/ml has been explored and demonstrated, challenging the requirement for high protein levels in recovery media.

Study design, size, duration: From July through August 2022, a prospective study was conducted in a university laboratory to compare the survival and implantation rates in Frozen Embryo Transfer cycles using vitrified blastocysts recovered in Continuous Single Culture–NX Complete (CSCM-NXC) (5mg/mL Human Serum Albumin (HSA)) or global HP[LW1] (8.8mg/mL HSA and 1.2mg/mL α-and ß-globulins). 50 blastocysts were warmed per condition. Comparative analyses included survival and implantation rates, beta HCG levels, and clinical pregnancy rates by fetal cardiac activity.

Participants/materials, setting, methods: To evaluate the effect of protein level in recovery medium for vitrified blastocysts, CSCM-NXC and global HP, with 5mg/mL and 10mg/mL total protein, respectively, were compared. Blastocysts were vitrified in Irvine Scientific Vit Kit – Freeze and were warmed in Irvine Scientific Vit Kit – Warm. Minimum recovery time was the same for all blastocysts (30 minutes), and is defined as the time from completion of the warming procedure to the time of embryo transfer.

Main results and the role of chance: In both study arms, one blastocyst was removed from the data analysis due to embryo transfer cancellation that was not related to embryo survival or suitability for transfer, resulting in N = 49 for each group. Chi-square analysis was performed to assess statistical significance of all compared parameters, and a p value < 0.05 was considered significant. Average patient age (years) for CSCM-NXC was 36.1 and for global HP, 35.0. There was no difference between blastocysts warmed in CSCM-NXC and global HP in average recovery time, i.e., 3.8 and 3.9 hours, respectively, or survival rates, 98.4% and 98.1% respectively. Comparison of patients with blastocysts warmed in CSCM-NXC reflected a trend towards higher outcomes compared to those in global HP with initial positive beta HCG rates of 64.6% vs 56.3% and ongoing clinical pregnancy rates of 52.1% vs 45.8%, respectively. Implantation rates were 56.1% and 57.7% in CSCM-NXC and global HP, respectively. Incidence of monozygotic twinning of 0% in CSM-NXC and 3.3% in Global HP was observed.

Limitations, reasons for caution: As this is a challenge to accepted formulation of recovery media for vitrified blastocysts, specifically with respect to protein supplementation - a strategy for minimizing osmotic shock and potential damage to warming embryos - more expansive and robust studies must be undertaken to increase the statistical power of this study.

Wider implications of the findings: As frozen embryo transfers are utilized more routinely in assisted reproductive treatment, the clinical finding that a commercial recovery medium containing of 5mg/mL protein, the standard for routine embryo culture, can support the recovery of vitrified embryos and lead to successful pregnancy could increase the efficiency in the laboratory.

Trial registration number: na

Abstract citation ID: dead093.503

P-139 Comparison of clinical outcomes between two hyaluronan-rich transfer medium

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Study question: Is there any difference in effect on clinical outcomes after single vitrified-warmed blastocyst transfer on day 5 using two hyaluronan-rich transfer medium?

Summary answer: There was no significant difference in rates of clinical pregnancy, implantation and miscarriage between two hyaluronan-rich transfer medium.

What is known already: Hyaluronan is one of the glycosaminoglycan that is naturally present in female reproductive organs and promotes the endometrial stroma and preimplantation embryo adhesion through CD44 glycoprotein receptors. Several studies demonstrated that use of hyaluronan-enriched transfer medium in women receiving either cleavage-stage or blastocyst-stage embryo improved clinical outcomes compared to the standard medium. However, studies on clinical outcomes according to two transfer medium containing different concentrations of hyaluronan are still insufficient.

Study design, size, duration: A retrospective study of 1047 vitrified-warmed blastocyst transfer cycles with normal responders (aged 23-35 years) was conducted from September 2018 to November 2022. All cycles with single vitrified-warmed blastocyst transfer on day 5 were evaluated. Only first IVF attempts cycles were included. All women had undergone a GnRH antagonist protocol. Cycles were divided into two groups: Group A (n = 511, EmbryoGlue®, Vitrolife) and Group B (n = 536, UTM™, Origio).

Participants/materials, setting, methods: All blastocyst were graded using Gardner and Schoolcraft's criteria. The vitrified-warmed blastocysts were equilibrated for an average of 47 minutes in two transfers medium until transfer. All blasotcyst were then loaded into an embryo transfer catheter (COOK® medical). The rates of clinical pregnancy, implantation and miscarriage were compared between the two groups. Statistical analyses were conducted using Chi-square test and Independent t-test using SPSS.

Main results and the role of chance: There were no significant differences in mean female age (31.6 ± 2.5 vs. 31.8 ± 2.3, P = 0.432), mean endometrial thickness (10.4 ± 1.4 vs. 10.3 ± 1.3, P = 0.478), mean oocytes retrieved (20.8 ± 6.8 vs. 20.5 ± 6.9, P = 0.470) and number of transferred good-quality blastocyst stage rate (≥BB grade, 74.8% vs. 72.6%, P = 0.423) between the two groups. We also observed similar rates of biochemical pregnancy (79.5% vs. 78.0%, P = 0.562), clinical pregnancy (71.4% vs. 69.2%, P = 0.434), implantation (72.4% vs. 70.3%, P = 0.459), ongoing pregnancy (67.5% vs. 66.2%, P = 0.659), miscarriage (5.5% vs. 4.3%, P = 0.463) and multiple pregnancy (1.4% vs. 1.6%, P = 0.782) in Group A and B, respectively.

Limitations, reasons for caution: This study is limited by its retrospective nature in single-center and absence of live birth information. We investigated only single vitrified-warmed blastocyst on day 5 to have data reliability. Therefore, further studies are needed to evaluate our data with vitrified-warmed blastocyst transfer on day 6 or day 7.

Wider implications of the findings: The present data showed that clinical outcomes were similar between two transfer medium containing different concentrations of hyaluronan. Our findings could have significant clinical implication for selection of the appropriate commercial embryo transfer medium according to IVF laboratory conditions.

Trial registration number: not applicable

Abstract citation ID: dead093.504

P-140 Relationship between re-expansion rate of vitrified blastocysts during recovery culture post-warming and pregnancy rate after single blastocyst transfers

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Study question: What is the relationship between re-expansion rate of vitrified-warmed blastocysts and pregnancy rate after single blastocyst transfers (SVBT)?

Summary answer: Re-expansion rate of vitrified-warmed blastocysts was higher with increasing the number of TEs and yielded higher pregnancy rate after SVBT.

What is known already: The increased use of blastocyst transfer has emphasized the importance of selecting high-quality embryos. Pre-vitrification morphological grading: Gardner's criteria or time-lapse imaging of embryo morpho kinetics and developmental speed, is established a tool for selection of high-quality blastocysts. The recovery culture of vitrified blastocysts post-warming is performed before embryo transfer. Embryos that have dehydrated and shrunk during vitrification are restored to their pre-vitrification state during the recovery culture. Developmental competence in vivo of re-expanded blastocysts after thawing was higher than that of non-expanded blastocysts, but it is not clear the relationship between the re-expansion rate and the pregnancy rate, quantitatively.

Study design, size, duration: We analyzed 1034 vitrified-warmed blastocysts in 758 patients from July 2017 to June 2021. All embryos were monitored by a time-lapse system (EmbryoScope+, Vitrolife). Expanded blastocysts which reached the blastocoel cavity of 160 μm or more with obvious ICM were vitrified and cryopreserved. The blastocysts were transferred in the following cycle or later. Fetal heartbeat was detected as the pregnancy confirmation.

Participants/materials, setting, methods: The re-expansion rate was defined as the percentage of re-expanded blastocyst diameter after the recovery culture to the blastocyst diameter at the time of vitrification. The time of recovery culture was about 3 hours. The re-expansion rate and pregnancy rate were analyzed by logistic regression analysis. We also examined the association between re-expansion rate and the number of trophectoderm cells at equatorial region (eTE) or iDAScore: the parameters for selecting high-quality embryos.

Main results and the role of chance: Logistic regression analysis showed a significant relationship between re-expansion rate and pregnancy rate ($P < 0.0001$), with an AUC of 0.58 and a cutoff value of 88.5%. Pregnancy rates in re-expansion rates ≥ 88.5% were significantly higher than those in < 88.5% (51.7 vs. 37.4%). Therefore, we classified re-expansion rates ≥ 88.5% as positive and < 88.5% as negative, and performed logistic regression analysis of women age, vitrification time of blastocysts, eTE, and iDASCore. The results showed that women age, vitrification time, and iDAScore all did not differ significantly from the re-expansion rate. The number of eTE was significantly higher with increasing re-expansion rates ($P < 0.0001$), with an AUC of 0.58 and a cutoff value of 7.5.

Limitations, reasons for caution: We used only the expanded blastocysts with 160 μm or more in diameter and obvious ICM for vitrification. In addition, this study was retrospective, and further research is needed to confirm the reproducibility of the study in other clinics with different vitrification protocols and patient populations.

Wider implications of the findings: Quantitative analysis of re-expansion rate of vitrified blastocysts indicated that higher re-expansion rate (≥ 88.5%) had high pregnancy rate. It is useful to exploring the influence factors of re-expansion for producing high-quality embryos. Here, we found that the number of eTE is as an important factor for re-expansion.

Trial registration number: not applicable

Abstract citation ID: dead093.505

P-141 Independent external pre-clinical validation of iDAScore v1.0 in 1232 PGT-A cycles with 3604 biopsied blastocysts and 808 euploid transfers.

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Study question: Is iDAScore v1.0 associated with euploidy and live-births (LBs) after euploid transfers? How often would it have affected embryologists' clinical choices in a blinded analysis?

Summary answer: iDAScore v1.0 was associated with both euploidy (AUC:0.60) and LBs after euploid transfers (AUC:0.66). iDAScore v1.0 and embryologists would have frequently disagreed on embryo ranking.

What is known already: Embryo assessment/ranking to improve IVF efficiency (LB/transfer) is still challenging. The widely used static morphological evaluation suffers from subjectivity and intra-/inter-operator variability. Preimplantation genetic testing for aneuploidies (PGT-A) discriminates euploid/aneuploid embryos, improving IVF efficiency. Nevertheless, it requires manipulation and expertise and ~50% euploid blastocysts still fail to implant. Time-lapse (TL) technology allows continuous undisturbed monitoring of embryo morphokinetics. The software iDAScore v1.0 is a deep learning algorithm trained on TL videos from implanted/non-implanted blastocysts to generate a score that should predict their implantation potential (score:1.0-9.9, from the lowest to the highest, respectively).

Study design, size, duration: Retrospective independent external pre-clinical validation of iDAScore v1.0 in PGT-A cycles (N = 1232) with fresh own oocytes and ≥1 biopsied blastocyst (N = 3604) (April-2013 to August-2022). The AI-based tool was investigated for associations with embryologists' assessment, blastocysts' karyotype, and LBs. Two simulations were then conducted to estimate how often iDAScore v1.0 would have ranked (i) euploid blastocysts first in presence of aneuploid as well, (ii) reproductively-competent blastocysts before incompetent in presence of ≥2 euploid and ≥1 LB.

Participants/materials, setting, methods: Embryos were cultured in EmbryoScope (Vitrolife). Only the first PGT-A cycles were included (maternal age 38.7 ± 3.4 years). The day of biopsy was defined based on the hours-post-insemination (hpi) and blastocyst morphology based on Gardner. ROC curve analyses were conducted to calculate the AUC for euploidy and LB discrimination based on embryologists' assessment or iDAScore v1.0.

Main results and the role of chance: iDAScore v1.0 was associated with ICM-quality (A-grade, N = 2107, score: 7.5 ± 1.8; B-grade, N = 833, score: 5.6 ± 1.9; C-grade, N = 664, score: 4.4 ± 1.7; $p < 0.01$), trophectoderm-quality (A-grade, N = 1988, score: 7.5 ± 1.8; B-grade, N = 951, score: 5.9 ± 1.9; C-grade, N = 664, score: 4.3 ± 1.6; $p < 0.01$), and day (≤120hpi, N = 1462, score: 8.2 ± 1.5; 121-144hpi, N = 1874, score: 5.6 ± 1.7; >144hpi, N = 268, score: 3.9 ± 1.4;$p<0.01$).

Euploid blastocysts showed the highest score (N = 1443, 7.0 ± 2.1) versus both single-aneuploid (N = 1194, score: 6.5 ± 2.2, $p < 0.01$) and complex-aneuploid (N = 967, score: 5.8 ± 2.1, $p < 0.01$). AUC of 0.60 (95%CI 0.59−0.62) and 0.66 (95%CI 0.64−0.68) were reported for iDAScore v1.0 and embryologists' assessment association with euploidy, respectively.

Blastocysts resulting in a LB showed higher score (N = 361, 7.6 ± 1.8) than non-implanted/miscarried (N = 447, score: 6.5 ± 2.2; OR: 1.3, 95%CI 1.2-1.4, $p < 0.01$). AUC of 0.66 (95%CI 0.62-0-69) and 0.64 (95%CI 0.60-0-67) were reported for iDAScore v1.0 and embryologists' assessment association with LBs, respectively.

The simulations revealed that (i) across 587 cycles with both diagnoses, iDAScore v1.0 would have ranked euploid blastocysts as "top-quality" in 63% of the cases; (ii) across 202 cycles with sibling euploid blastocysts and a LB, it would have been equally, less, and more effective than embryologists, in 52%, 3% and 15% of the cases, respectively. Nonetheless, in 29% of the cases, this comparison was not doable because top-ranked euploid blastocysts were not transferred but a LB was achieved with worse-ranked blastocyst.

Limitations, reasons for caution: iDAScore v1.0 performance might be suboptimal on a dataset of mostly advanced-maternal-age women and on day-7/poor-quality blastocysts, due to their poor representation in the algorithm training. Although euploidy is a proxy of LB, iDAScore v1.0 was trained only to predict the latter. A prospective randomized study is now warranted

Wider implications of the findings: iDAScore v1.0 was significantly associated with euploidy and LB with results comparable to embryologists' performance. Nevertheless, iDAScore v1.0 is more objective and reproducible than embryologists' assessment. Embryo selection workflows, scientific debate, and patient counselling practice might all benefit from AI-based standardization of blastocyst evaluation.

Trial registration number: not available

Abstract citation ID: dead093.506

P-142 Alterations in mitochondrial DNA levels in luteal granulosa cells may affect the morphology of mature oocytes and subsequently their fertilization potential: a retrospective multicenter study

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Study question: Do alterations in mitochondrial DNA levels in luteal granulosa cells (LGCs) affect the quality of mature oocytes in infertile women?

Summary answer: Alterations in the mitochondrial DNA levels in LGCs may modify the characteristics of the first polar body in oocytes and subsequently decrease their fertilization potential.

What is known already: Assisted reproductive treatment outcomes are closely linked to the oocyte quality as shown in several systematic reviews and meta-analyses. Particularly, the oocyte quality is highly affected by the surrounding luteal granulosa cells (LGCs). This is mainly due to the crucial role of the mitochondria in the LGCs in fueling the metabolic processes required for oocyte maturation. Therefore, modifications in the quality of LGCs may have direct effects on the developmental competence of oocytes. However, the exact mechanisms by which this could happen are not fully understood.

Study design, size, duration: A retrospective multicenter study was conducted on 303 mature oocytes retrieved from 51 women undergoing intracytoplasmic sperm injection (ICSI). It was conducted in Lebanon at Al Hadi IVF center and Azoury IVF clinic, between January 2019 and January 2020. G-Power 3.1 was used to determine the sample size for Generalized Linear Mixed Models through a one-sample t-test power analysis with alpha 0.05, power 0.8, medium effect size ($f^2 = 0.15$), and 5 predictors. Resulting sample size: 43.

Participants/materials, setting, methods: This study excluded cases of premature ovarian failure, severe oligozoospermia (<2 x 10^2/ml), or cases using frozen gametes. Mature oocytes, from young women (< 36 years old), were injected and cultured in the Embryoscope where morphometric measurements were performed. LGCs vitality and mitochondrial DNA (mtDNA) levels were analyzed using trypan blue exclusion dye and next-generation sequencing, respectively. Possible associations between these independent variables and the size and integrity of the first polar body were evaluated.

Main results and the role of chance: The included women presented with primary infertility. Their mean age was 29.98 ± 5.5 years old and their mean body mass index was 23.33 ± 3.32 Kg/m^2. 10% of the included women had polycystic ovary syndrome, and 48% were smokers. Regarding the LGCs parameters, a statistically significant negative correlation was found between the mtDNA levels in LGCs and their vitality percentage ($r = -0.313$, $p = 0.029$). Interestingly, the average size of the first polar body (PBI) was 346.67 µm. Here we found that the PBI size decreased by 15.05 units when the mtDNA level in LGCs increased by one unit above its average. In parallel, the median percentage of oocytes having a fragmented PBI was 33.33 (0-100). This percentage increased by 4.26% for every one unit increase in the mtDNA level in LGCs ($p < 0.0001$). In contrast, this percentage decreased by 0.86% for every unit increase in the percentage of LGCs vitality ($p < 0.0001$). Moreover, the mean percentage of fertilization rate was found to be 70.19 ± 26.7. A higher percentage of fragmented PBI (38.3%) was found among oocytes that did not fertilize compared to those that did successfully fertilize (24%) ($p = 0.019$).

Limitations, reasons for caution: This study only analyzed the effects of alterations in mtDNA levels in LGCs on oocytes. It would be worthwhile to also analyze the effects on foetal development and offspring health. Furthermore, it would be interesting to study the relationship between alterations in LGCs and oocyte quality within individual follicles.

Wider implications of the findings: Several studies have suggested that abnormal size and fragmentation of the PBI may impair embryo development. Therefore, treating factors that affect LGCs, such as obesity and polycystic ovary syndrome, may benefit infertile women. This highlights the need for new clinical trials in this context.

Trial registration number: Not applicable

Abstract citation ID: dead093.507
P-143 Will Use of Testicular sperm in ICSI cycles increase Embryo Aneuploidy?

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Study question: Will use of surgically retrieved Testicular Sperms (TESA) in Intra-Cytoplasmic Sperm Injection (ICSI) cycles increase embryo aneuploidy?

Summary answer: Use of TESA sperms for ICSI seems safe and doesn't increase embryo aneuploidy. TESA performed for various indications also showed no variation with embryo aneuploidy.

What is known already: Invention of ICSI and improved surgical sperm retrieval techniques have helped men with severe male factor infertility to father their own genetic child. Sperms retrieved from seminiferous tubules of testes are considered immature as they haven't completed the process of spermiogensis. It is hypothesized that use of TESA sperm for ICSI can increase embryo aneuploidy, which is still uncertain.

Study design, size, duration: This retrospective study conducted at a private fertility clinic from 2014–2022 (n = 265 patients; total of 860 embryos evaluated). Study populationTESA sperm used and PGT-A done (n = 66). TESA group was further sub-divided into 2 groups based on the indication for TESA,Non-Obstructive Azoospermia(n = 26) and Raised SDF(n = 40). Control Population Ejaculated sperm used and PGT-A done (n = 199) Only younger women(<37yrs) with self-gametes and RIFconsidered in this study. RIF was defined as women with 2 failed IVFcycles in thepast with at least 4 blastocystsbeing transferred.

Participants/materials, setting, methods: All fertilized oocytes inseminated by ICSI and subjected to extended blastocyst culture. TESA done as per our clinic's SOP and for control group sperm preparation done by density gradient method. Trophectoderm biopsy was performed and sent for PGT-A to the genetic lab. Next-Generation sequencing (NGS) was the Comprehensive Chromosomal technique (CCS) used for assessing embryo ploidy status. Aneuploidy across all groups compared.

Main results and the role of chance: Following were the Aneuploidy rates of TESA sperm, Ejaculate sperm group and TESA done for NOA and SDF respectively.

Ejaculate Sperm – Aneuploidy%- 42%

TESA Sperm (irrespective of the indication)- Aneuploidy%- 44% ($p = 0.6260$)

TESA Sperm (done for NOA)- Aneuploidy%- 44% ($p = 0.7504$)

TESA Sperm (done for raised SDF) – Aneuploidy%- 32% ($p = 0.05$)

Embryo aneuploidy was comparable between Ejaculate and TESA sperm group. Though TESA sperm is considered immature, it didn't seem to increase embryo aneuploidy. Embryo Aneuploidy from TESA sperm ICSI cycles done for NOA or raised SDF also showed no altering trend. Data from this study is suggestive that TESA sperm doesn't alter embryo ploidy status.

Limitations, reasons for caution: Data is retrospective with small sample size and unequal distribution. A well designed Randomized Trial might help test the inference of this study further.

Wider implications of the findings: Incidence of male infertility and use of ICSI is on a rise globally. Interventions to optimize embryo implantation and reproductive outcomes will help with better success rates and shorten duration to conception. Further research is warranted with severe male factor infertility to improve sperm selection techniques and reproductive outcomes.

Trial registration number: not applicable

Abstract citation ID: dead093.508
P-144 An artificial intelligence algorithm demonstrates optimal performance for evaluating embryo genetic status at 120 hours post-fertilization

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Study question: What is the effect of time-point on performance of a non-invasive artificial intelligence (AI) algorithm for evaluating embryo genetic status?

Summary answer: While predictive ability was maintained across different time-points on day 5, optimal performance for ranking and selecting euploid embryos was observed at 120 hours post-fertilization.

What is known already: Studies have shown that it is possible to develop computer vision-based AI algorithms capable of predicting embryo ploidy status using single images of blastocyst-stage embryos. The genetic status of embryos is linked to morphokinetic development, with aneuploidy generally resulting in earlier arrest. Given the dynamic nature of embryo development, it might be expected that the time-point selected for analysis could influence AI performance. The key questions remaining to be answered are to what extent is AI analysis affected by expansion grade, and what does this mean for selection of a time-point for evaluation?

Study design, size, duration: 2,683 images of day 5 blastocyst-stage embryos with matched ploidy outcomes from pre-implantation genetic testing for aneuploidies (PGT-A) were provided by 10 IVF clinics in the USA, Australia, Malaysia, and India. A subset of 182 embryos had images provided at 110, 115, and 120 hour time points (GERI and EmbryoScope time lapse systems).

Participants/materials, setting, methods: Images were analysed by a previously developed AI algorithm which evaluates the likelihood of an embryo being euploid according to PGT-A (Diakiw et al, 2022. Hum Reprod, Jul 30;37(8):1746-1759). Evaluation was performed on embryos of each expansion grade, and at three time-points on day 5. Correlations were assessed using Chi-squared test for trend, with pair-wise comparisons conducted using Student's t-test. Performance was evaluated using ROC-AUC, and a simulated cohort ranking analysis method.

Main results and the role of chance: AI scores positively correlated with expansion grade, and expansion grade likewise correlated with an increasing proportion of euploid embryos. AI scores also increased over time on day 5, consistent with continued embryo expansion. Scores for grade 4 (expanded)

embryos increased more than for grade 5 (hatching) embryos (+2.2-fold and +0.8-fold, respectively), indicative of continued expansion becoming limited at later stages. Despite the valid association of AI scores with expansion grade, results showed the AI could predict euploidy even amongst embryos of the same expansion grade (ROC-AUC ranging from 0.61−0.69).

While predictive ability was maintained at each time-point on day 5, ROC-AUC values were highest at 120 hours (0.64, 0.64, and 0.68 for 110, 115, and 120 hours, respectively). Simulated cohort ranking analyses also showed that the AI performed best at 120 hours, selecting a euploid embryo as the top-ranked embryo in 77.1% of patient cohorts (71.4%, 75.1%, and 77.1% for 110, 115, and 120 hours, respectively). These results suggest that regardless of expansion grade, all embryos should be assessed at the same time-point on day 5, preferably closer to the 120 hour time-point.

Limitations, reasons for caution: The time-point analyses were conducted on a relatively small dataset, and therefore findings should be validated on a larger dataset including embryos of other expansion grades. The analysis was limited to day 5 post-fertilization, however it would be interesting to extend the study to embryos on day 6 also.

Wider implications of the findings: These results suggest that the AI is providing additional information regarding embryo genetic status, over and above that provided by known morphological parameters. The dynamic nature of AI score related to expansion is of interest as it relates to the optimal time-point for conducting analyses for selection of euploid embryos.

Trial registration number: Not applicable

Abstract citation ID: dead093.509

P-145 Direct-ICSI results in good embryological outcomes. A time-lapse study

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Study question: Is there a correlation between the method of sperm injection and morphokinetic and morphologic characteristics?

Summary answer: Results were comparable between the groups whereas the rate of utilizable embryos was significantly higher using Direct-ICSI. This suggests safety and efficacy using this technique.

What is known already: Maintaining high fertilization- and embryo development rates are key objectives of the IVF-laboratory. Little effort has been put into studying the effects of different manipulation techniques when performing manual microinjections. Specifically, we wanted to compare two methods for achieving cell-membrane puncture: Traditional cytoplasm aspiration technique versus membrane stretching and injection without aspiration –the so-called Direct ICSI method. This method has been proposed to give higher fertilization rates and is being implemented in IVF-laboratories to try to increase ICSI-results. As far as we are aware, no detailed comparison between the two methods on embryo development using time-lapse documentation has been published.

Study design, size, duration: The study included 44 ICSI-treatments from April 2020 to October 2021 with 119 oocytes injected with Direct-ICSI and 349 sibling oocytes using the conventional aspiration-ICSI technique. In this way, each patient contributed oocytes for both test- and control groups. However, as this was a pilot study, typically only a minority of the oocytes for each patient was injected using the Direct-ICSI method. All fertilized oocytes were included in the assessments up to the blastocyst stage.

Participants/materials, setting, methods: The center is a public funded University clinic performing >500 ICSI cycles annually. Inclusion criteria were treatment cycles using fresh own oocytes with ejaculated sperm and at least 5 injectable oocytes after denudation. Time lapse culture was performed using the Embryoscope+ with GTL culture media, both supplied by Vitrolife. Clinical and laboratory procedures were performed according to standard practice. Fisher's exact test (categorical variables) and Mann-Whitney U test (continuous variables) were used for statistical analysis.

Main results and the role of chance: In total, 314 oocytes (67,1%) were fertilized and included in the morphology and morphokinetic assessments up to the blastocyst stage (day 5 or day 6 post-fertilization). Time-lapse parameters such as timing of cell division events and time to morula, time to blastocyst and similar were not significantly different between the groups. Rates of categorical classification of embryological anomalies such as degree of fragmentation, rate of multinucleated cell events, uneven cell size and irregular cell division events also did not differ significantly between the groups. Interestingly however, although fertilization rates did not differ between the two groups (68,1% in the test group vs 67,6% in the control group), the rate of utilizable embryos per injected oocyte was significantly higher in the Direct-ICSI group (40,3% vs 29,5%, $p < 0.03$), whereas the pregnancy rates did not differ between the groups.

Limitations, reasons for caution: The present study is a small pilot study with a limited number of cycles, however with 468 oocytes included. The study utilized internal (sibling) controls although oocytes were not truly randomized between the groups. To fully test the efficiency of Direct-ICSI a larger randomized study should be performed.

Wider implications of the findings: Our results indicate that Direct-ICSI is a safe method with no visible indication of irregularities in preimplantation cell division events using time-lapse monitoring. Aspirating an excess amount of cytoplasm during ICSI might impact embryo development, possibly explaining the increase of utilizable embryos in the Direct-ICSI group, where aspiration is avoided.

Trial registration number: Not applicable

Abstract citation ID: dead093.510

P-146 Larger time intervals between pronuclear fading and the first mitosis are strongly associated with trichotomous mitoses and cleavage arrest in human embryos

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Study question: Does the duration of the interval between pronuclear fading and first cell division affect preimplantation embryo developmental competence?

Summary answer: Larger pronuclear-fading to first-division intervals (t2-tPNf) are associated with greater risk of direct unequal cleavage (DUC) and lower blastulation rate, but not of blastocyst aneuploidy.

What is known already: During the last decade, time lapse technology (TLT) has made possible detailed and dynamic observation of in vitro embryo development, offering a powerful non-invasive tool for improving embryo selection. Recent evidence suggests that the phase between pronuclear fading and the first cleavage is a perilous bridge between the zygote and the cleaving embryo. Following pronuclear breakdown, delayed progression through mitosis caused by incomplete DNA replication may result in chromosome lagging, whole and segmental segregation errors, and breakage. In this analysis, we specifically tested the hypothesis that delays in a short final phase of fertilization have an impact on embryo development.

Study design, size, duration: Retrospective study of 1316 zygotes cultured until day 5 using TLT and derived from first ICSI cycles (111 no PGT-A and 94 PGT-A) performed between 2018 and 2022. Testicular sperm or cryopreserved gametes cycles were excluded. DUC was defined as the cleavage of one zygote or blastomere into three daughter blastomeres or cell cycle interval shorter than five hours at the first (DUC1), second (DUC2) or third (DUC3) cell division cycle.

Participants/materials, setting, methods: t2-tPNf intervals were clustered into quartiles (Q1, <2hpi; Q2, 2–2.5hpi; Q3, 2.5–3hpi and Q4, >3hpi), which were compared based on embryo development and live birth

by linear and logistic regression. The Q1 cluster was set as control, while all other quartiles groups were considered as dummy variables. Multivariate analysis was conducted using the generalized estimating equations, to control for variable numbers of zygotes from each patient. The primary outcome was DUC rate.

Main results and the role of chance: Overall, rates of DUC1, DUC2 and DUC 3 rates were 10.3%, 8% and 2.9%, respectively. After adjusting for major confounders (maternal age, paternal age, maternal body mass index, cause of infertility, anti-Müllerian hormone, follicle stimulating hormone) we observed a higher DUC 1 and DUC 2 rate with increasing times of t2-tPNf. We observed abnormal distribution of DUC1 and DUC2 rate between quartiles groups. Specifically, 89% of DUC1 was shown among Q3 (21%) and Q4 (78%) and 61% of DUC2 among Q3 (22%) and Q4 (39%). The percentage of DUC1 was higher for Q3 (multivariate-OR = 3.29, 95%CI 0.99-10.85, adjusted-p = 0.01) and Q4 (multivariate-OR = 22.79, 95%CI 7.80-66.62, adjusted-p<0.01]. The percentage of DUC2 was higher only in Q4 (multivariate-OR = 2.58, 95%CI 1.13-5-57, adjusted-p = 0.01). Moreover, we observed a reduced blastulation rate in Q3 (multivariate-OR = 0.68, 95%CI 0.49-0.95, adjusted-p = 0.01) and Q4 (multivariate-OR = 0.27, 95%CI 0.19-0.38, adjusted-p<0.01). No significant differences were observed in DUC3 rate, top quality blastocyst, euploidy rate (PGT-A cycles analysis) and live birth rate.

Limitations, reasons for caution: The retrospective single-centre design and the limited sample size are limitations of the study

Wider implications of the findings: The study is consistent with the emerging view that the final phases of fertilization - although short - are crucial for development. Delays in progression towards mitosis probably express chromosome integrity loss and perturbances of chromosome segregation. In most embryos, such perturbances appear to cause trichotomous mitoses and cleavage arrest.

Trial registration number: Not applicable

Abstract citation ID: dead093.511
P-147 Comparison of Fertilization rate, Blastocyst rate and number of Top Quality Embryo with Short (2 hours) and conventional co-incubation (16-20 hours)of gametes

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Study question: Does short Co-incubation of gametes increase quality and quantity of embryos by eliminating suboptimal culture conditions like increased reactive oxygen species seen with overnight incubation?

Summary answer: Although short co-incubation group had lower fertilization rate it yielded more blastocysts and top quality embryos on day 3 and 5.

What is known already: Successful fertilization can occur 20 minutes after co-incubation of gametes. Incubation time of 18 hours has been used as a routine combining it with fertilization check. However it is known to produce reactive oxygen species (ROS) released by cellular metabolism of inseminated sperms which affect embryo development and cause zona pellucida hardening. Studies have shown reduced oocyte exposure time caused improved embryo development. However, other studies have reported lower fertilization rate with no such advantage. Not many studies have been done with short co-incubation without denudation which might allow optimum fertilization and at the same time prevent increase in ROS.

Study design, size, duration: It is prospective controlled study. 62 patients (921 oocytes) were recruited. The sibling oocytes from each patient were divided into 2 groups. Group 1(Control) conventional co-incubation of gametes (16-20 hours) and group 2 short co-incubation (2 hours) after which they were transferred to a fresh media. Study period was from January 2022 to September 2022. Institutional scientific and ethical committee approval and written consent of couple taken.

Participants/materials, setting, methods: Couples undergoing IVF in tertiary infertility centre were recruited. Women under 40 years who yielded minimum 6 oocytes and males with normal semen parameters were included. Those excluded were couples requiring ICSI, poor responders with <6 oocytes retrieved and couples requesting day 3 transfer. Primary outcome was number and percentage of top quality embryos (TQE) and blastocyst rate. Secondary outcome was fertilization rate. SPSS used for data analysis. Results expressed as mean +/-SD or percentage

Main results and the role of chance: Total oocytes studied 921 in 62 patients. Since control and study group were from sibling oocytes of same patient they were matched for age BMI, ovarian reserve, stimulation protocol, semen parameters and laboratory handling. Extra oocytes in odd number oocytes were randomized to 2 groups. Mean age was 31 years. Group 1 had 425 out of 455 (93.4%) oocytes fertilized and group 2 had 412 out of 466 (88.4%) oocytes fertilized (p value<0.05). TQE on day 3 were 251 of 425 (59.05%) fertilized in group 1 and 277 of 412 (67.23%) fertilized in group 2 (p = 0.013). Blasts formed in group 1 were 41 of 425 (9.64%) and in group 2 were135 of 412 (32.76%) (p = 0.001). TQE on day 5 were 28/425 (6.67%) in group 1 (0.45 +/−0.9 blasts per patient) and 95/412 (23.05%) in group 2 (1.53+/-1.86 blasts per patient). (P = 0.001) Percentage of grade 2 embryos on day 3 and 5 was significantly higher in short co-incubation group. Although fertilization was higher in group 1 many embryos did not progress beyond day 2 as both grade 1 and 2 embryos on day 3 and 5 were less in percentage and absolute per patient value in this group.

Limitations, reasons for caution: Pregnancy rate could not be calculated as some patients wanted 2 embryos transferred and one group had only a single blast. For their transfer blasts were taken from both groups. Very poor responders (<6 oocytes) not studied. Further studies needed to establish best time duration for short co-incubation (1-8 hours)

Wider implications of the findings: Practice for co-incubation after 16-20 hours needs to be re-evaluated. The study shows more top quality blasts with short insemination period. This may become the practice if it is established by further studies assuming that TQE are known to yield better pregnancy rates and IVF results

Trial registration number: not applicable

Abstract citation ID: dead093.512
P-148 The importance of filopodia during early human embryonic development

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Study question: The aim of this study was to examine the relation between the presence of filopodia and the embryo viability in human *in vitro* fertilised embryos.

Summary answer: The presence of filopodia related to a faster blastocyst development, a better blastocyst quality and higher morphokinetic scores which indicates a higher embryo viability.

What is known already: Filopodia are involved in numerous cellular processes, for example cell migration, neuronal extension growth and during embryonic development. There is no uniform name to describe these cytoplasmic string structures in the literature. Beside the frequent presence in different cell functions, we have limited information about its function in human embryos. In the early 2000s, the occurrence of filopodia or cytoplasmic strings at the blastocyst stage was classified as a negative sign of embryo viability, but more recently, it has been described as a positive feature, however, the importance and clinical significance of this structure is still controversial.

Study design, size, duration: Evaluation of the morphokinetic data on the development of 208 embryos from 78 IVF cycles. Examination was carried out at the Division of Assisted Reproduction, Department of Obstetrics and Gynaecology, Semmelweis University, Budapest between December 2020 and March 2021.

Participants/materials, setting, methods: Embryos from both conventional IVF (cIVF) and intracytoplasmic sperm injection (ICSI) treatments were analysed and evaluated. The dynamics of embryo development, embryo morphology and morphokinetic scores generated by time-lapse system was compared between the embryos with filopodia (FP+) and embryos without filopodia- (FP-) groups.

Main results and the role of chance: 81.2% of the embryos had filopodia in blastocyst stage. Embryos created by cIVF had 77%, while embryos fertilised by ICSI had 86% of filopodia presence ($p = 0.08$). FP+ embryos developed in a greater number into a higher quality blastocyst (52.1% vs 20.5%, $p = 0,02$), their KIDScore was higher (6.1 ± 2.1 vs 4,7 ± 2,07, $p < 0.001$) and they showed a tendency of higher implantation rate (39.7% vs 14.3% $p = 0.16$) than the FP- group. The dynamic of the early embryo development was similar between the two groups, however, FP+ embryos reached blastocyst stage significantly earlier (tB: 103.9 hours vs. tB: 107.6 hours; $p = 0.007$). Based on our results, there was a higher number of embryos with filopodia than without, and the presence of it is not related to the fertilisation method. These embryos developed faster into a blastocyst stage, their morphokinetic parameters were better. KIDScore and iDAScore embryo evaluation systems also recommend in a greater number the FP+ blastocysts for ET. We could also observe vesicle like transport along the filopodia, which leads us to believe that there might be a molecular communication between the differentiating cell populations.

Limitations, reasons for caution: Due to the low number of cases, the higher implantation rate is not significant.

Wider implications of the findings: The examination of this feature improves our knowledge of the early embryonic development and may help us to make decisions about the embryos with higher implantation potential.

Trial registration number: Not applicable

Abstract citation ID: dead093.513
P-149 Time is over: time-lapse technology to set time cut-offs defining embryo developmental incompetence

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Study question: Can time-lapse technology (TLT) discriminate developmentally incompetent embryos through time cut-offs of fertilization and cleavage stages?

Summary answer: TLT identifies developmentally incompetent embryos with high precision, through cut-offs of tPNa, tPNf, t2, t4 and t8. No clinically-applicable cut-offs were found for blastocyst euploidy.

What is known already: TLT is instrumental for continued and undisturbed observation of embryo development. This has produced morphokinetic algorithms aimed at selecting embryos able to generate a viable pregnancy. Such efforts have had limited success. Regardless, the potential of this technology for improving multiple aspects of the IVF process remains considerable. Specifically, TLT could be harnessed to discriminate developmentally incompetent embryos: i.e., those unable to develop to the blastocyst stage or affected by full-chromosome meiotic aneuploidies. If developed, this application would prevent the non-productive use of such embryos, improving laboratory and clinical efficiency and reducing costs derived from unproductive embryo transfer and cryopreservation.

Study design, size, duration: The training dataset involved embryos of PGT-A cycles cultured in Embryoscope with single- media (836 euploid blastocysts, 1179 aneuploid and 1874 arrested embryos; 2013-2020). Selection criteria were ejaculated sperm, fresh own eggs, trophectoderm biopsy, and comprehensive-chromosome-testing to diagnose non-mosaic aneuploidies. Out-of-sample (30% of training), internal (299 euploid blastocysts, 490 aneuploid and 680 arrested embryos; 2021- 2022) and external (97 euploid, 110 aneuploid, 603 untested blastocysts and 514 arrested embryos, 2018 to early-2022) validations were conducted.

Participants/materials, setting, methods: A training dataset (70%) was used to define thresholds. Several models were generated by fitting outcomes to each timing (tPNa-t8) and maternal age. ROC-curves pinpointed in-sample classification values associated with 95%, 99% and 99.99% true-positive-rate for predicting incompetence. These values were integrated with upper limits of maternal age ranges (<35, 35-37, 38-40,41-42 and >42 years) in logit functions to identify time cut-offs, whose accuracy was tested on the validation datasets through confusion matrices.

Main results and the role of chance: For developmental (in)competence, the best performing (i) tPNa cut-offs were 27.8hpi (error-rate: 0/743), 32.6hpi (error-rate: 0/934), 26.8hpi (error-rate: 0/1178), 22.9hpi (error-rate: 1/654,0.1%), and 17.2hpi (error-rate: 4/423,0.9%) in the <35, 35-37, 38-40, 41-42 and >42 years groups; (ii) tPNf cut-offs were 36.7hpi (error-rate: 0/738), 47.9hpi (error-rate: 0/921), 45.6hpi (error-rate: 1/1156,0.1%), 44.1hpi (error-rate: 0/647), and 41.8hpi (error-rate: 0/417) in the <35, 35-37, 38-40, 41-42 and >42 years groups; (iii) t2 cut-offs were 50.9hpi (error-rate: 0/724), 49hpi (error-rate: 0/915), 47.1hpi (error-rate:0/1146), 45.8hpi (error-rate: 0/636), and 43.9hpi (error-rate: 0/416) in the <35, 35-37, 38-40, 41-42 and >42 years groups; (iv) t4 cut-offs were 66.9hpi (error-rate: 0/683), 80.7hpi (error-rate: 0/838), 77.1hpi (error-rate:0/1063), 74.7hpi (error-rate: 0/590), and 71.2hpi (error-rate: 0/389) in the <35, 35-37, 38-40, 41-42 and >42 years groups; (v) t8 cut-offs were 118.1hpi (error-rate: 0/619), 110.6hpi (error-rate: 0/772), 140hpi (error-rate: 0/969), 135hpi (error-rate: 0/533), and 127.5hpi (error-rate: 0/355) in the <35, 35-37, 38-40, 41-42 and >42 years groups.

tPNf and t2 showed a significant association with chromosomal (in)competence, also when adjusted for maternal age. Nevertheless, relevant cut-offs were not clinically applicable.

Limitations, reasons for caution: Study limits are its retrospective design and datasets unbalanced towards advanced maternal age cases. The potential effects of abnormal cleavage patterns were not assessed. Larger sample size and external validations in other clinical settings are warranted.

Wider implications of the findings: If confirmed by independent studies, this approach could significantly impact on the efficiency of ART reducing the workload (extended culture, cryopreservation, and transfer) associated with embryos that ultimately are developmentally incompetent and should not be considered for treatment. Pending validation, these data might be applied also in static settings.

Trial registration number: N.A.

Abstract citation ID: dead093.514
P-150 Comparison of morphokinetic parameters of embryos generated from Capacitation In-vitro Maturation and Controlled ovarian stimulation-ICSI

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Study question: Is there any difference in the embryo morphokinetic parameters between embryos generated from Capacitation – In-vitro maturation (CAPA-IVM) and Controlled ovarian stimulation – ICSI (COS-ICSI)?

Summary answer: Embryos generated from CAPA-IVM cycles reached tPNa, t2, t4, t8, tSC, tM, tSB, and tB significantly later than COS-ICSI embryos.

What is known already: IVF with ovarian hyperstimulation has limitations in some subgroups of women at high risk of ovarian stimulation, such as those with polycystic ovary syndrome. IVM may be a feasible alternative to IVF in women with a high AFC. A recent randomized controlled trial produced statistically inconclusive findings regarding the noninferiority of the CAPA-IVM technique compared with COS-ICSI. Moreover, there is a lack of studies investigating the impact of CAPA-IVM on the morphokinetic parameters of embryos.

Study design, size, duration: This is a retrospective cohort study conducted from March 2021 to March 2022. The study included 115 couples (68

couples used CAPA-IVM, 47 couples used COS-ICSI) who had their embryos cultured to the blastocyst stage using a time-lapse system.

Participants/materials, setting, methods: The study was conducted at My Duc Phu Nhuan hospital, Ho Chi Minh City, Vietnam. Couples aged ≤40 years old, had blastocyst culture using time-lapse system were included in the study. Couples used oocyte or sperm donation, PGT treatment, had severe male factors, and fertilization failure in previous cycles were excluded. We analyzed 392 blastocysts from 115 couples for morphokinetic parameters including tPNa, tPNf, the onset of t2 to t8, tSC, tM, tSB, and tB.

Main results and the role of chance: There was no difference in tPNf, t3, and t5 between embryos from CAPA-IVM and COS-ICSI. However, embryos generated from CAPA-IVM reached tPNa, t2, t4, t8, tSC, tM, tSB, and tB significantly later than COS-ICSI embryos [tPNa (9.3 2.0 vs 8.0 1.9; $P < 0.001$), t2 (27.4 3.7 vs. 25.5 3.1; $P < 0.001$), t4 (38.7 4.5 vs. 37.3 4.3; $P = 0.004$), t8 (58.9 9.2 vs. 56.3 8.4; $P = 0.011$), tSC (76.2 11.1 vs. 71.4 10.4; $P < 0.001$), tM (90.4 8.6 vs. 85.5 10.0; $P < 0.001$); tSB (101 8.1 vs. 97.1 8.3; $P < 0.001$) and tB (108 7.6 vs 106 7.7; $P < 0.05$)]. The frequency of direct cleavage in the first and the second cell division cycle was similar between two groups (58.6% vs. 30.0%; 41.4% vs. 70%, respectively, $P > 0.05$).

Limitations, reasons for caution: The retrospective cohort study design could limit the ability to eliminate sources of bias. The characteristics of participants may influence the study findings, i.e. women in the CAPA-IVM group were PCOS while women in the COS-ICSI were not. In addition, the study population's single-ethnicity nature limits the results' external generalizability.

Wider implications of the findings: This study found that embryos from CAPA-IVM reached tPNa, tPNf, t2, t4, t8, tSC, tM, tSB, and tB significantly later than COS-ICSI. The findings of this study provide evidence for further studies to evaluate CAPA-IVM embryos and to improve the CAPA-IVM culture system.

Trial registration number: Not Applicable

Abstract citation ID: dead093.515
P-151 In women with recurrent implantation failures, can hyaluronan enriched media optimize reproductive outcomes?

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Study question: To evaluate the efficacy of Hyaluronan Enriched Media (HEM) to optimize Reproductive Outcomes in women with **Recurrent Implantation Failures (RIF)?**

Summary answer: In women with RIF, HEM seems to offer beneficial and safer reproductive outcomes.

What is known already: HEM has shown to enhance the implantation process of an embryo in-utero. Efficacy of HEM to optimize reproductive outcomes is still not proven.

Study design, size, duration: This retrospective study comprises data of 430 women with two failed IVF cycles or had RIF undergoing infertility treatment between 2016 to 2021. Study group included Young women (<37yrs) with RIF who opted elective-single blastocyst transfer (eSET) with HEM (n = 228). Control group had young women with RIF and eSET without use HEM (n = 202). Only self-gamete cycles with No PGT-A screening were included in this study. Women with known uterine pathologies were excluded from the study.

Participants/materials, setting, methods: All women had ICSI - freeze all culture strategy. In a FET, blastocyst showing 100% survival post warming was considered transfer. In study group, embryos were incubated in pre-equilibrated HEM for 10minutes before the intended embryo transfer. In control group embryo was transferred in single step media. ET procedure done as per the clinic's SOP. Outcomes measured: Clinical Pregnancy Rates (CPR), Implantation rates (IR), Miscarriage Rates(MR), Live Birth rates(LBR) and Neonatal outcomes.

Main results and the role of chance: Following were the outcomes measured in HEM group Vs Control group:
CPR- 66.22% Vs 61.38% ($p = 0.2938$)
IR – 49.56% Vs 31.68% ($p = 0.0002$; Significant)
MR – 11.4% Vs 16.33% ($p = 0.1359$)
LBR – 54.82% Vs 45.05% ($p = 0.0418$; Significant)

There was no significant adverse events with neonatal outcomes between the groups and data looked comparable.

Use of HEM at embryo transfer for women with RIF seemed to be a beneficial intervention. It showed significant improvement with embryo implantation and live birth. Data in this study has also looked at Neonatal outcomes. Since HEM group had no reported adverse neonatal outcomes, it not only aids in improving reproductive outcomes but also is a safe intervention and can be considered for routine clinical use for indicated cases.

Limitations, reasons for caution: Retrospective data with small and unequal sample size.

Wider implications of the findings: Efficacy of various therapies is still unknown for women who had RIF. PGT-A and ERA (invasive therapies) have been tried for such cases, but its effectiveness is still up in question. Development for newer & safer therapies to improve embryo implantation and live birth for these patients is still awaited.

Trial registration number: Not applicable

Abstract citation ID: dead093.516
P-153 Clinical outcome of different embryo transfer strategies after late rescue ICSI procedure: A cohort study based on over 140 thousand ART cycles

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Study question: How effective and safe is freeze-all blastocyst transfer in combination with late rescue intracytoplasmic sperm injection (r-ICSI)?

Summary answer: Freeze-all blastocyst stage embryo transfer (ET) serves as an optimal strategy to support late r-ICSI.

What is known already: Late r-ICSI has not been widely adopted due to the time-dependent in vitro deterioration of oocyte quality and endometrial growth not being synchronised with embryo development.

Study design, size, duration: This was a retrospective cohort study. All participants received treatment between 2009 and 2019. 2,270 patients in the aggregate encountered unexpected total fertilisation failure (TFF) during 149,054 cycles of in vitro fertilisation (IVF) and adopted a late r-ICSI procedure, whilst meeting the inclusion criteria for this study.

Participants/materials, setting, methods: Patients were grouped according to transfer strategies (926 women in Group 1 underwent fresh ET, 365 women in Group 2 underwent freeze-all ET, 716 women in Group 3 experienced blastulation failure, and 263 women did not have available D3 embryos). Patients received different ET strategies after r-ICSI, with the main outcome measures included live birth rate (LBR), cumulative live birth rate (cLBR), and conservative cLBR.

Main results and the role of chance: TFF occurred in 7.4 % of all IVF cycles. Group 1 tended to be older at oocyte retrieval [33.00 (30.00–37.00) vs. 31.00 (29.00–35.00), $P < 0.001$], with more infertile years [5.00 (3.00–8.00) vs. 3.00 (2.00–6.00), $P < 0.001$], higher follicle-stimulating hormone (FSH) levels [7.10 (5.71–8.69) vs. 6.61 (5.25–7.95) mIU/mL, $P < 0.001$], higher gonadotropin consumption [2850.00 (1950.00–4125.00) vs. 2325.00 (1800.00–3150.00) IU, $P < 0.001$], and fewer oocytes retrieved [10.00 (6.00–15.00) vs. 13.00 (9.00–19.00), $P < 0.001$]. Group 2 exhibited considerably better LBRs following the first ET cycle (37.53 % vs. 4.64 %) and cLBR (52.60 % vs. 8.21 %). After adjustment for covariates using binary logistic regression analyses, Group 2 still showed better obstetric performance in LBR (OR:11.77, 95 % CI (8.42–16.45)], cLBR (OR:11.29, 95 % CI (7.84–16.27)], and conservative cLBR (OR:2.55, 95 % CI (1.83–3.55)]. Additionally, the two groups showed similar miscarriage rates, whilst no new-borns with malformations or congenital diseases were reported.

Limitations, reasons for caution: This study was based on a retrospective cohort, which is inevitably associated with a skewed data distribution and inherent biases.

Wider implications of the findings: Our study highlights r-ICSI as a safe and effective alternate solution for unexpected TFF to aid frustrated couples. However, for women with limited oocytes available for r-ICSI use, weighing the benefits against the costs of the procedure might be prudent before implementing in vitro blastulation.

Trial registration number: NA

Abstract citation ID: dead093.517

P-154 Is there an impact of oocyte vitrification on embryological and clinical outcomes in PGT-A cycles? A propensity score matching-based study

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Study question: Does oocyte vitrification affect euploid blastocyst rate (EBR) per cohort of inseminated oocytes and/or clinical outcomes per euploid blastocyst transfer?

Summary answer: Vitrified-warmed oocytes are subject to ≈90% cryo-survival and lower blastulation rates than fresh cohorts, but similar euploidy and clinical outcomes per euploid blastocyst transfer.

What is known already: Cryopreservation was a game-changer in IVF. It is an essential step of the IVF journey with a plethora of clinical (e.g., reduction of OHSS while fully-exploiting the ovarian reserve, implementation of chromosomal/genetic testing, cumulative perspective in defining IVF success), logistic (e.g., higher flexibility), and social (e.g., fertility preservation) advantages. Vitrification represents the gold-standard protocol. Studies in sibling oocytes showed that vitrification may slightly reduce blastocyst development without impact on embryo chromosomal constitution. Here we aimed at measuring the impact (if any) of oocyte vitrification on EBR per cohort of inseminated oocytes and clinical outcomes per euploid blastocyst transfer.

Study design, size, duration: We gathered a dataset of first PGT-A cycles with vitrified-warmed (N = 54) or fresh (N = 3916) inseminated MII-oocytes (April-2013 to July-2022). Patients were matched 1:1 for maternal age at retrieval (37.2 ± 3.4 versus 37.1 ± 3.4 years) and MII-oocytes used (8.7 ± 4.1 versus 9.0 ± 5.1) through propensity-score-matching resulting in 49 cycles per arm. The primary outcome was EBR per cohort of inseminated MII-oocytes. The secondary outcomes were all embryological and clinical outcomes, including cumulative-live-birth-rate (CLBR) per concluded warming/fresh cycle.

Participants/materials, setting, methods: ICSI of all (cryo-survived) MII-oocytes, trophectoderm biopsy without day3 zona-drilling, and comprehensive-chromosome-testing to assess full-chromosome non-mosaic aneuploidies were performed. Cryopreservation was performed via vitrification. Only euploid blastocyst transfers were conducted. The groups were comparable also for BMI and sperm factor. The reasons for oocyte vitrification were supernumerary oocytes (N = 22, 45%; days between vitrification and warming: 573.7 ± 503.9), postponement of sperm collection (N = 7, 14%; 69.3 ± 113.4 days) and fertility preservation (N = 20, 41%; 1160 ± 779.7 days).

Main results and the role of chance: 8.7 ± 4.1 oocytes were warmed and 7.9 ± 3.7 survived (survival rate: 90.3 ± 14.7%). The zygotes were 5.4 ± 2.9 and 6.5 ± 4.0 in the vitrified-warmed and fresh groups, respectively (p = 0.2; fertilization-rates: 69.7 ± 22.3% versus 73.6 ± 18.4%, p = 0.5). The blastocysts were 2.1 ± 1.8 and 3.0 ± 1.9 (p = 0.01; blastulation-rates per zygotes: 34.9 ± 26.2% versus 49.4 ± 26.7%, p = 0.02). The euploid blastocysts were 1 ± 1.2 and 1.4 ± 1.6 (p = 0.2; euploidy-rates per biopsied blastocysts: 49.7 ± 34.3% versus 45.7 ± 36.3%, p = 0.5). The EBR per inseminated oocytes were 12.7 ± 13.3% and 17.5 ± 18.1% (p = 0.3). AA-blastocyst rates were also similar (N = 48/101, 47.5% versus N = 77/149, 51.7%, p = 0.6), while the day5-blastocysts rate was lower among vitrified-warmed cohorts (N = 12/101, 11.9% versus N = 51/149, 34.2%, P < 0.01). Nonetheless, the clinical outcomes per euploid transfer (LBR: N = 15/34, 44.1% versus N = 16/38, 42.1%, p = 0.99; miscarriage-rate per clinical pregnancy: N = 1/16, 6.3% versus N = 4/20, 20%, p = 0.4), gestational age (37.8 ± 1.5 versus 38.4 ± 1.4 weeks, p = 0.32) and birthweight (3471.7 ± 575 versus 3153.8 ± 449.3 g, p = 0.1) were comparable. 85.7% (N = 42/49) and 77.6% (N = 38/49) of the warming/fresh cycles were concluded with similar CLBR: 33.3% (N = 14/42) versus 39.5% (N = 15/38; p = 0.6). Among the 22 cohorts of vitrified-warmed oocytes because supernumerary, the EBR per inseminated oocytes was similar to their sibling fresh cohorts (11.7 ± 13.4% versus 13.8 ± 11.1%; p = 0.6). Also, 68.1% warming cycles were concluded (N = 15/22) eliciting 20% CLBR (N = 3/15), comparable to their sibling fresh cohorts (N = 4/22, 18.2%).

Limitations, reasons for caution: Retrospective design with limited sample size. About half of the patients in the vitrified-warmed group cryopreserved oocytes because supernumerary. These women were characterized by better ovarian reserve markers and response to ovarian stimulation than controls. Nonetheless, these parameters do not affect our primary outcome.

Wider implications of the findings: Oocyte vitrification is crucial in IVF. It involves higher flexibility with clinical (full ovarian reserve exploitation, oocyte accumulation), social (fertility preservation), and ethical (cryo-storage of supernumerary oocytes) benefits. Beyond a moderate impact (degeneration after warming, lower blastulation-rates), euploidy-rates and clinical outcomes are not affected by this procedure.

Trial registration number: not applicable

Abstract citation ID: dead093.518

P-155 MicroRNA-155 (miR-155) expression profile and developmental characteristics of bovine early embryos are altered when cultured in group versus individually

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Study question: Culturing bovine preimplantation embryos in group versus individually has an impact on the expression profile of miR-155 and developmental characteristics.

Summary answer: MiR-155 was significantly greater in its expression when embryos were cultured in groups versus singly. Developmental characteristics of blastocysts were similar regardless of culture condition.

What is known already: MiRNAs are short non-coding RNAs that play important roles in many biological pathways including embryogenesis and are potent effectors of post-transcriptional gene silencing. It has been estimated that approximately 60% of all proteins-coding genes are regulated by miRNAs. MiR-155 is involved in early events of embryogenesis; however, it has not been characterized in bovine embryos and this knowledge has not yet been clinically translated. Existing literature indicates that group culture yields superior embryo development as embryos can benefit from autocrine signaling and paracrine communication but embryos in groups may be exposed to negative effects of dying or delayed embryos.

Study design, size, duration: The study consisted of two groups: zygotes cultured to blastocysts in group and those cultured individually. Each sample consists of 3 blastocysts. Droplet digital PCR (dd-PCR) was used to examine the expression of miR-155. Gene Set Enrichment Analysis (GSEA) of miRNAs was carried out to identify functional Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways that are enriched for the targets of one or more miRNAs.

Participants/materials, setting, methods: Oocytes were aspirated from antral follicles of bovine ovaries and matured in vitro. Twenty-four hours post retrieval, matured oocytes were inseminated with sperm from similar bull. Presumptive zygotes were cultured either in groups or individually until

blastocyst stage, Blastocyst were collected and purified for RNA. Analysis for KEGG and GO Biological Process, Cellular Component and Molecular Functions were carried out on the identified target genes. Results were plotted according to level of enrichment for miR-155.

Main results and the role of chance: Five hundred and seventy-five oocytes were retrieved from 120 bovine ovaries. Cleavage rate and blastocyst formation after IVF were compared between those blastocysts cultured in groups versus individually. There was no significant difference among the two groups with regards to cleavage rate (69% vs. 54%) and blastocyst formation (42% vs.35%). ddPCR assessment showed significantly greater MiR-155 expression in those blastocysts cultured in groups ($p = 0.002$). The highest level of miR-151 expressed was 20 copies/sample. Every sample, regardless of the culture conditions, had detectable miR-155 level. The interaction of predicted miR-155 target clusters based on the GO Biological Processes shows that miR-155 plays a role in DNA binding, transcription factor binding, regulates transcription and affects cell adhesion. KEGG analysis revealed an association between miR-155 to steroidogenesis and controls apoptosis pathways.

Limitations, reasons for caution: The number of copies expressed for this miRNA was relatively low, which could suggest that miR-155 may not have a functional role at the blastocyst stage as the effectiveness of a miRNA depends on the relative number of binding site and target molecules. Blastocysts were pooled based on morphological assessment.

Wider implications of the findings: MiRNAs offer a novel mean to assess embryonic fitness. Further validation and more complex study designs are required to implement the profiling of embryonic miRNAs as a diagnostic test for embryo selection. Group culture increased miR-155 expression, possibly reflecting differences in cellular behavior between group cultured embryos and individually ones.

Trial registration number: Not applicable

Abstract citation ID: dead093.519
P-156 A statistical model to pinpoint oocyte donors subject to IVF outcomes significantly poorer than expected: a tool for egg banks' management and Research

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Study question: What is the prevalence of egg donors resulting in oocyte maturation/fertilization/blastulation rates significantly poorer than expected?

Summary answer: 6.7% of the egg donors (N = 34/504) elicited outcomes significantly poorer than expected mostly due to low oocyte maturation rates (N = 27/34, 79.4%).

What is known already: 5-10% of donor oocyte cycles produce no blastocyst involving challenging follow-up counseling. Recent research outlined a statistical model identifying women subject to IVF outcomes poorer than expected. These infertile women underwent whole-exome-sequencing (WES) to identify genes involved in oocyte-maturation-defects (OOMD) and/or preimplantation-embryo-lethality (PREMBL). Oocyte donors are the ideal population to apply this model, because they are young, theoretically-fertile, and undergo multiple retrievals of numerous oocytes. This model can find clinical application in improving egg banks' management, social application in pinpointing young women potentially-infertile because of oocyte defects, and improving knowledge on the molecular pathways involved in human oocyte/embryo competence.

Study design, size, duration: Retrospective study involving oocyte donors recruited at three private IVF centers in 2020-2021. 504 donors (25.6 ± 4.7 years; range: 18-35) undergoing 1684 retrievals (N = 32056 cumulus-oocyte-complexes, 24288 metaphase-II) and whose metaphase-II were used (N = 6996 to date) in 487 fresh and 445 frozen ICSI cycles, respectively, were included. Recipient couples affected from severe-male-factor infertility were excluded. Blastocyst culture was systematically conducted. We leveraged clinical/embryological key-performance-indicators to pinpoint outliers subject to outcomes significantly poorer than expected.

Participants/materials, setting, methods: We quantitatively described donors' performance as oocyte maturation, fertilization and blastulation rates. The outlier identification was based on the probabilistic modeling of the rates as independent binomial processes, whose baseline success probabilities were estimated from the whole dataset and accounted both for donor age and oocyte type (fresh/vitrified-warmed). The model assigns low probability to individuals with unusually large numbers of immature/unfertilized oocytes or arrested embryos. Donors with low probability ($p < 0.001$) were designated as outliers.

Main results and the role of chance: Oocyte donors underwent 3.3 ± 1.9 retrievals (1-8) collecting 63.6 ± 44.4 cumulus-oocyte-complexes (5-241), of which 48.2 ± 34.6 (3-162) metaphase-II (maturation-rate: 75 ± 13%,19-100). 13.9 ± 10.5 (3-75) metaphase-II were used during 1.8 ± 1.3 (1-9) cycles per donor, 1.0 ± 1.0 (0-6) cycles with 7.1 ± 7.6 (0-45) fresh metaphase-II oocytes and 0.9 ± 1.0 (0-7) cycles with 6.8 ± 8.1 (0-59) vitrified-warmed metaphase-II oocytes. Overall, 10.1 ± 8.0 (0-58) 2PN-zygotes were obtained per donor (fertilization-rate: 72 ± 16%,0-100), 5.1 ± 5.8 (0-34) in fresh (fertilization-rate: 72 ± 17%,0-100) and 5.0 ± 6.1 (0-41) in frozen cycles (fertilization-rate: 74 ± 16%,20-100). Overall, 5.5 ± 5.0 (0-31) blastocysts were obtained per donor (blastulation-rate: 55 ± 25%,0-100), 3.0 ± 3.8 (0-25) in fresh (blastulation-rate: 59 ± 25%,0-100) and 2.5 ± 3.6 (0-31) in frozen cycles (50 ± 24%,0-100). The model applied to this dataset designated 34 outliers (N = 34/504,6.7%, $p < 0.001$, FDR<0.1%). Most of them (N = 27) showed oocyte maturation-rates significantly lower than expected (51 ± 10%,19-66). These women were 26.9 ± 5.1 years old (19-35) and underwent 4.3 ± 1.7 retrievals, collecting 91.2 ± 45.0 cumulus-oocyte-complexes (25-210), of which 49.1 ± 31 (8-127) were metaphase-II oocytes. Three outliers showed fertilization-rates significantly poorer than expected (19 ± 17%,0-31). They were 21 ± 4.4 years old (18-26) and their metaphase-II oocytes (12.7 ± 4.9,7-16) were inseminated in 1.3 ± 0.6 donation cycles (1-2) resulting in 3 ± 2.6 (0-5) 2PN-zygotes. Four outliers showed blastulation-rates significantly poorer than expected (13 ± 7%,7-22SAME). They were 26.3 ± 6.2 years old (19-34) and their oocytes resulted in 28.5 ± 20.5 (14-58) 2PN-zygotes across 4.5 ± 3.1 (2-9) donation cycles that developed into 4.5 ± 5.7 (1-13) blastocysts.

Limitations, reasons for caution: Euploidy, another pivotal measure of oocyte competence, was not assessed. Larger studies are warranted, also accounting for putative additional confounders on all embryological outcomes under investigation. Nonetheless, gathering well-structured data on the cumulative performance of donor oocytes collected across multiple retrievals and through multiple IVF cycles is challenging and remarkable.

Wider implications of the findings: These outlier oocyte donors could benefit from follow-up investigation aimed at underpinning OOMD/PREMBL-related genetic factors. The development of genetic prediction tools to this end will improve egg donation cycles' management and equity and might pave the way to preventive screening strategies and precision reproductive interventions in theoretically-fertile young women.

Trial registration number: not applicable

Abstract citation ID: dead093.520

P-157 Improved embryo development and clinical outcome using bicarbonate buffer as the oocyte holding medium during Intracytoplasmic sperm injection (ICSI) compared to MOPS buffer

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Study question: Does bicarbonate buffer used as the oocyte holding medium during ICSI result in improved embryo development and clinical outcome compared to the zwitterionic buffer MOPS?

Summary answer: Pre-equilibrated bicarbonate buffer is acceptable for ICSI use in atmospheric conditions, and results in superior sibling-oocyte cohort study outcomes compared to ICSI with MOPS buffer.

What is known already: Maintaining proper pH is of the utmost importance during IVF micromanipulation procedures. However, currently used zwitterionic buffers, HEPES and MOPS, may significantly influence intrinsic biological mechanisms outside pH maintenance, impacting oocyte competence, embryo development, and clinical outcome. Zwitterionic buffer influence is of concern during ICSI because piercing the oolemma allows the influx of the surrounding buffer into the oocyte (Mendola unpublished data, 2022). Bicarbonate is the physiological buffer for pH maintenance in all mammals providing numerous benefits for cellular development.

Study design, size, duration: An iSTAT handheld blood analyzer was used to analyze the pH shift of pre-equilibrated bicarbonate buffer dishes to determine the acceptablity for use during ICSI in atmospheric conditions. A clinical sibling-oocyte cohort study consisting of 115 patients and 1,997 oocytes (1,016 bicarbonate/981 MOPS) was analyzed to compare the embryo development and clinical outcomes of bicarbonate buffer vs. MOPS as the oocyte-holding medium during ICSI. Morphokinetic time points for all PGT-A tested embryos were also compared.

Participants/materials, setting, methods: We analyzed the pH shift of pre-equilibrated bicarbonate buffer in atmospheric conditions using 50x9mm culture dishes (Falcon, cat#351006) comparing light and high viscosity paraffin oil overlay, OvOil and Heavy OvOil (Vitrolife), after 5-minute, 10-minute, and 15-minute intervals. We analyzed all data points in triplicate. We analyzed the sibling-oocyte cohort study comparing fertilization rate, abnormal fertilization rate, useable blastocyst rate, mosaicism rate, euploidy/aneuploidy rate, morphokinetic time points, pregnancy rate, and ongoing pregnancy rate.

Main results and the role of chance: Pre-equilibrated (6.5%CO2/5.0%O2/7.301pH) bicarbonate buffer in 50 × 9mm ICSI culture dishes, with 5.0mL of OvOil overlay, maintained proper pH (7.301/7.328/7.354pH) for the time required to complete the ICSI procedure in atmospheric conditions (0min/5min/10min). The use of the high viscosity paraffin oil overlay, Heavy OvOil, reduced pH drift of the bicarbonate buffer ICSI dishes (7.301/7.323/7.342pH). In this clinical sibling-oocyte cohort study, ICSI was performed in pre-equilibrated bicarbonate buffer culture drops (6μL) in the 50x9mm dishes with 5.0mL Heavy OvOil overlay.

Utilization of bicarbonate buffer as the oocyte-holding medium during ICSI is superior to MOPS buffer. Bicarbonate buffer used with ICSI resulted in higher fertilization rates (85.4% vs. 78.8%, $P<0.0001$), higher useable blastocyst rate per ICSI oocyte (50.6% vs. 46.2%, $P<0.05$), lower abnormal fertilization rate (4.5% vs. 6.7%, $P<0.05$), and a lower whole chromosome mosaicism rate (4.2% vs. 8.2%, $P<0.03$) compared to the MOPS buffer. Pregnancy rates were higher in the bicarbonate group compared to MOPS but not statistically significant. Morphokinetic analysis revealed a slight delay in all MOPS developmental time points compared to bicarbonate, but only statistically significant in the time to compaction ($P<0.03$).

Limitations, reasons for caution: During this study, different genetic testing companies, with differing mosaicism thresholds and decisions on included ploidy results, were used for PGT-A analysis of patient samples.

Wider implications of the findings: We determined that pre-equilibrated bicarbonate buffer can be used for ICSI in atmospheric conditions, therefore avoiding the potential detrimental effect of zwitterionic buffer after influx into the oocyte from the ICSI procedure. Bicarbonate buffer used during ICSI was determined to be superior to MOPS buffer.

Trial registration number: Not applicable

Abstract citation ID: dead093.521

P-158 Effect of women body mass index on in vitro embryos development and effectiveness of «Assisted reproductive technologies»

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Study question: Does a woman's body mass index (BMI) affect the development of an embryo in vitro?

Summary answer: Evaluation of the development of embryos indicates that the overweight affects the development of the embryo and associated with a lower rate of live births.

What is known already: Excess weight and obesity - as a cause of infertility, occures against the background of existing metabolic disorders. Severe thinness and, like obesity, often lead to violations of the interval and reproductive functions of a woman. An increase in adipose tissue of more than 20% or more leads to dysfunction of the hypothalamic-pituitary-ovarian system. With obesity and emaciation of the body, a woman has various forms of menstrual disorders, the frequency of uterine bleeding and also endometrial pathology increases.

Study design, size, duration: This study included the retrospective analysis of Assisted Reproductive Technologies (ART) cycles using statistical data on the development of embryos in the Embryological laboratory, pregnancy and childbirth was carried out from 2016 to 2018. Morphological studies were carried out using Time-Lapse embryo imaging.

Participants/materials, setting, methods: In this study, morphological and biostatistical methods were used. The cohort of patients aged 20 to 45 years was divided into four groups: 1623 embryos from 243 underweight women; 19542 embryos from 2270 normal weight women (control group); 9701 embryos from 1069 overweight women; and 2668 embryos from 343 obese women.

Main results and the role of chance: In the group of underweight women the pregnancy rate is 46%, which is 3% less than in overweight and obese group of women, while the percentage of the control group is 47.5%. However, the results of live births in group of women with obesity is 7% less than in women of normal weight, which is 29 and 36 percent respectively. In addition, a comparative analysis of the number of fertilized eggs showed that at a normal BMI, 6.3 ± 0.4 oocytes were fertilized, while in the group of women with second-degree obesity only 4.2 ± 0.3 oocytes. Based on the results of the T-test, it can be concluded that these indicators have differences with a significance level of less than 0.05. Also, the results obtained indicate differences in the maturity of embryos at the blastocyst stage, where blastocysts of excellent quality matured in the control group of 3.4 ± 0.4 embryos, and in the experimental group with obese women 2.4 ± 0.1 embryos. Furthermore, the frequency of live births showed a relationship between the norm and high BMI. In the control group, the result was 0.7 ± 0.02, while in first-degree obesity, the result was 0.2 ± 0.06.

Limitations, reasons for caution: The main limitation of this study is a verity of women with different anamnesis, which were not taken into account. The grading of blastocysts is subjected to a human factor. The limitation of the Time-lapse technology is the inability to rotate the embryos making it very difficult to observe.

Wider implications of the findings: This method needs further investigations about limitations, such as the age of patients, type and heaviness of infertility, existing diseases and previous pregnancies in anamnesis.

Trial registration number: Not Applicable

Abstract citation ID: dead093.522
P-159 Impact of the symbiotic metabolites D-amino acids on preimplantation development

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Study question: Are the metabolites derived from the symbiotic microbiota of reproductive tract involved in preimplantation development?

Summary answer: The metabolites derived from the symbiotic microbiota of reproductive tract, D-amino acid(D-AA), contributed to preimplantation development.

What is known already: It has been known that *Lactobacillus* exist in the vagina as symbiotic bacteria. Though it was thought that *Lactobacillus* keep the pH environment acidic and prevent infection by pathogenic bacteria, thereby keeping the uterus and fallopian tubes sterile in mammal, we have identified *Staphylococcus aureus* in oviductal lavage fluid from female mice and confirmed that the antibiotics disrupt the implantation pattern.

In human, the vaginal microbiota is reported to be dominated by *Lactobacillus*, which produces D-amino acids (D-AA). These findings suggest the involvement of bacterial metabolites D-AA in the establishment of human pregnancy.

Study design, size, duration: 23 women undergoing *in vitro* fertilization (IVF) treatment at Keio University Hospital from 2019 to 2021 were assessed for eligibility. 3 were excluded due to spontaneous pregnancy or infectious disease. Twenty subjects were allocated to the successful pregnancy group (S-group (8); mean ± standard deviation 35.5 ± 2.8 years) defined as gestational sac detected on ultrasound at 5 weeks' gestation; and to the unsuccessful pregnancy group (U- group (12); 35.5 ± 2.7 years)).

Participants/materials, setting, methods: Genomic DNA (gDNA) extracted from vaginal and intrauterine samples at each menstrual cycle was used for metagenome analysis at the genus and the species levels by next-generation sequencing, and by quantitative real-time PCR. Amino acids were analyzed using a two-dimensional HPLC.

Immunocytochemical staining by a polyclonal antibody to D-AA oxidase (DAO), which catabolizes D-AA, was performed using B6D2F1 mice and mice with a natural point-mutation G181R in DAO gene, which abolishes DAO activity.

Main results and the role of chance: Both vaginal/uterine bacterial counts were higher in the luteal phase than in the follicular phase. Vaginal bacterial counts in the S-group were lower than those in the U- group. The composition of microbiota of the vaginal samples (N = 23) shifted from the follicular to the luteal phase, with *Lactobacillus spp*. predominating. However, there was no significant difference in the ratio of *Lactobacillus spp*. between the S- and U-groups. The intrauterine samples of the S-group contained a high abundance of *L. crispatus, L. gasseri*, and *L. ultunensis*, while the U-group contained a high abundance of *L. jensenii*.

Next, we analyzed the *Lactobacillus* metabolite, D-AA, and detected D-Ser, D-Ala, D-Asn, and D-Pro in the vaginal, uterine, and follicular fluid of the S-group. Follicular fluid that developed into blastocysts contained more D-AA than that of arrested embryos (N = 2). To investigate the significance of D-AA in the preimplantation development, mouse embryos were cultured in amino acid-depleted medium. All of them arrested (0.0%: 0/78), whereas 41% (25/61) of the embryos with D-AA added to the amino acid-depleted medium developed into blastocysts. We confirmed the expression of DAO in oocytes and blastocysts. DAO-null mice had fewer births than wild type.

Limitations, reasons for caution: The main limitation is the differences of symbiotic microbiota in reproductive tract and embryonic development between mice and humans; it may be difficult to apply the results of this analysis to humans.

Wider implications of the findings: Our results display a correlation between species-level microbiome profiling and IVF outcomes. In addition, microbiota-derived metabolites D-AA are suggested to be crucial mediators of embryo development. These findings could give rise to the development of new diagnostic techniques and drug.

Trial registration number: Not applicable

Abstract citation ID: dead093.523
P-160 Trophectoderm biopsy techniques with and without laser use and their contribution to PGT-A results in sibling embryos

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Study question: Do the methods used in trophectoderm biopsy and the use of laser affect the genetic results of the harvested cells and the embryos thus examined?

Summary answer: The different methods of trophectoderm biopsy showed no differences in terms of euploidy rate and the specific type of chromosomal abnormalities.

What is known already: Next generation sequencing (NGS) is a comprehensive method for the genetic screening of preimplantation embryos and is now favored by many clinics. The method can detect structural abnormalities on all chromosomes and offers better reproductive success compared to earlier tests. However, as the test is very sensitive, biopsy of the embryo and treatment of the biopsied embryos and harvested cells are important to improve diagnostic success. Contamination of the biopsied sample can affect the results of the genetic test and lead to misdiagnosis.

Study design, size, duration: A prospective, randomized controlled trial of 44 couples undergoing infertility treatment with pre-implantation genetic screening (PGT-A, NGS) at the British Cyprus IVF Hospital between October 2021 and December 2022.

Participants/materials, setting, methods: PGT-A cases with more than two blastocysts were included in the study. Randomization was performed on days 5 and 6 of embryo development and embryos were divided into two groups before the biopsy. In group 1, embryos were biopsied using the flicking method without laser assistance. In group 2 embryos, cells were removed by pulling the desired cells with 3 to 5 laser shots at the edge of the selected cell group.

Main results and the role of chance: PGT-A results of 288 blastocyst-stage embryos from 44 cases were analyzed. 136 embryos were in group 1 (flicking) and 152 to group 2 (laser). The embryos in the groups were siblings, so the basic patient characteristics, number of eggs retrieved, sperm characteristics, and early embryonic details were identical between the groups. The mean number of retrieved cells was 6.19 ± 0.915 and 6.14 ± 0.849 ($p = 0.764$), the number of euploid embryos was 49/122 (40.2%) and 57/137 (41.6%) ($p = 0.814$), the number of embryos with whole chromosome aneuploidy was 46/73 (63%) and 58/80 (72.5%) ($p = 0.209$) and embryos with complex aneuploidy (more than two abnormal chromosomes) were 30/73 (41.1%) and 40/80 (50%) ($p = 0.268$) in group 1 and group 2, respectively.

Limitations, reasons for caution: As this is a preliminary research result, the small sample size is the major limitation. Mosaicism, which is common in preimplantation embryos and has a significant impact on the results, was not investigated.

Wider implications of the findings: Although the present data showed no differences between the two biopsy procedures in terms of PGT-A test results, invasive embryo biopsy procedures can damage embryos or misdirect genetic test results. Therefore, validation and universalization of the method for embryo biopsy are needed, which is emerging in recent publications.

Trial registration number: not applicable

Abstract citation ID: dead093.524

P-161 Impact of physical activity (PA) on oocyte quality: a prospective study among IVF/ICSI patients

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Study question: To determine if various levels of PA have an influence on oocyte quality, ovarian response to controlled stimulation and IVF/ICSI success rate.

Summary answer: Globally, PA has no influence on ovarian response in IVF cycles. However, in certain subgroups (endometriosis, disovulation, normal-BMI) high PA is associated with higher response.

What is known already: Lifestyle is considered a key factor concerning health. According to WHO, moderate PA is an effective way to lower the risk of many pathologic conditions, although the impact of PA on female fertility is still unknown.

There is scientific evidence that vigorous exercise can be detrimental for female fertility Studies evaluating the effect of PA on oocyte quality are lacking. However, murine models show that PA may have a positive impact on it.

Study design, size, duration: Prospective observational study.

PA was evaluated before the IVF cycle, using the International PA Questionnaire (IPAQ) (n = 617), assessing frequency and duration of PA in the previous week, and using accelerometer (n = 102). Mean age of women was 35.

Patients were classified into three levels of PA: low, moderate, high. The following outcome measures were analysed: number of collected oocytes, mature oocytes, ovarian response, biochemical pregnancy rate.

Size: 617 infertile women undergoing IVF/ICSI cycles

January 2019 - October 2020

Participants/materials, setting, methods: 617 participants voluntarily fulfilled the IPAQ prior to IVF/ICSI cycle. Ethical approval was obtained from the Clinical Research Ethics Committee (CEIC E19/06).

IPAQ's results were measured in metabolic equivalent of task (METs-min/week) (Walking = 3.3 METs; Moderate PA = 4 METs; Vigorous PA = 8 METs). 1 MET = resting metabolic rate.

In 102 participants PA was objectively measured for 7 consecutive days, prior to IVF cycle, using triaxial accelerometry.

Setting: Human Reproduction Unit of a Universitary Hospital (Spain)

Main results and the role of chance: The number of collected oocytes was similar in all three groups according IPAQ (9.23 ± 7.72; 8.35 ± 5.57; 8.82 ± 6.38). Something similar happened with the number of mature oocytes (MII) (6.97 ± 5.99; 6.84 ± 4.85; 7.05 ± 5.61) and fertilized oocytes (3.72 ± 3.84; 4.16 ± 3.35; 3.98 ± 3.88).

Biochemical pregnancy rate was slightly superior in high PA vs moderate vs low (38% vs 34.8% vs 29%), but without statistical significance.

In the subgroup of patients having endometriosis the number of MII oocytes was significantly superior in high and moderate vs low PA ($p = 0.024$). In the group of disovulating women there were also more MII oocytes in high vs moderate vs low PA ($p = 0.038$).

When performing the analysis according to accelerometer, even though the number of total collected oocytes (9.93 ± 9.1; 7.93 ± 5.38), MII oocytes (8.36 ± 7.64; 6.79 ± 4.63) and fertilized oocytes (5.25 ± 6.41; 3.91 ± 2.82), tended to be slightly superior in high vs moderate PA, there were no significant differences.

In women with normal BMI, high PA was associated with a greater number of collected oocytes ($p = 0.005$), MII oocytes ($p = 0.004$) and fertilized oocytes ($p = 0.007$).

Limitations, reasons for caution: This study was performed in infertile women undergoing IVF/ICSI cycles. Hence, it is not possible to determine if PA has an impact on natural fertility.

The self-administration of IPAQ has some disadvantages as subjectivity, memory bias or overestimation of PA. Subgroups' sample size was small.

Wider implications of the findings: In general it does not seem that modifying short-term PA in women undergoing IVF could improve oocyte quality. However in some subgroups of patients (endometriosis, disovulation, normal-weight women), perhaps changes in PA could improve oocyte quality and ART success rates. More prospective clinical studies are needed.

Trial registration number: Not applicable

Abstract citation ID: dead093.525

P-162 Numeric Embryo Quality Scoring Index (NEQsi) is Predictive of IVF Cycle Outcome at any Endometrial Thickness

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Study question: Is it possible to use NEQsi to determine the statistical contributions of embryo quality and endometrial thickness (ET) to IVF cycle outcome?

Summary answer: Yes. Analytics revealed that the likelihood of pregnancy is reliably predicted by the quality of the embryo at any endometrial thickness.

What is known already: ET is widely utilized to assess the quality of endometrial preparation in IVF cycles. The connection between endometrial thickness and cycle outcome is divided in the literature; studies report correlation between ET and cycle outcome while others find no link between the two. Regardless, the statistical interactions between ET and embryo quality remain understudied. NEQsi was recently developed as a method for converting Gardner embryo grades to interval variables for use in statistical modeling. The combination of NEQsi scores and endometrial thickness measurements provides an opportunity to assess the contributions of embryo quality and ET on IVF cycle outcomes.

Study design, size, duration: We conducted a retrospective analysis including IVF cycles conducted at a Canadian multi-clinician fertility care centre (n = 1623). Data were acquired from a chart review of deidentified patient files from 2014 – 2021.

Participants/materials, setting, methods: Participants were all patients who underwent single embryo transfer and had endometrial thickness measurements, Gardner embryo grading, and ultrasound based endometrial receptivity testing (usER) as part of their care program. No interventions were made. Gardner embryo grades on file were created using EmbryoScope (Vitrolife, Göteborg Sweden) and converted to NEQsi scores. We conducted multivariate statistical modelling analysis to determine the combined contribution of embryo grade and endometrial thickness on IVF cycle outcome.

Main results and the role of chance: NEQsi scores ranged from 3 – 11 (mean 8.32 ± 1.78 SD). Endometrial thicknesses ranged from 3.1 mm to 20.2 mm (mean 9.69 ± 2.23 SD). A positive linear relationship was observed between NEQsi score and probability of pregnancy. Multivariate logistical regression determined that embryo grade was a highly significant predictor of cycle outcome ($p < 0.0001$). Endometrial thickness was not a statistically significant predictor of cycle outcome ($p = 0.585$).

Limitations, reasons for caution: We acknowledge that the single centre design is a limitation of this study. Future investigation with more cycles and multiple centres will strengthen the study and interpretation of the data.

Wider implications of the findings: The application of NEQsi was simple and this work confirms the utility of the NEQsi in conducting statistical analyses with embryo quality as a covariate. In addition, our findings imply that

cancellation of embryo transfers based on ET measurements may not be warranted.

Trial registration number: Not applicable

Abstract citation ID: dead093.526
P-163 Three-year follow-up of babies born derived from mono-pronuclear zygotes

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Study question: Are neonatal outcomes normal up to 3 years of ages from the transfer of blastocysts derived from mono-pronuclear(1PN) zygotes?

Summary answer: There was no effect on growth up to 3 years of ages in babies born from 1PN-implanted blastocysts.

What is known already: In human ART, 1PN zygotes are observed at low rates. We have previously reported that 80.7% of 1PN zygotes had a biparental chromosome using a Live-Cell imaging technique, and some of these developed to the blastocyst stage (Tokoro et al. ASRM 2013). Furthermore, we have reported that these blastocysts can result in a viable pregnancy and healthy live birth (Tsuji et al. ASRM 2020), and there was no effect on growth up to 18-months (Tsuji et al. ESHRE 2021). However, there is no information on the long-term prognosis of babies derived from1PN-zygotes.

Study design, size, duration: This was a retrospective study which included 86 cases where there was a live birth after single embryo transfer of a blastocyst derived from 1PN zygote. The incidence of birth defects, birth weight were recorded as well as a physical development survey of 19 children who responded to a 3-years follow-up survey. The time period was 108 months (January 2013 to December 2021).

Participants/materials, setting, methods: Patients seeking fertility treatment at an established private IVF clinic. We compared the birth weight, 3-years old height and weight of children born from 1PN zygotes with data from a control, 2PN group. Statistical significance was determined using the t-test (level of $P < 0.05$).

Main results and the role of chance: The incidence of birth defects in 1PN embryo-derived infants was 1.2% (1/86). The average birth weight of boys in the 1PN group was 3147.6+/-408.7 g, which was not significantly different from 3070.5+/-479.5 g in the 2PN group. In girls, the average birth weight was 3048.1+/-510.7 g in the 1PN group, which was not significantly different from the 2PN group (2966.2+/-462.7 g). The average height at 18-months, was 81.3+/-2.5 cm vs 80.9+/-3.4 cm for boys; 79.5+/-2.2 cm vs 79.5+/-3.0 cm for girls in the 1PN and 2PN groups, respectively. The average body weights of the 1PN and 2PN groups were 11.0+/-1.2 kg vs 10.8+/-1.1 kg for boys; 9.8+/-1.0 kg vs 10.2+/-1.1 kg for girls, respectively. The average height at 3-years, was 95.2+/-3.1 cm vs 93.8+/-4.5 cm for boys; 91.5+/-2.9 cm vs 92.5+/-4.9 cm for girls in the 1PN and 2PN groups, respectively. The average body weights of the 1PN and 2PN groups were 14.7+/-1.2 kg vs 14.1+/-1.5 kg for boys; 13.2+/-1.9 kg vs 13.6+/-1.5 kg for girls, respectively. There was no significant difference in average height and weight up-to the 3-years follow-up survey.

Limitations, reasons for caution: The incidence of 1PN- zygotes that develop to blastocysts resulting in births is low and the study was limited to cases of single blastocyst embryo transfer.

Wider implications of the findings: The incidence of congenital anomalies in Japan is around 1.7 to 2%, and the incidence was similar in the 1PN group. Also, there was no difference in 3-years follow-up survey of the 1PN compared with the 2PN. These data are reassuring for the clinical utility of 1PN derived embryos.

Trial registration number: not applicable

Abstract citation ID: dead093.527
P-164 Promoting embryo plating and outgrowth with extracellular vesicles

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Study question: Can extracellular vesicles promote embryo plating and improve embryo outgrowth?

Summary answer: The addition of extracellular vesicles to the media and the use of assisted hatching can promote embryo plating and outgrowth.

What is known already: Effective and synchronous embryo-maternal cross-talk is required for proper implantation of the embryo into the receptive endometrium. Extracellular vesicles (EV) are imperative in embryo-maternal crosstalk and successful implantation (Das and Kale, 2020). Nowadays, embryo implantation still has some unexplainable and unsolvable issues which further leads to implantation failure in the field of reproductive medicine research which may result from the lacking of the EV between the endometrium and the embryo communication.

Study design, size, duration: Total 35 mice with total 176 embryos were utilised. Each run, embryos were cultured to blastocyst stage, total 4 experiment run and 3 control run were conducted. The embryo cell culture was performed on the 96-well cell culture plate coated with matrigel and treated with cleavage media and extracellular vesicles additive. Each run, the size of the embryo outgrowth area were recorded and measured every 24 hours for 4 consecutive days.

Participants/materials, setting, methods: We performed 4 types of assisting hatching (AH) to the embryo: 25%AH, 50%AH, single shot AH (1AH) and with an embryo hatching control (without AH performed) then cultured in 4 different culture treatments: 10% foetal bovine serum (FCS) as culture control and 3 different extracellular vesicles concentrations: 0%EV, 2.5%EV, 5.0%EV. The outgrowth area of plated embryos were measured. The significance in outgrowth size at 4 different time point was estimated by repeated 2-way ANOVA.

Main results and the role of chance: Results are divided based on the treatment and the assisted hatching type, 2.5%EV media additive resulted in significantly larger outgrowth areas at 96 hours post plating ($p = 0.0463$) which proves that with the addition of the extracellular vesicles in the culturing can promote the embryo outgrowth. And within 4 AH types, all showed significantly larger outgrowth at 96 hours post plating (a.$p = 0.0009$, b.$p < 0.0001$, c.$p < 0.0001$, d.$p < 0.0001$) which prove that with the use of assisting hatching to the embryo can improve the embryo outgrowth.

Limitations, reasons for caution: Experiment limitation appeared in the embryo orientation, where we could not control the embryo direction when it plated to the matrigel which affected the outgrowth direction, some of our embryo's outgrowth showed vertical growth rather horizontal growth, which resulted a small outgrowth area when we were measuring it.

Wider implications of the findings: 5.0%EV had poorer outcome in the outgrowth. 50% zona breaching caused embryo death. Therefore, further studies can look into finding the most suitable EV and AH for the embryo and look into adding EV to the transfer media in murine model to assess implantation.

Trial registration number: not applicable

Abstract citation ID: dead093.528
P-165 Artificial removal of the zona pellucida at the pronuclear stage in patients with poor quality embryos with severe fragmentation

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Study question: Is artificial removal of the zona pellucida (ZP) at the pronuclear stage effective in patients with poor quality embryos with severe fragmentation?

Summary answer: Artificial removal of the ZP at the pronuclear stage significantly improved culture results in patients with poor quality embryos with severe fragmentation.

What is known already: In 2020, Yumoto et al. first reported that artificial removal of the ZP (ZP-free) at the pronuclear stage decreased the rate of fragmentation and improved the culture outcomes of 3 pronuclei (3PN) embryos reaching to the good blastocysts. Therefore, the ZP may not always be necessary for normal embryonic development after the pronuclear stage. The same group presented at the 37th ESHRE meeting in 2021, that the blastocyst transfer after ZP-free embryo of 2 pronuclei (2PN) resulted in normal pregnancy in two patients with poor-quality embryos resulting in live births.

Study design, size, duration: This study included 211 of 2PN embryos from 46 cycles for 30 couples obtained between July 2021 and December 2022, whose previous oocyte retrieval cycle showed massive cytoplasmic fragmentation at the first cleavage confirmed by a time-laps incubator (TLI). Average age of women was 38.8years. The embryos with artificial removal of the ZP at the pronuclear stage were cultured in a TLI up to 7 days. All blastocysts were cryopreserved for future embryo transfer cycles.

Participants/materials, setting, methods: All participants provided written informed consent, and Institutional Review Board approval was obtained. Artificial removal of the ZP at the pronuclear stage was performed by following procedure. 1) Embryos were placed in 0.125M sucrose-containing HEPES for cytoplasm shrinking. 2) The ZP without adhesion was removed with a laser system. 3) Ooplasms were completely separated from ZP by blowing the medium to adhesion point like a jet car wash. 4) ZP-free embryo was obtained.

Main results and the role of chance: Day 3 embryos were divided into 3 groups using the Veeck's classification: 8 cell embryos with grade 2 or higher were defined as good, embryos with grade 4 or 5 regardless of the number of cells were defined as poor, and others were defined as fair. We compared the culture results of ZP-free cycle and previous oocyte retrieval cycle (ZP-intact). For day 3 embryos, good, fair and poor grade were 19.4% (41/211), 62.6% (132/211) and 18.0% (38/211) in ZP-free embryos compared with 2.9% (15/561), 37.4% (210/561), and 59.9% (336/561) in the past, respectively ($p < 0.001$). Good-quality blastocysts were defined as 3BB or more on day 5 by the Gardner's classification. As to the blastocysts, blastocysts development and good-quality blastocysts rates were 45.0% (95/211) and 27.0% (57/211) in ZP-free embryos compared with 23.0% (112/488) and 6.8% (33/488) in the past, respectively ($p < 0.001$). Of these, 30 embryo transfers were performed resulting in 11 clinical pregnancies. We had 3 live births without any congenital anomalies and one miscarriage. One was delivered at 33 weeks of gestation with caesarian section, one mother had gestational diabetes.

Limitations, reasons for caution: This study was retrospective study without any control group and was conducted at only single fertility center. It is necessary to conduct sibling oocytes study to demonstrate the effectiveness of ZP-free culture, and to create criteria who are benefit from ZP-free culture. Moreover, safety of ZP-free culture should be evaluated.

Wider implications of the findings: ZP-free culture reduced fragments and improved culture outcomes in patients with poor quality embryos with severe fragmentation. ZP-free culture might bring light to the patients who have difficulty obtaining good-quality embryos with severe fragmentation.

Trial registration number: Not Applicable

Abstract citation ID: dead093.529

P-166 The association between pronuclei parameters and blastocyst formation — a pixel-level time-lapse imaging study based on AI segmentation

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Study question: Do the morphokinetics of pronuclei in zygote associate with embryo developmental potential?

Summary answer: The position, distance to the central, size and fading time point of pronuclei were associated with blastocyst formation.

What is known already: Pronucleus is the nucleus formed by sperm or oocyte during fertilization containing the genetic material, which is important for further embryo development. We previously developed an AI-based morphology segmentation system for time-lapse imaging and it is able to capture the morphology changes and movement of pronuclei at the pixel level with high accuracy. In this study, we analysed the pixel-level morphokinetics of pronuclei with the blastocyst formation of the embryo under time-lapse incubation.

Study design, size, duration: The study was conducted in a university-affiliated IVF clinic from 2017 to 2019. There were 631 normal fertilized embryos with known blastulation results. They were cultured in the time-lapse incubator and taken images every 10 minutes from fertilization to embryo transfer/embryo freezing/discard. The images were extracted and the area of each pronucleus was segmented by the AI system. We reconstructed the segmented area of the pronucleus and count the morphokinetics parameters.

Participants/materials, setting, methods: The parameters included size, distance to the central, distance between each pronucleus, the movement pathway of each pronucleus and the fading time point. Quartiles of the parameters were divided into 25th (Q1), 50th (Q2), 75th (Q3) and 100th (Q4). In each quartile, the median of this quartile was presented with IQR. The chi-square test was used for the distribution comparison of the blastocyst formation. The FDR method was used to adjust the multi-comparison *P*-value.

Main results and the role of chance: We defined the pronucleus that was close to cortical as pn2 while the other was pn1. For the distance from each pronucleus to the zygote's geometric centroids (pn1_dist and pn2_dist), though pn1_dist did not show any significance, the pn2_dist showed a higher blastulation rate in Q2 (Q2:29.10% vs 17.90%). For the distance between the pronucleus (pn1_pn2_dist), the closer of PN got less blastocyst formation outcomes (Q1: 21.39% vs 31.44%). The results in the size of pronuclei (pn1_sz and pn2_sz) both showed that there was less blastocyst formation in small PN size (pn1_sz: Q1:19.90% vs 34.06%; pn2_sz: Q1:20.15% vs 33.19%). The duration from fertilization to pronuclei fading also had different blastulation rates. In the longest duration, there were the least blastocyst formation outcomes (Q4: 18.66% vs 35.81%). During the pn2 movement to the pn1, it might have an appropriate distance for the PN fusion. The result of the pn1_pn2_dist also showed that if the male and female pronuclei were too close (less than 14.98-17.24 μm), the proportion of blastocyst formation was lower. If the movement of pn2 showed a decrease to the centre and then an increasing distance during development, more blastocyst formation was expected.

Limitations, reasons for caution: This was a retrospective and descriptive study and the end point of embryo developmental potential was blastocyst formation. The further association of the pronuclei parameters needs further endpoints to dig out. The diagnostic value of the pronucleus parameters needs further study to validate.

Wider implications of the findings: The pronucleus parameters are associated with blastocyst formation, which could serve as a non-invasive morphological tool to assess embryo developmental potential. Our study showed the explainable application of AI for enhancing the morphokinetics study. We

believe both the pronucleus parameters and the AI tool could shed the light on embryology.

Trial registration number: ChiCTR1900025776

Abstract citation ID: dead093.530

P-167 Bacterial contamination of embryo culture medium in conventional in vitro fertilisation versus ICSI

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Study question: Our aim is presenting a clinical case of pregnancy using intracytoplasmatic sperm injection with bacterial contamination of embryo culture after failed conventional in vitro fertilisation.

Summary answer: Intracytoplasmatic sperm injection (ICSI) can be an effective and safe method to prevent embryo culture infection in case of microbial contamination of semen.

What is known already: Contamination of embryo culture is rare, but can be detrimental. The most common sources are semen and follicular fluid. We currently have no standard protocols for monitoring biological fluids. Semen often contains bacteria even if hygiene rules for collection are followed. Most studies found no significant difference in the effectiveness of in vitro fertilisation treatment in the presence or absence of bacteriospermia. This can be explained by sperm preparation techniques, antibiotic content of culture media and use of ICSI. Most centres advise against contaminated embryo transfers, but some achieved pregnancy after removing the zona pellucida or using ICSI.

Study design, size, duration: This is a case report of two consecutive in vitro fertilisation - ICSI cycles at the Division of Assisted Reproduction, Department of Obstetrics and Gynecology, Semmelweis University, Budapest in 2022.

Participants/materials, setting, methods: First we performed conventional in vitro fertilisation in a couple with secondary infertility because of oligoasthenozoospermia. For the second time we suggested ICSI due to bacterial growth observed in the embryo culture medium. Microbiological analyses were carried out. We video-recorded the cytoplasmic infection of one oocyte following ICSI with time-lapse microscopy.

Main results and the role of chance: After conventional in vitro fertilisation we observed a proliferation of rods in the embryo culture medium. The control culture medium was sterile. The semen and infected embryo culture medium contained Escherichia coli and Staphylococcus hominis. The oocytes did not fertilize and degenerated. The husband was asymptomatic and did not have alteration in physical examination therefore he was not treated with antibiotics. In next cycle we performed ICSI. 2 of 14 oocytes fertilized normally and developed into high-quality blastocysts. One embryo was transferred resulting in live birth and one embryo was cryopreserved. We video-documented proliferation of bacteria in one fertilised oocyte but not in the others. Infected oocyte was removed from culture and its culture medium showed Escherichia coli and Cutibacterium acnes. Fungal culture and sexually transmitted diseases were negative. The semen only contained Escherichia coli. The cervix culture was negative. Escherichia coli was resistant to gentamicin contained in culture medium.

Limitations, reasons for caution: We only had one case of bacterial contamination in 30 years and therefore can not conclude to the routine use of ICSI if microbial infections of biological fluids are present.

Wider implications of the findings: ICSI can be offered to patients with bacterial contamination of semen and can help reducing the risk of infection of embryo culture. We can prevent the disadvantages of excessive antibiotic treatments. New guidelines are needed how to handle bacterial contamination of embryo culture media.

Trial registration number: not applicable

Abstract citation ID: dead093.531

P-168 Follow-up survey of deliveries derived from Day 7 blastocysts

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Study question: Does Day 7 blastocyst-transfer affect prognosis of babies born?

Summary answer: There was no effect on growth up to 3 years of age in babies from Day 7 blastocyst-derived births.

What is known already: Culture medium and technology have advanced, and the efficiency to culture up to the blastocyst stage in order to obtain viable embryos has increased dramatically. As a result, blastocyst transfer is associated with higher clinical pregnancy outcomes than cleavage transfer. In 1998, it was first reported that some embryos may develop into blastocysts on Day 7. However, there are few reports on neonatal outcomes from Day 7 blastocyst transfer. Therefore, the utility of Day 7 blastocysts is controversial.

Study design, size, duration: This was retrospective study which included 23 cases where there was a live birth after single embryo transfer of a Day 7-derived blastocyst. The incidence of birth defects, birth weight was recorded as well as a physical development survey. The height and weight were recorded in 10 children aged 18-months and 4 children aged 3 who responded to follow-up survey. The time period was from January 2013 to December 2021.

Participants/materials, setting, methods: Patient seeking fertility treatment at an established private IVF clinic. We compared the birth weight, and birth after 18-months and 3 years height and weight of children born to a Day 7 blastocyst with data from babies born from Day 5 and Day 6 derived blastocysts. Statistical significance was determined using Kruskal-Wallis test.

Main results and the role of chance: The incidence of birth defects was 2.4% (115/4828), 1.7% (16/954) and 0% (0/23) in the Day 5, Day 6 and Day 7 groups, respectively. The average birth weight of the Day 7 group was 3057+/-532 g, which was not significantly different from 3024+/-472 g and 3041+/-480 g in the Day 5 and Day 6 groups. The average height at 18-months, was 80.0+/-5.3 cm, 79.8+/-5.1 cm, 77.8+/-6.9 cm in the Day 5, Day 6 and Day 7 groups, respectively. The average weight at 18-months, was 10.5+/-1.2 kg, 10.4+/-1.1 kg, 10.5+/-1.4 kg. The average height at 3 years old, was 93.2+/-4.8 cm, 92.9+/-4.7 cm, 93.4+/-5.9 cm. The average weight at 3 years old, was 13.9+/-1.6 kg, 13.8+/-1.4 kg, 14.1+/-2.6 kg, respectively. There was no significant difference in average height and weight up-to the 3 years old follow-up survey.

Limitations, reasons for caution: The incidence of Day 7 blastocyst-transfer is low and the study was limited to cases of single blastocyst embryo transfer. The study was not separated by sex, because sample size was small.

Wider implications of the findings: There was no difference in growth up to a 3-year-old follow-up survey of the Day 7 blastocyst compared with the Day 5 and Day 6 groups. Transfer of a Day 7-derived blastocyst does not appear to affect infant development and should be considered in cases where blastocyst development is delayed.

Trial registration number: not applicable

Abstract citation ID: dead093.532

P-169 In vitro fertilization versus intrauterine insemination with ovarian stimulation for unexplained infertility: A Collaborative Meta-analysis with Individual Participant Data

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Study question: In couples with unexplained infertility, does IVF increase cumulative live birth rate and reduce multiple pregnancy rate compared to intrauterine insemination with ovarian stimulation (IUI-OS)?

Summary answer: There were no significant differences in cumulative live birth and multiple pregnancy rates between IVF and IUI-OS in couples with unexplained infertility.

What is known already: IVF and IUI-OS are widely used in managing unexplained infertility, especially in couples with a poor prognosis for natural conception. Although several randomized controlled trials (RCTs) have compared IVF versus IUI-OS, it remains inconclusive regarding which approach is more effective. Some of the RCTs did not define a time limit for follow up and others made the comparison on a per-cycle basis which provides a biased estimate in favour of IVF.

Study design, size, duration: We performed an individual participant data meta-analysis (IPD-MA) that synthesised available individual-level data comparing IVF and IUI-OS in unexplained infertility. We searched MEDLINE, EMBASE, CENTRAL, PsycINFO, CINAHL, and the Cochrane Gynaecology and Fertility Group Specialised Register of RCTs and included eligible RCTs that completed data collection before June 2021. We invited author groups of eligible studies to join the IPD-MA and share the deidentified IPD of their RCTs.

Participants/materials, setting, methods: RCTs that compared IVF/ICSI to IUI-OS in couples with unexplained infertility were included. The primary effectiveness outcome was cumulative live birth, defined by time to pregnancy leading to live birth. The primary safety outcome was the number of multiple pregnancies per participant. IPD were checked and standardised before synthesis. The quality of evidence was assessed using the Risk of Bias 2 tool. The analysis followed the intention-to-treat principle, and a two-stage IPD meta-analysis was performed.

Main results and the role of chance: Of eight potentially eligible RCTs, four shared individual-level data of 933 couples, of which 550 couples were allocated to IVF and 383 couples to IUI-OS. Two RCTs had a low risk of bias, one had some concerns, and one had a high risk of bias. Considering the time to pregnancy leading to live birth, the cumulative live birth rate was not significantly higher in IVF compared to that in IUI-OS (4 RCTs, 908 couples, 50.3% vs 43.2%, HR 1.10, 95% CI 0.54 to 2.21, $I^2 = 68.7\%$). For the safety primary outcome, the rate of multiple pregnancy was not significantly lower in IVF than in IUI-OS (3 RCTs, 923 couples, 3.8% vs 5.2%, OR 0.78, 95% CI 0.41 to 1.50, $I^2 = 0.0\%$). Clinical pregnancy (4 RCTs, 933 couples, OR 1.09, 95% CI of 0.78 to 1.53, $I^2 = 14.3\%$) and pregnancy loss (3 RCTs, 760 couples, OR 0.97, 95% CI 0.55 to 1.72, $I^2 = 0.0\%$) were comparable between IVF and IUI-OS. There were no significant differences in neonatal outcomes between the two interventions on gestational age and birth weight.

Limitations, reasons for caution: Four RCTs did not share IPD which may introduce the risk of data availability bias. Only two included RCTs collected data on neonatal outcomes. Three of the included RCTs predominantly or only included couples with poor prognosis of natural conception which limits generalisability.

Wider implications of the findings: IVF and IUI-OS are both viable options in terms of effectiveness and safety for managing unexplained infertility, especially for those with a poor prognosis of natural conception. The associated costs of interventions and the preference of couples are important in clinical decision-making.

Trial registration number: not applicable

Abstract citation ID: dead093.533

P-173 Neuregulin 1 supplementation and sequential stimulation improve post-IVM embryo production

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Study question: Can supplementation with neuregulin 1 during a pre-IVM culture step followed by sequential stimulation with FSH and amphiregulin improve IVM outcomes?

Summary answer: Supplementation with neuregulin 1 during a pre-IVM culture step followed by sequential stimulation with FSH and amphiregulin improves post-IVM bovine embryo production

What is known already: Evidence that gradual activation of the maturation cascade favours nuclear-cytoplasmic synchrony and cumulus-oocyte communication has motivated the investigation of pre-IVM culture steps. *In vivo*, the cumulus-oocyte complex (COC) is first exposed to increased FSH levels, after which final maturation is triggered by EGF-like factors secreted by granulosa cells. We have demonstrated that supplementation of the IVM medium with neuregulin 1 (NRG1), a modulatory EGF-like factor, improves oocyte developmental competence. Herein, we utilized the bovine model to assess the impacts of NRG1 supplementation during pre-IVM and sequential exposure to FSH and amphiregulin (AREG) during IVM on post-IVF embryo development

Study design, size, duration: Bovine COCs were subjected to a pre-IVM culture with (group NS) or without NRG1 (groups D and S). Subsequently, COCs were subjected to direct (group D) or sequential IVM with FSH followed by AREG stimulation (groups S and NS). Post-IVF production of total and high quality (expanded and hatched) blastocysts was compared between groups by ANOVA followed by the Fisher Protected test (n = 3 replicates, each with 20-25 oocytes per treatment-group)

Participants/materials, setting, methods: Germinal stage vesicle COCs were aspirated from 2-8mm follicles of abattoir bovine ovaries, pooled in groups of 20-25 and subjected to pre-IVM for 9h in the "Follicular System" (FS)-preIVM medium, with or without 1ng/mL NRG1, followed by IVM in the FS-IVM medium for 24h. In sequential IVM, COCs were first subjected to a 6h culture in the FS-IVM medium without AREG and, subsequently, to a 18h culture in the complete FS-IVM medium (AREG 100ng/mL)

Main results and the role of chance: Supplementation of NRG1 during pre-IVM combined with sequential IVM with FSH followed by AREG stimulation increased post-IVF embryo development as assessed by the percentage of the total oocytes subjected to pre-IVM/IVM generating total and high quality (expanded and hatched) blastocysts. Total blastocyst rates were 34.34 ± 1.66%, 40.26 ± 1.94% and 45.94 ± 3.04% for groups D, S and NS, respectively (P = 0.032). Rates of high quality blastocysts were 30.53 ± 1.47%, 29.71 ± 2.36% and 38.56 ± 1.91%, for groups D, S and NS, respectively (P = 0.036)

Limitations, reasons for caution: Our study is subjected to the intrinsic limitations of the use of an animal model. Treatment effects may vary with different biological activities associated with different batches and suppliers of EGF-like factors

Wider implications of the findings: Our findings contribute for a better understanding of the mechanisms regulating oocyte developmental competence and constitute novel parameters for the improvement of IVM efficacy. Therefore, our data may contribute for the development of infertility treatment strategies with reduced hormonal cost and patient discomfort

Trial registration number: Not applicable

Abstract citation ID: dead093.534

P-174 Do culture conditions alter the efficacy of embryo selection algorithms using time-lapse technology? Development of novel embryo selection model with embryos cultured in different conditions

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Study question: Is the efficacy of embryo selection models altered by the conditions employed for embryo culture?

Summary answer: The predictive capacity of morphology and morphokinetics-based selection models might be dependent on the incubator and culture conditions employed.

What is known already: Morphological assessment remains the gold-standard for embryo selection. However, since the introduction of time-lapse technology to the incubators used for embryo culture, many selection algorithms based on morphokinetic annotations have been developed, adding objectivity to the embryo selection process. The efficacy and reproducibility of these algorithms have been questioned by other authors, being potentially influenced by characteristics of the patients and differing culture conditions. Nowadays, there are several incubators with time-lapse technology in the market, some of them providing novel features such as culture in high humidity conditions, which has been related to differences in the morphokinetics of embryo development.

Study design, size, duration: Retrospective external validation of an embryo selection algorithm based on morphokinetic annotations, in a set of 555 transferred blastocysts cultured in a time-lapse system in dry (DC, n = 281) or humid conditions (HC, n = 274), and comparison with selection by morphological criteria. A novel selection model was developed considering morphokinetic annotations of our embryo dataset, including blastocysts cultured in DC and HC. Embryos belong to autologous and oocyte-donation ICSI cycles performed in a clinic over 3 years.

Participants/materials, setting, methods: Embryos were cultured in a Geri incubator (Genea Biomedx) and automatically annotated (Connect&Assess2.0). The efficacy of the algorithm published by *Motato et al, 2016*, and ASEBIR morphological grading was assessed by Generalized Estimating Equations (GEE), considering possible confounders. Efficacy was quantified by the Area Under the ROC Curve (AUC), its 95% confidence interval (CI) and statistical significance was assessed by the Mann-Whitney test. A novel algorithm was developed aided by the visual tool FertAI (Merck).

Main results and the role of chance: Transferred blastocysts with known implantation data were classified A-D in base of the algorithm published by *Motato et al, 2016*, according to the optimal range of 2 morphokinetic parameters, tEB and s3, empirically-obtained in embryos cultured in an Embryoscope incubator. The algorithm had an AUC = 0.591, 95%CI(0.542−0.64), resulting significantly predictive of implantation ($P<0.001$), but lower than the efficacy reported by the authors (AUC = 0.602, 95%CI(0.559−0.645)). The efficacy was different in the two culture conditions: **AUC(DC) = 0.608**, 95%CI(0.54−0.676), $P = 0.002$; **AUC(HC) = 0.588**, 95%CI(0.518−0.657), $P = 0.103$. The morphological evaluation (3 categories: A (best) to C (worse)) resulted statistically predictive of implantation: AUC of 0.596, 95%CI(0.547−0.646), $P<0.001$. Again, its efficacy was different in **DC (AUC = 0.626**, 95%CI(0.559−0.693), $P<0.001$) and **HC (AUC = 0.589**, 95%CI(0.52−0.657), $P = 0.013$). The lower efficacy shown by these algorithms might be associated with different morphokinetic development of embryos cultured in a different incubator and different culture conditions. Hence, a **novel scoring model** was developed with empirically-determinated optimal ranges of three morphokinetic parameters, **considering**, for the first time, **embryos cultured in DC and HC**: tEB<113.874; (t5-t3)/(t5-t2) = [0.521, 0.554] and cc2 = [10.34, 11.58], yielding a score from 0 to 3. The selection model resulted in an AUC = 0.637, 95%CI(0.59−0.684), and was equally efficient in DC and HC: **AUC(DC) = 0.645**, 95%CI(0.579−0.712); **AUC(HC) = 0.645**, 95%CI(0.579−0.711); $P<0.001$).

Limitations, reasons for caution: This is a primary approach to a development of a selection algorithm using morphokinetic data of embryos cultured until blastocyst stage in a Geri incubator. The efficacy and reproducibility of the model must be validated in a different dataset.

Wider implications of the findings: The lower efficacy shown by a selection algorithm developed in a different incubator supports the necessity of adjusting selection tools for the specific culture conditions employed by each IVF laboratory. This is the **first scoring model** developed for selection of **blastocysts** using morphokinetic parameters recorded in a **Geri** time-lapse incubator.

Trial registration number: Not applicable

Abstract citation ID: dead093.535

P-175 Human cumulus cell telomere length and its association with assisted reproduction outcomes

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Study question: Is there any relationship between the relative telomere length (RTL) within cumulus cells (CCs) and the outcome of assisted reproductive treatment using the corresponding oocyte?

Summary answer: Lower RTLs in CCs were significantly associated with embryos chosen for transfer or cryopreservation. In contrast, embryos considered non-viable (discarded) tended to have higher RTLs.

What is known already: Cumulus cells fulfil vital roles in support of oocyte development, including the transduction of external signals and the provision of resources via transzonal projections. Given their essential role in the acquisition of oocyte developmental competence, the biology of CCs is of clinical relevance. Telomeres are specialised structures protecting the ends of chromosomes, composed of repetitive DNA sequences and associated proteins. Telomeres shorten with each mitotic division, as well as due to oxidative damage, eventually reaching a critical threshold at which point cellular senescence occurs. Currently, published data on CCs telomere length and relationship with oocyte potential are conflicting.

Study design, size, duration: The study involved 182 human CC samples collected from 52 IVF patients. Quantitative PCR (qPCR) was used to measure the relative telomere length in each of the CC samples. Telomere lengths were assessed for associations with various patient characteristics (e.g. age, body mass index, infertility diagnosis). Additionally, potential relationships with clinically relevant oocyte/embryo features were investigated (fertilisation; development/morphology), as well as the eventual fate of the associated embryo (transferred; cryopreserved for potential future use; discarded).

Participants/materials, setting, methods: Real-time quantitative PCR was carried out using PCR primers specific for the telomere repeat. A single-copy gene was also amplified from each CC sample. Quantification of this gene was used for normalisation of the telomere data, allowing control for variation in the number of cumulus cells in each sample. Associations between RTL in CCs and patient, embryonic, and clinical factors were assessed using various statistical methods, with P-values <0.05 considered significant.

Main results and the role of chance: No associations were identified between RTL and any patient characteristics, except for BMI. The amount of CC telomeric DNA tended to be greater for patients with higher BMI (P = 0.002). When considering links between RTL and oocyte or embryonic factors, a significant relationship was detected between the quantity of telomeric DNA in CCs and whether the corresponding embryo was considered

non-viable (discarded) or whether it was transferred or cryopreserved (P = 0.019). This finding raises the possibility that measurement of RTL in CCs could provide a pre-conception, non-invasive assessment of oocyte quality. In the context of fertility preservation, RTL measurement could assist in evaluating a cohort of oocytes, indicating whether the cryopreserved eggs are likely to be sufficient or whether additional cycles to generate more would be advisable. If future studies confirm that CC RTL has a strong predictive value, the possibility of limiting fertilisation to oocytes considered to have high likelihood of viability could also be considered for routine IVF cycles. It is unclear why shorter CC telomeres might be associated with oocytes of superior potential, but one possibility is that the cells may have undergone a greater proliferation (more mitoses), resulting in a more extensive cumulus mass supporting the enclosed oocyte.

Limitations, reasons for caution: Before drawing definitive conclusions, confirmation of the findings within a larger, independent data set is necessary. Even if confirmed, determination of the true clinical value of telomere assessment will require further, appropriately designed studies. The current study was not powered to evaluate relationships between CC telomere lengths and IVF outcomes.

Wider implications of the findings: Currently, simplistic morphological evaluation is the only method for assessing oocyte competence prior to fertilisation. If CCs telomere measurement is confirmed to have predictive value, a preconception test of oocyte potential could be offered. This would be extremely valuable for patients cryopreserving oocytes for fertility preservation and for donor banks.

Trial registration number: NA

Abstract citation ID: dead093.536
P-176 Is there a relationship between ovarian reserve, oocyte and embryo quality in patients undergoing PGT-A?

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Study question: Is there a relationship between ovarian reserve, oocyte and embryo quality in patients undergoing PGT-A?

Summary answer: Our data revealed no relationship between percentage of aneuploid blastocysts in patients with hormonal signs of reduced ovarian reserve.

What is known already: Poor ovarian reserve, as determined by serum anti-mullerian hormone (AMH) and antral follicle count (AFC) levels, is a known risk factor for poor outcomes in in vitro fertilization (IVF). Ultrasound imaging is considered to be the most reliable method for measuring ovarian reserve, as it provides a direct and accurate assessment of the number of ovarian follicles. However, the relationship between ovarian reserve and oocyte quality is not well understood, and further research is needed to fully understand the implications of these test results for IVF outcomes.

Study design, size, duration: The present retrospective case-control study, performed between 2015 and 2021, aims to investigate the association between ORTs and the rate of euploid embryos conceived through ICSI. 680 patients underwent in vitro fertilization procedures that included blastocyst biopsy and 23-chromosome aneuploidy testing. Regardless of cycle day, a level greater than 1,2 ng/mL was regarded as normal, less than 10 antral follicles in total were associated with diminished ovarian reserve according to current knowledge.

Participants/materials, setting, methods: AMH and AFC were studied to determine their relationship with rates of abnormal embryo development. Patients were divided into two groups based on their baseline hormone levels. The AMH group (n = 680) had 562 individuals with normal ovarian reserve in Group 1 and 118 with lower reserve in Group 2. A similar division was made in the same group based on AFC values, resulting in 210 patients with low AFC and 348 with normal AFC.

Main results and the role of chance: Low AMH Group was made up of slightly older patients, as would be expected (39,9 compared with 40,41 years for group 1; $P = 0{,}193$). Patients with low AMH shown FSH levels significantly higher ($P = 0{,}022$) compared to those with normal ones. Not surprisingly, aspirated follicles, oocytes retrieved, pre-ovulatory follicles, fertilizable oocyte, fertilized oocytes and evolutionary embryos appeared significantly higher in people with normal levels of AMH (respectively $P<0{,}01$; $P<0{,}01$; $P = 0{,}004$; $P = 0{,}013$; $P = 0{,}009$; $P = 0{,}015$). This means the latter had a better oocyte quality even though they shown a comparable non-evolutionary embryo mean ($P = 0{,}047$). Regarding euploid embryos, it didn't result in a statistically significant difference between the 2 groups ($P = 0{,}14$) AMH cohort and ($P = 0{,}12$) AFC cohort. We obtained the almost the same results taking into account AFC cohort. As expected, regarding protocols and gonadotropins used to stimulate women, our data shown a statistically significant value ($P = 0{,}016$) due to personalized protocols for women predicted as poor or normal/high responders.

Limitations, reasons for caution: There are many points affecting diminished ovarian reserve (DOR), linked to a variety of etiologies. However, the genetic determinants of DOR remain largely unknown. All these features don't allow making an easy model for better studying this condition.

Wider implications of the findings: This study can help clinics provide more accurate and informative counseling to patients undergoing IVF, by giving them a better understanding of the factors that affect their chances of success and how they can impact their treatment.

Trial registration number: None

Abstract citation ID: dead093.537
P-177 Is AI the future of ART? Key barriers and drivers to AI adoption in clinical practice from the views of 144 fertility professionals

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Study question: What are the barriers, needs and views of fertility professionals towards using AI based decision tools in clinical practice?

Summary answer: There is an overall positive view towards using AI. The low implementation of AI in clinical practice is resultant of insufficient experience, knowledge and validation.

What is known already: Increased use of AI in fertility is aimed at improving clinical decisions and lab techniques by fertility professionals and improving standards of care experienced by patients. Despite the increasing presence of AI in peer-reviewed publications, the real views of fertility professionals regarding AI tools in fertility care are still unknown. Especially, with regards to whether any barriers in implementing these tools are experienced by fertility professionals or whether all required needs are met. This is the first comprehensive study that specifically focuses on understanding the views, needs and barriers faced by fertility professionals regarding utilising AI in fertility clinical practice.

Study design, size, duration: A structured questionnaire was distributed to 1419 fertility professionals. Respondents received the questionnaire online. In person interactions were also made at the ESHRE conference (2022) to encourage participation. Fertility professionals were identified from the ESHRE members list, with an equal distribution between countries as represented by the number of IVF cycles in those countries. With 144 responses from 37 countries, the response rate was 10%. Responses were collected between 13th May and 14th July 2022.

Participants/materials, setting, methods: The questionnaire consisted of 28 questions, split into 4 sections: (i) the demographics of the participant, (ii) their knowledge of AI, (iii) current use and (iv) unmet needs towards AI in clinical practice. Perceptions were graded (1:Strongly Against, 2:Against, 3:Neutral, 4:For, 5:Strongly For).

Main results and the role of chance: Respondents expressed positive views towards AI in clinical practice as highlighted by fertility professionals having felt more positive towards using 'AI' (average score = 3.9) compared to using 'decision support tools' (3.7,p = 0.002). Most participants had positive/neutral views regarding 'patient confidence towards them when implementing AI decision tools in treatment plans' (77%,n = 125). 78% of participants were intrigued in finding out more about the possible implementation of AI in clinical practice. Contrastingly, only 53% believed that AI could help reduce their clinical burden in decision making (1:7%,2:13%,3:37%,4:40%,5:13%). Age, source of funding and occupation did not affect perceptions. Furthermore, inexperience and lack of knowledge of AI tools were major barriers experienced: 14/29 participants that disagreed with whether utilising AI could reduce clinical burden, had never even used AI tools in the first place. Similarly, 11% indicated discomfort in using AI tools in clinical practice, of whom 79% self-described as having poor knowledge of AI. Evidence and data with improved live birth rate was identified by most respondents (120/144) as the most critical/important unmet need by fertility professionals. The preferred manner of implementation was directly to the equipment used in clinical practice (50%).

Limitations, reasons for caution: Despite attempts to have invitees' global distribution matching global cycle numbers, the actual respondents did not reflect the proportion of IVF cycles in each country due to an increased response rate from UK, France and Europe and a reduced response rate from China and Asia.

Wider implications of the findings: This is the first study to assess perceptions of fertility professionals towards the use of AI in fertility care, highlighting the need to increase education of fertility professionals and validation standards to support fertility professionals in the responsible use of this new type of technology.

Trial registration number: N/A

Abstract citation ID: dead093.538

P-178 The interaction between spermatozoa and cumulus cells: a more physiological approach to the selection of good quality spermatozoa for assisted reproduction

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Study question: Does selection by cumulus cells (CCs) improve sperm quality in terms of acrosome integrity and mitochondrial functionality compared to standard techniques?

Summary answer: The sperm-CCs interaction method appears to be effective in selecting sperm with an intact acrosome and functioning mitochondria

What is known already: In nature, the selection of the best quality sperm is very demanding and involves high morphological qualities and dynamic features. Semen preparation in ART has long been carried out using either the swim-up or density gradient method; although both methods provide motile sperm, they cannot really replicate the physiological selection processes. As a result, several methods have been proposed to mimic the natural selection that occurs in the female reproductive tract, allowing the most competent sperm to provide the paternal contribution to the zygote. However, the literature on these techniques is limited and does not provide consistent conclusions

Study design, size, duration: Proof-of-principle study conducted at the New Fertility Group (NFG) in Rome and the Department of Molecular and Developmental Medicine at the University of Siena (Italy) from July 2022 to December 2022, including 9 semen samples. Comparison of the CCs selection model with the standard swim-up by assessing mitochondrial and acrosome integrity. In addition, mitochondrial activity was assessed by oxygraphic analysis in spermatozoa exposed to immortalised human granulosa cells (hGL5) compared to standard swim-up sorted spermatozoa

Participants/materials, setting, methods: The CCs-model was prepared by running two channels of medium, one with CCs (study group) and one with medium alone (control). Spermatozoa were added to each channel and incubated at 37 °C for 1 hour. Moreover, spermatozoa were also added on hGL5 and incubated for 1.5h. The collected spermatozoa were compared with controls for acrosomal integrity, assessed by FITC-labelled PSA, and mitochondrial membrane potential, assessed by Mitotracker. Finally, sperm oxygen uptake was measured by oxygraphic analysis

Main results and the role of chance: Assessment of sperm acrosome integrity showed a higher percentage of responding acrosomes in the CCs model compared to swim-up and controls (61% ± 0.3; 39% ± 0.4; 19% ± 0.4 $p < 0.05$). The CCs model also showed 83% ± 0.3 of spermatozoa with active mitochondria compared to 65% ± 0.3 in the control group ($p < 0.005$). A similar pattern was observed in the swim-up group. Quantitative analysis of mitochondrial membrane potential (MMP) confirmed that CCs and swim-up groups had higher MMP compared to the control group ($p < 0.005$). Evaluation of mitochondria in the hGL5 treated group showed active mitochondria in 78% ± 0.49 of the sperm compared to 59% ± 0.65 in the control group ($p = 0.1$). The same profile was detected by fluorescence quantification of MMP in the hGL5 group compared to the control group ($p < 0.01$). Oxygraphic analysis, as an index of oxidative phosphorylation and consequently of ATP production, was performed on hGL5 exposed spermatozoa, swim-up selected spermatozoa and controls. hGL5 exposed and swim-up selected spermatozoa showed a similar O2 consumption, about 4 times higher than unselected spermatozoa ($p < 0.05$)

Limitations, reasons for caution: The limited sample size and the heterogeneity of the samples were the main limitations of this study. Although CC and hGL5 sperm selection models appear to be more physiological, further studies on other parameters such as sperm DNA integrity are needed.

Wider implications of the findings: Both the CCs model and the hGL5 model appear to be effective in selecting good quality sperm. As these models avoid sperm centrifugation, further research is needed to understand their efficacy and reliability and whether they are a valid alternative to conventional sperm selection methods in terms of IVF outcomes.

Trial registration number: none

Abstract citation ID: dead093.539

P-179 Euploid blastocysts rate in pre-implantation genetic testing (PGT) cycles with high levels of sperm DNA fragmentation does not improve after Magnetic Activated Cell Sorting (MACS)

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Study question: Is there any benefit when using MACS before PGT to increase the euploid blastocysts rate in patients with high sperm DNA fragmentation?

Summary answer: Euploid blastocysts rate was not improved after the use of MACS in PGT patients where the male partner has high DNA fragmentation.

What is known already: The presence of apoptotic markers in spermatozoa is highly correlated with the failure of assisted reproduction treatments. Under normal physiological conditions, apoptotic sperm cells with externalized phosphatidylserine (PS) residues on the plasma membrane can be recognized and efficiently eliminated in the female genital tract, preventing the fertilization of the oocyte by a spermatozoon with alterations in its DNA integrity. MACS eliminates apoptotic sperm with PS residues using magnetic

microbeads conjugated with Annexin V. This technique reduces the proportion of sperm with high rates of sperm DNA fragmentation and can be used to maximize assisted reproduction technique (ART) outcomes.

Study design, size, duration: This retrospective cohort study included 54 PGT cycles with high sperm DNA fragmentation between March 2018 and November 2022. Among them, some cycles used MACS as a selection tool for non-apoptotic sperm. The control group consisted of cycles that did not perform MACS due to the patients' decision. We compared the euploid blastocysts, fertilisation and blastocysts formation rates.

Participants/materials, setting, methods: PGT cycles using their own oocytes and with high sperm DNA fragmentation were separated into two groups according to the use of MACS as a selection technique ($n = 25$) or not ($n = 29$). Both groups were comparable regarding patients' age, number of mature oocytes collected and cleavage embryo quality. Semen samples were considered with high sperm DNA fragmentation after a TUNEL test $\geq$ 20%. Data was compared through Chi-square test with significance set at $P < 0.05$.

Main results and the role of chance: The primary outcome was the euploid blastocysts rate, while secondary outcomes included fertilisation and blastocysts formation rate. No differences between MACS and control groups were observed regarding the euploid blastocyst rate [45.3% (34 euploid blastocysts/75 total blastocysts) vs. 43.4% (63/145)] or the blastocysts formation rate [43.6% (75 total blastocysts/172 normal fertilized oocytes) vs. 28.6% (18/63)]. Fertilization rate was better in the control group [72.9% (172 fertilized oocytes/236 inseminated oocytes) vs. 80.1% (145/181)], $P < 0.05$.

Limitations, reasons for caution: These results need confirmation with a bigger population size. As with any retrospective study, the potential for residual confounding factors exists. Even though no significant differences were observed in terms of blastocysts formation and euploidy, other parameters such as embryo quality or birth results should be evaluated in the future.

Wider implications of the findings: These data suggest that MACS in PGT cycles offers no clinical benefit to increase the euploid blastocyst rate. The use of add-ons must be well justified when introduced in the laboratories' everyday work.

Trial registration number: Not applicable

Abstract citation ID: dead093.540
P-180 Hyaluronidase used in oocyte denudation inhibits the proliferation and the viability of cumulus cells through the inhibition of midkine

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Study question: Does hyaluronidase (HAse) have possible negative or positive effects on cumulus cells (CCs) during oocyte denudation before ICSI by altering midkine (MK) levels?

Summary answer: Hyaluronidase inhibits the proliferation of CCs via the inhibition of MK activity which may result in poor embryo quality and low ICSI success.

What is known already: MK is abundantly expressed in ovarian follicles. MK secreted from the cumulus-granulosa cells that surround oocytes was shown to promote the cytoplasmic maturation of oocytes. This effect of MK could be mediated via its' anti-apoptotic effect and some other mechanisms. The removal of CCs during oocyte denudation (OD) is done in order to select and grade the oocytes before ICSI. HAse is applied for ICSI to break down hyaluronic acid, which is present at high levels in the cumulus-oocyte complex during OD. To date, there have only been a few reported cases of the toxic effects of hyaluronidase on oocytes.

Study design, size, duration: This study is a prospective, randomized study done with 90 healthy women between September 2017 and September 2018.

Participants/materials, setting, methods: This study was done with women diagnosed as a male factor between the ages of 21 and 40 who underwent ICSI. HAse was applied to the cultured CCs at concentrations of 0.1 IU/ml, 1 IU/ml, and 10 IU/ml. The proliferation and apoptosis indices (Flow cytometry), structures [Transmissive electron microscopy (TEM)], and MK levels (ELISA) of CCs were evaluated every 24 hours for 48 hours. One way-Anova was used and $p < 0.05$ was considered statistically significant.

Main results and the role of chance: The application of HAse at all concentrations decreased cumulus cell numbers for 48 hours ($p < 0.05$). The highest decrease in cell number and cell viability with the highest number of apoptotic cells were detected at the 48th hour at the application of the highest concentration of HAse ($p < 0.05$). The highest concentration of HAse application caused the highest decrease in MK levels at the end of 48 hours ($p < 0.05$).

Limitations, reasons for caution: The effect of HAse on the CCs of women aged below 21 and above 40 could not be evaluated. Therefore, the overall resistance and fragility of CCs to these HAse concentrations could not be evaluated. In addition, the HAse effects should be evaluated in female infertility cases such as PCOS.

Wider implications of the findings: This is the first report to examine the effect of HAse on MK activity. 2-3 lines of CCs are commonly left during oocyte denudation in order to protect the oocyte from stress-induced reactions. The low number of CCs means low MK levels resulting in a loss in oocyte competence.

Trial registration number: Tthe Ethics Committee Directive on Non-Interventional Studies of Biruni University with the permission numbers 2017/5-1.

Abstract citation ID: dead093.541
P-181 The impact of high proportion of immature oocyte in a cohort on the reproductive outcome following icsi

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Study question: Whether high proportion of immature oocyte in a cohort impacts outcome of sibling mature oocyte in ICSI cycles.

Summary answer: Study demonstrate that if immature oocyte retrieved was more than 50% in a cycle then it reduces reproductive outcome with the remaining mature sibling oocytes.

What is known already: The selection of competent oocyte is crucial to assisted reproductive technique procedure outcome. In ICSI procedure where only metaphase II oocytes (MII) are injected correlates with number of MII oocytes available, embryo development and pregnancy outcome. Percentage of immature oocyte {Metaphase I (MI) and Germinal Vessicle (GV) } retrieved in a cycle is always a concern for stimulation protocol, patient response which affects IVF laboratory outcome.

Study design, size, duration: A prospective study was conducted from 1st January 2020 to 1st December 2022 in the Department of Reproductive medicine at a tertiary infertility centre in India.

Participants/materials, setting, methods: Patients were divided into 3 groups on the basis of percentage of immature oocyte retrieved in a single cycle. Group A ($n = 100$), where percentage of immature oocyte (MI and GV) was between 0 to 20%, while Group B ($n = 60$) where percentage of immature oocyte was between 21 to 50% and Group C ($n = 20$) where percentage of immature oocyte was between 51 to 100%.

Main results and the role of chance: This study included all normal responder patients and cycles using in-vitro fertilisation (IVF) and poor responders were excluded. All blastocyst formed were vitrified and transferred in frozen embryo replacement cycle. All the 3 groups were compared on the basis of fertilisation rate, blastocyst formation rate, clinical pregnancy rate and miscarriage rate.

Group A and group B had no significant difference in fertilisation rate (82% vs. 78%, $p > 0.5$), blastocyst formation rate (60% vs. 58%, $p > 0.5$), clinical

pregnancy rate (58% vs. 55%, $p > 0.5$) and miscarriage rate (12% vs. 11%, $p > 0.5$).

While significant reduction was observed in group C (>50% MI & GV oocyte) in fertilisation rate (40%, $p < 0.1$), blastocyst formation rate (28%, $p < 0.1$), clinical pregnancy rate (15%, $p < 0.1$).

Limitations, reasons for caution: Larger randomised control studies are needed to strengthen these results.

Wider implications of the findings: Percentage of immature oocyte can help in predicting the success of assisted reproductive technique. This will help in counselling patient about outcome.

Trial registration number: NA

Abstract citation ID: dead093.542

P-182 Validation of single-step warming for human blastocysts shows successful results are independent of the sucrose concentration used

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Study question: Can warming of blastocysts in a single step be successfully performed using different sucrose concentrations?

Summary answer: Blastocyst warming in a single step using sucrose concentrations between 0.25 M and 1.0 M shows similar rates of survival, re-expansion and development in vitro.

What is known already: Since the introduction of human blastocyst vitrification, excellent survival rates have been reported. Warming procedures usually involve three to five steps with exposure to solutions with decreasing concentrations of non-penetrating cryoprotectants. Successful blastocyst warming procedures are described using high as well as low starting levels of sucrose. Recent data show that a single step warming procedure can be equally effective in terms of survival and pregnancy rates. Moving towards simpler procedures can support further optimization of laboratory procedures.

Study design, size, duration: Blastocysts donated and consented by patients were used to evaluate a shorter warming procedure. Warming was performed in a single step using different sucrose concentrations. After warming, embryos were assessed for morphological survival and cultured in a time-lapse incubator to monitor re-expansion and development in vitro for 24 h. Three series of tests were performed. A control group using standard warming procedure was included in the first test

Participants/materials, setting, methods: For warming, carriers containing a single blastocyst were plunged into a warming solution at 37 °C, containing 0.25, 0.5 or 1.0 M sucrose and stayed in the solution for 1 or 2 minutes. Three series of experiments were performed, testing different sucrose concentrations or volume of warming solution. Warmed blastocysts were cultured following several rinsing steps and assessed for morphological survival. Re-expansion after 2 h, embryo characteristics and development were monitored until 24 h post warming.

Main results and the role of chance: Results of the different tests are summarized in the table, including information on sucrose levels, exposure times, volume of warming medium, numbers of blastocysts warmed, recovered, meeting criteria for embryo transfer, full re-expansion after 2 hours, viable after 24 h culture.

No differences were observed between the different groups. Overall, 98 % of warmed blastocysts would be considered transferable after single step warming and 96 % were viable after 24 h culture. Results confirm blastocysts can be warmed at 37 °C in a single step using 0.25 M, 0.5 M or 1 M sucrose with no effect on survival or development in vitro.

Limitations, reasons for caution: This is a preclinical validation on use of a reduced warming time for vitrified blastocysts. Further evaluation and clinical validation of the findings is required to confirm safety of a simplified warming procedure. Working temperature rather than warming medium volume may be critical when aiming for shorter warming procedures.

Wider implications of the findings: Reducing the warming time and number of handling steps minimizes exposure of blastocysts to a suboptimal environment and operator or handling related stresses. It also allows further optimization of laboratory protocols and workflow. When confirmed clinically, these findings encourage investigating similar changes for other reproductive cells.

Trial registration number: not applicable

Abstract citation ID: dead093.543

P-183 Blastocyst speed versus blastocyst look: Which is the better predictor of clinical pregnancy and live birth following time-lapse culture and single blastocyst transfer?

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Study question: To compare predictive value for pregnancy and live birth of morphological versus morphokinetic assessment of blastocysts after time-lapse culture and single blastocyst transfer.

Summary answer: Blastocyst morphokinetic assessment was not superior in predicting pregnancy and live birth compared to blastocyst morphology, but provides additional information, particularly for morphologically poorer-quality blastocysts.

What is known already: Blastocyst culture with time-lapse technology enables evaluation of both morphokinetic developmental speed and classical morphology including Gardner scoring of the blastocyst. One approach to assess the developmental speed of blastocysts is to use the morphokinetic classification proposed by Campbell, Fishel, et al., based on time to start of blastulation (tSB) and the duration of forming the blastocyst (dB). However, beyond the advantages of undisturbed embryo culture, the value of the kinetic data from time-lapse is debated.

Study design, size, duration: EDCOS (Embryo Developmental profiles from Controlled Ovarian Stimulation cycles using follitropin alfa) is a multicentre, prospective cohort study of patients undergoing IVF/ICSI whose embryos were cultured in time-lapse incubator devices in 10 ART centres throughout Denmark and Sweden. Patients undergoing IVF/ICSI (including donor oocyte cycles) who had ovarian stimulation with follitropin alfa (Bemfola®) and had zygotes and embryos available for monitoring in the EmbryoScope™ time-lapse incubator device (Vitrolife) were invited to join this study.

Participants/materials, setting, methods: Morphological and morphokinetic prognostic classifications for single blastocyst transfers (SET) were determined for three study subpopulations: fresh autologous SET (311 women), frozen autologous SET (246 women; 345 transfers) and frozen donor SET (38 women; 59 transfers). All blastocysts were graded morphologically in five grades from Amorph (best) to Emorph (worst) based on their Gardner score and morphokinetically in three grades from Akin (best) to Ckin (worst) based on tSB and dB.

Main results and the role of chance: Overall ongoing pregnancy and live birth rates (OPR/LBR) for morphokinetic classifications Akin, Bkin and Ckin were 36.1%, 30.0% and 16.3% ($P = 0.021$) and 30.4%, 21.8% and 9.1% ($P = 0.000$), respectively. OPR and LBR for morphological classifications Amorph, Bmorph, Cmorph, Dmorph and Emorph were 41.9%, 37.8%, 30.8%, 19.0% and 15.6% ($P = 0.000$) and 36.6%, 29.1%, 15.6%, 16.5% and 8.3% ($P = 0.002$), respectively. For Dmorph blastocysts, OPR and LBR for morphokinetic classifications Akin, Bkin and Ckin were 50%, 21.4% and 7.1% ($P = 0.009$) and 33.3%, 10.8% and 0% ($P = 0.003$), respectively. Receiver

operating characteristic curve analyses achieved areas under the curve for fresh autologous SET of 55.7%, 58.9% and 67.2% for the morphokinetic, morphological and combined classifications, respectively; and 53.4%, 64.0% and 65.6%, respectively, for frozen autologous SET.

Limitations, reasons for caution: This was an observational study with no control group, conducted under usual clinical practice. Data were collected prospectively, although clinics could deselect and rank blastocysts according to their own procedures. However, the outcome of subsequent frozen blastocyst transfer cycles was also analysed.

Wider implications of the findings: Selection of a blastocyst for transfer based on morphokinetic assessment at the blastocyst stage was not superior to classical blastocyst morphological assessment after time-lapse culture but is a viable option. Combining morphokinetic and morphological evaluation provides additional supporting information to guide in blastocyst selection, especially for morphologically poorer-quality blastocysts.

Trial registration number: Not applicable

Abstract citation ID: dead093.544
P-184 Morphokinetic analysis of cases with vitrified-warmed oocytes in oocyte donation program

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Study question: Does vitrification of oocytes in oocyte donation program alter the morphokinetic pattern of human embryos?

Summary answer: Both the extrusion of the second polar body (tPB2) and the appearance of the pronuclei (tPNa) occur earlier in vitrified oocytes without affecting further development.

What is known already: Vitrification of oocytes has been a breakthrough in oocyte cryopreservation and significantly altered practices in IVF labs. Oocyte vitrification/warming has become an integral part of daily routine and thorough assessment of its efficiency is crucial. Though, studies of vitrified-warmed oocytes have shown similar fertilization, cleavage, blastulation, pregnancy and live birth rates to fresh oocytes, the morphokinetic pattern of embryos originating from vitrified oocytes has not been thoroughly studied. According to some studies vitrification may be related to delayed blastocyst formation while others failed to see any alteration in the morphokinetic pattern between embryos derived from fresh or frozen oocytes.

Study design, size, duration: This retrospective observational study was performed at Embryolab Fertility Clinic, in Thessaloniki, Greece between April 2020 and September 2021 and included 590 vitrified oocytes from 68 oocyte donation cycles. Control group consisted of 31 good prognosis cases (oocyte donation cycles or homologous oocyte cycles of women under 35 years old).

Participants/materials, setting, methods: 68 vitrified oocyte donation cycles and 31 fresh control cycles were analyzed. All oocytes after ICSI were cultured in Embryoscope time-lapse incubator up to blastocyst stage. Key time parameters and dynamic events were analyzed using generalized estimating equations (GEE) regression analysis for the non-independent nature of the data. Moreover, the following comparisons were performed between groups: fertilization, cleavage, top cleavage, blastocyst, top blastocyst and pregnancy rates.

Main results and the role of chance: There was no significant difference in fertilization rates between control and frozen oocytes (77.79% ± 15.73 vs 71.26% ± 21.50 respectively, $p = 0.15$). Cleavage rate and top cleavage rate (more than 6 cells, equal size, with no or minor fragmentation), were higher in frozen oocytes with this difference being significant ($p = 0.02$ for cleavage rate and $p = 0.015$ for top cleavage rate). Blastocyst rate and top blastocyst (2AA, 3AA, 3AB, 4AA, 4AB according to Gardner criteria) rate, though, showed similar rates with no statistically significant difference (Blastocyst rate: 74,15% ± 17.19-control vs 76.42% ± 30.75-frozen oocytes, $p = 0.6$ & Top blastocyst rate: 45.54% ± 24.04-control, 45.30% ± 32.04-frozen oocytes, $p = 0.82$). Pregnancy rates were not significantly different (control:85.19%, frozen oocytes:75.38%, $p = 0.29$). The comparison for key time parameters and dynamic events that were analyzed (tPB2, tPNa, tPNf, t2, t3,t4, t5, t6, t7, t8, t9+, tSC, tM, tSB, tB, tEB, S2, S3, S5, CC1, CC2, CC3,Compaction and Blastulation) showed significant difference in 2 time parameters: tPB2 (4.62 hours-control, 3.67 hours-frozen, Coef: -1.09, $p = 0.015$) and tPNa (9.07 hours-control, 8.25 hours-frozen oocytes, Coef: -1.06, $p = 0.043$).

Limitations, reasons for caution: The fact that the oocytes under comparison were not sibling as well as its retrospective nature, constitute the main limitations of the present study. Moreover, the number of cases included in this study was limited and the available information about ongoing pregnancy and live births is still pending.

Wider implications of the findings: tPB2 and tPNa seem to occur earlier in vitrified oocytes without affecting further development, implying a need for reviewing timings between OPU/vitrification/warming/ICSI. Moreover, vitrified oocytes displayed higher cleavage and top cleavage rates with unaffected blastocyst or pregnancy rates. Overall, vitrification of oocytes seems to be safe and reliable.

Trial registration number: N/A

Abstract citation ID: dead093.545
P-185 Performing Assisted Hatching on Day 4 next to the area of early cavitation reduces the chances inner cell mass (ICM) herniation and simplifies trophectoderm biopsy

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Study question: Can the location of the opening in the zona pellucida during Assisted Hatching reduce the herniation of cells in the ICM and simplify trophectoderm biopsy?

Summary answer: Performing Assisted Hatching on D4, next to an area of the zona pellucida (ZP) close to first embryo cavitation, reduces the hatching of ICM cells.

What is known already: Trophectoderm biopsy is performed on blastocysts to perform preimplantation genetic testing of mutations and/or aneuploidies. To simplify the excision of trophectoderm cells, Assisted Hatching (AH) is typically performed on D3 at the cleavage/morula stage by creating a hole in the ZP through which some cells will herniate. This opening is traditionally performed on a random area of the ZP and, consequently, predicting whether the trophectoderm or the ICM cells will hatch first is not possible. Should the latter be the case, the biopsy procedure would need to be adjourned until trophectoderm cells become accessible.

Study design, size, duration: Regular AH was performed on mouse embryos on D3 by creating a 10µm opening on a random area of their ZP. A second group of embryos was cultured until D4 and the opening of the ZP was performed in a directed way (AHD) close to where the first cavitation of the cells was observed. Both groups were compared with a third control group to which no opening was made.

Participants/materials, setting, methods: Thirty mouse embryos were allocated into each test group ($n = 90$) and cultured in an Embryoscope time-lapse incubator to better determine the first cavitation of the cells. Laser-assisted AH/AHD was performed on the Embryoslide dish. Blastocyst formation, quality and hatching rates were compared among all groups, and the belongingness of the first herniated cells to the trophectoderm/ICM was annotated individually by D5/D6. Statistical analyses were performed by Fisher's exact test, and significance set at 5% ($\alpha = 0.05$).

Main results and the role of chance: The blastocyst formation and hatching rates were close to 100% in all three groups with no differences among any of them, indicating that neither AH nor AHD had a detrimental effect on embryo development between D3-D6 or blastulation. In more than a third of the blastocysts of the non-treated control group, the first cells to herniate out of the ZP belonged to the ICM (38.2%), similar to what was observed in the random AH group (44.4%). By performing AHD on day 4 in the ZP next to the area of first embryo cavitation, the incidence of the

ICM hatching first was significantly reduced to only 11.1% of the cases ($p \leq 0.01$).

Interestingly, the ratio of blastocysts that were able to completely hatch out of the ZP by day 6 was reduced both in the AH and AHD (2.8% and 5.6%, respectively) compared to the control group with no opening on the ZP (42.9%, $p \leq 0.0002$).

Limitations, reasons for caution: The present study has been performed on the mouse model and AH/AHD timings may need to be adjusted if used in the human. Time-lapse technology has eased the recognition of early cavitation stages to perform AHD, which could result more complex in a regular culture setting.

Wider implications of the findings: Trophectoderm biopsy depends on the unique rate of development of each blastocyst, which complicates its integration in the laboratory workflow. The results of this study could improve the quality and simplicity of the biopsy technique by directly targeting the appropriate subgroup of cells and making them easier to be excised.

Trial registration number: not applicable

Abstract citation ID: dead093.546

P-186 Combination of proteomics and automatic scoring using artificial neural networks to detect aneuploid embryos

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Study question: How powerful is the combination of artificial neural networks that combine embryo's protein profile with its automatic score provided by time-lapse videos to predict ploidy?

Summary answer: An artificial neural network (ANN) that considers proteomics and automatic score assigned by deep learning achieves 71% accuracy in distinguishing between euploid and aneuploid embryos.

What is known already: Currently the most widely used technique for detecting chromosomal abnormalities involves biopsy of the developing embryo. However, it has several disadvantages related to invasiveness, technical difficulty, high economic costs, etc. Therefore, different non-invasive techniques are being studied for the detection of aneuploid embryos. The discovery of cell-free DNA (cfDNA) released by the embryo to the culture media during its development marked the beginning of a new era of noninvasive PGT (niPGT) but some factors require adaptation for the analysis. Also, artificial Intelligence (AI) represents a valuable alternative to developing new models for predicting PGT outcomes without disturbing the embryo.

Study design, size, duration: This study included 294 samples of culture medium from 81 treatments of the PGT-A program. Out of the total, 23 were control samples (medium in which no embryos had been cultured) and 271 were samples where there was a developing embryo. Embryos were cultured until the blastocyst stage in EmbryoScope systems (Vitrolife, Sweden) with single-step medium (Gems, Genea) and automatically scored by iDAScore v2 algorithm from 1 to 9.9.

Participants/materials, setting, methods: The spent culture medium was collected on day 5/6 of embryo development and chromosome analysis was performed using next-generation sequence technology (Juno Genetics, Valencia). The relative concentrations of 92 proteins were analysed using Proseek Multiplex Assays (Olink Bioscience) using 1 µl of each sample. The final assay readout was presented as Normalized Protein eXpression (NPX) values. Finally, we developed our own ANN algorithms considering protein profile and automatic embryo score.

Main results and the role of chance: For euploid embryos (n = 101), 35 protein samples analysed had different NPX values between conditioned and control media*. The relative concentration was reduced for 11 proteins (consumption by the embryo) and 24 proteins increased their levels (secretion). For aneuploid embryos (n = 170), 33 protein samples analysed had different NPX values between conditioned and control media*. The relative concentration was reduced for 4 proteins and 29 proteins increased their levels. Out of the total, only 6 proteins had on average different concentrations between normal and abnormal embryos*: MCP_1, IL_17A, CXCL1, IL18, IL_22RA1 and CSF_1. Additionally, the automatic embryo score provided by iDAScore v2 algorithm was higher for euploid embryos than for aneuploid embryos (5.9 ± 2.7 vs. 5.1 ± 2.6)*. For ploidy prediction, three architectures of ANN were developed with different input data ANN1 (six discriminatory proteins), ANN2 (automatic embryo score) and ANN3 (six discriminatory proteins and automatic embryo score). Our dataset was divided into 68% training, 16% validation and 16% test. The accuracy, sensibility and specificity for the test phase were as follows: 63.6%, 76.9% and 44% for ANN1; 61%, 11.8% and 95.8% for ANN2; and 71.1%, 62.5% and 77.3% for ANN3.

Limitations, reasons for caution: Only one laboratory by using single step culture medium from one brand was involved in this study.

Wider implications of the findings: Our study showed a new approach to avoid transferring aneuploid embryos in cases where embryo biopsy is not performed. In addition, further studies on this field may result in a new non-invasive methodology for detecting aneuploidy.

Trial registration number: PI21/00283

Abstract citation ID: dead093.547

P-187 Vitrified-warmed donor oocytes' cryo-survival rate is not associated with subsequent embryological outcomes and clinical pregnancy rate per first transfer

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Study question: Is the survival rate of a cohort of donor oocytes used for each recipient associated with the outcomes of the cycle?

Summary answer: Oocyte cryo-survival rates (benchmark = 95-100%, competency = 85-95%, below-competency = 70-85%, and poor = 50-70%) are not associated with embryological outcomes and clinical pregnancy in the first transfer of the cycle.

What is known already: The use of vitrified-warmed donor oocytes allows overcoming many logistic challenges, but it is hindered by the loss of oocytes due to degeneration after warming. The 2012 Vienna Consensus established the benchmark rate of oocyte survival at 95-100%, competency level at > 85%. Results <85% cryo-survival are considered below competency, and <70% can be considered poor. Below-benchmark rates of survival may not only limit the number of oocytes, but also could translate into lower cycle KPIs.

Study design, size, duration: We analyzed 2190 vitrified-warmed donor oocyte recipient cycles, encompassing a total 20280 vitrified oocytes from 1036 donors. Cycles were carried out in 6 private IVF units from the same group. Cycles from 2018 to 2022 were included in the analysis. Cycles with PGT-A and spermatozoa from testicular biopsy were excluded. The outcome of the first transfer of each cycle was analyzed.

Participants/materials, setting, methods: Vitrification was performed using open carriers and two different commercial media (Irvine-Scientific and Kitazato). In all cases, fertilization was carried out by ICSI, and embryos were cultured to the blastocyst stage at low oxygen tension. Blastocysts with good or fair quality (A, B & C by ASEBIR Criteria) were considered usable, and the usable blastocyst rate was the sum of all blastocysts transferred and cryopreserved, divided by the number of zygotes on day1.

Main results and the role of chance: The mean number of warmed oocytes per cycle was 9.3, with a survival rate of 93.7%. A pairwise comparison of fertilization rates with Bonferroni correction revealed that the benchmark survival rate group had similar results to the 85-95%, 70-85% and the

50-70% groups (95-100%: 73.7%; 85-95: 72.1%, 70-85%: 70.9%, 50-70%: 73.1%, p = 1 in all cases).

The usable blastocyst rate was similar between the benchmark survival rate and the 85-95% and 50-70% groups, but higher than the 70-85% group (95-100%: 49.0%; 85-95: 48.5%, p = 1.00; 70-85%: 43.5%, p = 0.012, 50-70%: 51.3%, p = 1.00).

Of 1368 Day5, SET, first-attempt transfers, 53.5% had a clinical pregnancy. The mean age of recipients undergoing embryo transfer was 42.6 ± 4.5 years and they had undergone 1.1 ± 1.8 previous IVF cycles. Adjusted-OR of clinical pregnancy was only significantly lowered in comparison to the benchmark survival rate in the 70-85% survival group (OR:0.49; 95%CI:0.34−0.71), but not it in the 50-70% group (OR:1.50, 95%CI: 0.92-2.46) nor the 85-95% group (OR:1.00, 95%CI: 0.70-1.42). The use of a sperm donor was positively correlated with clinical pregnancy (OR:1.39; 95%CI:1.04-1.88). Recipient age, male factor, and number of previous IVF cycles were not correlated with clinical pregnancy.

Limitations, reasons for caution: Retrospective study. Only open carriers were used for vitrification. Cumulative outcomes were not assessed.

Wider implications of the findings: Sub-benchmark levels of oocyte cryo-survival were not directly associated with worse laboratory outcomes or clinical pregnancy rates. These results are encouraging for cases with lower cryo-survival and useful to counsel patients in this regard. Nevertheless, striving to meet benchmark cryo-survival rates is essential.

Trial registration number: Not Applicable

Abstract citation ID: dead093.548
P-188 Cleavage stage morphokinetic timings can predict the likelihood of aneuploidy in blastocysts

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Study question: Can morphokinetic analysis be used as a tool to predict the ploidy status of blastocysts?

Summary answer: A blastocyst is more likely to be aneuploid when it divides slowly to two cells and eight cells but quickly to three cells.

What is known already: Existing data suggests morphokinetic analysis and PGT-A both have advantages and limitations when used independently. However, the hope is that if strong associations can be found linking morphokinetic qualities of embryos and their ploidy status, then this can be used as a predictive tool. Currently, morphokinetic analysis cannot be used as a predictive tool for euploidy. However, there is some existing data that suggests that clinics can correlate morphokinetics and ploidy status using their in-house data.

Study design, size, duration: The study was retrospective and involved the assessment of blastocysts that had undergone PGT-A with next generation sequencing (NGS) and had a confirmed outcome of aneuploid or euploid between January 2021 and June 2022. Only patients who obtained genetic results showing they had at least one aneuploid embryo and at least one euploid embryo were included to allow for a matched case-control study – this resulted in 89 aneuploid and 89 euploid embryos being analysed.

Participants/materials, setting, methods: All embryos were cultured in the same conditions, including the same incubators, media (both from Vitrolife, SWE), and standard operating procedures. Morphokinetic data was exported and analysed from annotations made on the EmbryoScope annotation software. Embryos that could not be fully annotated were excluded from analysis. A conditional logistic regression was performed in order to ascertain the effects of each morphokinetic parameter on the likelihood that the embryo will be aneuploid or euploid.

Main results and the role of chance: From 89 patients, 178 embryos were analysed, of which half were euploid and half were aneuploid. The morphokinetic time points assessed were time to pronuclei fading (tPNF), time to 2-cell (t2), time to 3-cell (t3), time to 4-cell (t4), time to 5-cell (t5), time to 8-cell (t8), time to start of blastulation (tSB) and time to blastulation (tB). The duration of the second cell cycle (cc2) and its synchrony (s2) were also analysed. When euploid and aneuploid case-control matched embryos were compared, t2, t3 and t8 were the best predictors of aneuploidy when analysed together ($P = 0.06$, $P = 0.074$, $P = 0.071$ respectively). There are higher odds of a blastocyst being aneuploid if a later t2 (OR = 1.361), an earlier t3 (OR = 0.784) and a later t8 (OR = 1.047) have occurred. A later t2 ($P = 0.044$, OR = 1.361) is statistically significant when alongside an earlier t3 ($P = 0.122$, OR = 0.828). A later t8 is statistically significant, irrespective of t2 and t3 ($P = 0.05$, OR = 1.043). In addition to these results, there is a general trend for aneuploid embryos to develop to each time point slower than euploid embryos. Furthermore, there is more variation in the range of values for each time point in aneuploid embryos compared to less variation in euploid embryos.

Limitations, reasons for caution: There was a small number of embryos included in the study, so a larger cohort would increase the statistical power. A multi-centre study would also be beneficial to incorporate factors such as larger patient demographics, multiple embryo culture environments and variable clinical protocols.

Wider implications of the findings: Utilising morphokinetic data to predict aneuploidy can help us to counsel the patient on selecting embryos for biopsy, and to make a predictive tool that would be applicable within our laboratory and patient settings. A secondary impact could be supporting the use of non-invasive methods to predict embryo ploidy.

Trial registration number: Not applicable

Abstract citation ID: dead093.549
P-189 Systematic management and confirmation of safety (Safety management manual; 7 CHA IVF laboratory instructions; Field training; Lecture; Inspection) can prevent embryo mix-up in IVF laboratory

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Study question: Is systematic patient safety management training effective in improving awareness in safety confirmation and preventing errors in Embryo mix-up for embryologists?

Summary answer: From survey for 116 embryologists, they responded that they followed safety management instructions and regulations, and that they improved their safety work capability and awareness

What is known already: The cases of embryo mix-up known through the media include errors of sperm change during IUI, fertilization, Freeze-thawing, PGT, and Embryo transfer process, but there will be more due to statistical difficulties. Errors mostly occur because clinics do not have the right protocol. Currently, there aren't any regulations or policies to track eggs, sperm, embryos or frozen embryos during ART. For this reason, infertile couples have to choose clinics with high pregnancy rates and safe clinics on their own to perform assisted reproductive techniques.

Study design, size, duration: This study was conducted through a survey of 116 embryologists who worked at five clinics of CHA fertility centers. The patient safety management system was created from the CHA fertility Center itself. From August 2021 to December 2022, safety confirmation training was conducted for 16 months. All embryologists were anonymously surveyed twice with 32 questions. The first survey was conducted in April 2022, and the second survey was in December 2022.

Participants/materials, setting, methods: The survey was conducted in 32 items with 10 categories. Interviewees' made response for each item in five steps. Each item asked 1) Learning in the standard safety manual 2) Changes in safety awareness before and after training, 3) Compliance to the seven safety instruction (double checks) for each work part, 4) Level of difficulty in education, 5) Effects of education and 6) Suggestions for safety confirmation.

Main results and the role of chance: In the first survey, 61 out of 112 responded (54.5%) 60 out of 116 (51.7%) in the second. The answers were scored using a 5-point Likert scale (1 = strong disagree, 5 = Strong agree).

1. The mean score for work satisfaction was 3.1 ± 0.8 out of 5 in the first survey and 3.1 ± 0.7 out of 5 in the second. Work satisfaction remained unchanged.
2. The perception of patient safety confirmation was 3.7/5 before training and 4.0/5 after training ($p < 0.03$) in the second which improved the perception of safety confirmation.
3. The safety confirmation ability through training was improved to 4.1/5($p < 0.01$) after training compared to 3.8/5 in both the first and second surveys.
4. The necessity and importance of safety training were considered important in the first survey as it scored 3.6/5 and 4.5/5 ($p < 0.001$) in the second survey.
5. In the course of work, they responded that their safety management skills were improved (1st: 3.4/5, 2nd: 4.5/5($p < 0.05$)) by the effects of training.

As for CHA IVF lab, the embryologists were well aware of the guidelines (scoring 4.5/5 in the first and second survey) to know seven safety instructions (which are double check, outloud, RI witness, monitoring, document, information) should be followed well.

Limitations, reasons for caution: Embryo mix-up during ART procedures can be a major mistake to be avoided. The confirmed documentations can make errors any time. Establishing the safety protocol of the same process as ART procedure, the system, and education are important, but the most important is the safety management capability of the manager.

Wider implications of the findings: The safety management manual, the 7 CHA IVF laboratory patient safety instructions, online lecture training, field training, and inspection have raised awareness of the safety errors and the importance of double checking. Errors in safety can be prevented through systematic system development and repeated safety training.

Trial registration number: non-clinical trials

Abstract citation ID: dead093.550

P-190 Spontaneous blastocyst collapse during pre-vitrification equilibration is related to a lower live birth rate: a prospective cohort study

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Study question: This report provides updated data from a study investigating the association between the spontaneous collapse patterns of blastocysts during pre-vitrification equilibration and pregnancy success.

Summary answer: Live birth rates were lower for completely collapsed blastocysts, suggesting the possibility for including blastocyst collapse pattern as a criterion for selecting embryos for transfer.

What is known already: Previous time-lapse studies have found that blastocysts exhibiting strong contraction during culture have a low probability of hatching in animals and implanting in humans, suggesting blastocyst contraction has a negative impact on reproductive outcomes. Conversely, artificial shrinkage of human blastocysts before vitrification is considered to improve embryo survival and/or pregnancy rates by promoting cryoprotectant permeation inside the blastocoel. However, shrinkage is not induced in some vitrification protocols, including ours, as the viability is high; this demonstrates the potential of existing protocols to achieve sufficient cryoprotectant permeation. Moreover, the Alpha consensus meeting on cryopreservation did not issue recommendations regarding artificial shrinkage.

Study design, size, duration: This study included 798 patients who were undergoing their first autologous IVF/ICSI cycle followed by a freeze-all strategy, including blastocyst vitrification, at our clinic between June 2018 and November 2021. For patients with multiple vitrified blastocysts, embryos for transfer were selected hierarchically based only on morphological scoring during culture. To reduce bias, only data from a single blastocyst (day 5, Gardner score 4, excluding CC) transfer from each patient's first warmed cycle was analyzed.

Participants/materials, setting, methods: Blastocysts were vitrified-warmed in in-house-prepared solutions using Rapid-i carriers. Prior to vitrification, intact blastocysts were equilibrated in 10% ethylene glycol (15 min, 37 °C). Their spontaneous collapse patterns were assessed under an inverted microscope before they were vitrified in 15% ethylene glycol + 15% dimethylsulfoxide + 0.5 M sucrose. Blastocyst collapse was defined as the separation of the trophectoderm cells from the zona pellucida. Collapsed blastocysts with/without a blastocoel cavity were defined as partially/completely collapsed blastocysts.

Main results and the role of chance: Non-collapsed (Nc), partially collapsed (Pc), and completely collapsed (Cc) blastocyst transfers (embryo survival rate, 99.6%) constituted 27.1%, 54.6%, and 18.3% of the 798 cycles, respectively. Live birth rates differed significantly between groups (Nc, 54.2%; Pc, 46.6%; Cc, 34.2%; $P < 0.001$). Regarding perinatal outcomes of 366 singletons, the female rate tended to differ between groups (Nc, 35.3%; Pc, 44.3%; Cc, 53.1%; $P = 0.084$). Significant differences in maternal age (years: Nc, 34.6 ± 4.0; Pc, 35.4 ± 3.7; Cc, 36.4 ± 4.0; $P < 0.001$), MII oocyte number (Nc, 13.0 ± 6.2; Pc, 12.0 ± 6.6; Cc, 10.8 ± 6.9; $P = 0.009$), blastocyst diameter (μM: Nc, 184 ± 18; Pc, 181 ± 20; Cc, 173 ± 23; $P < 0.001$), and good-quality (4AA/4AB/4BA) blastocyst percentage (Nc, 70.4%; Pc, 61.2%; Cc, 47.9%; $P < 0.001$) were found between groups. Upon logistic regression analysis including these potential confounders, only maternal age (odds ratio (OR), 0.89; 95% confidence interval (CI), 0.86–0.93; $P < 0.001$) and collapse pattern (Cc/Nc: OR, 0.59; 95% CI, 0.37–0.93; $P = 0.023$) significantly impacted the live birth rate, whereas blastocyst morphology (good/not: OR, 1.37; 95% CI, 0.97–1.94; $P = 0.069$) tended to impact it.

Limitations, reasons for caution: The cryoprotectant concentration of our pre-vitrification equilibration solution is relatively low. Thus, caution is required when assessing the collapse pattern, as the solution osmolarity can influence blastocyst shrinkage. Follow-up studies with more participants are warranted to confirm these results and the health of the children born after vitrified-warmed embryo transfers.

Wider implications of the findings: A negative relationship was found between spontaneous blastocyst collapse during pre-vitrification equilibration and live birth rate. Studying blastocyst collapse pattern may assist selection of the most viable blastocysts after vitrification in ethylene glycol and dimethylsulfoxide—a widely used permeable cryoprotectant combination—thus, increasing IVF success rates.

Trial registration number: Not applicable

Abstract citation ID: dead093.551

P-191 The impact of resveratrol supplementation as antioxidant on embryonic development in older women (over 40years) undergoing IVF

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Study question: Does the resveratrol supplementation in human embryo culture medium improve embryo quality and pregnancy outcomes in older women?

Summary answer: Resveratrol significantly improved good quality embryo rates. These results are thought to be due to the biological activities of resveratrol as an antioxidant.

What is known already: Reproductive aging involves age-related declines in ovarian function and development capacity. After initiating meiosis, germ cells arrest at the dictyate stage of prophase I in the ovary for 10-50 years in women. Accumulated reactive oxygen species (ROS) affect oocytes adversely. Antioxidants reduce ROS-induced damage to oocytes and resveratrol is an important antioxidant polyphenolic compound. Resveratrol has positive effects on fertility, it improves the development of blastocysts in aged animal and it could enhance the maturation and quality of oocytes from aged

women. However, there are just a few studies there are clarified relationship between resveratrol and embryos in older women.

Study design, size, duration: The study retrospectively included 966 patients with aged over 40 years to evaluate the impact of resveratrol on embryo quality and IVF pregnancy outcomes between January 2019 and December 2021.

Participants/materials, setting, methods: The oocytes from aged female (over 40years) were divided into 2 groups, fertilized and cultured in MRC media (MRC#D13 (day 1∼3) and MRC#D46 (day 4∼6 culture medium); Maria Medical Foundation, South Korea) in the presence or absence of resveratrol (0.5 μM; Sigma-Aldrich, USA). Fertilization, good quality embryo, clinical pregnancy, and implantation rate after embryo transfer were compared among between the two groups. Data analyzed using the SPSS and T-test p value <0.05 considered statistically significant.

Main results and the role of chance: There was no significant difference in female age (42.5 ± 2.2 vs. 42.3 ± 2.0, P = 0.358), No. of previous ART cycles (3.6 ± 2.3 vs. 3.7 ± 2.5, P = 0.680), thickness of endometrium (9.2 ± 1.5 vs. 9.4 ± 1.4, P = 0.312), maturation rate per oocytes (74.6% vs. 75.8%, P = 0.290), fertilization rate (81.5% vs. 80.0%, P = 0.165), and No. of transferred embryos (2.4 ± 0.6 vs. 2.4 ± 0.6, P = 0.818). However, good quality embryo rate (44.0% vs. 36.4%, p < 0.05) in the resveratrol group was 7.6% higher than the control group. Pregnancy rate (26.8% vs. 22.7%, P = 0.167) and implantation rate (11.9% vs. 9.9%, P = 0.131) were slightly higher than control group but this was not statistically significant.

Limitations, reasons for caution: This is a retrospective study that generates strong conclusions, but it could be interesting to test it in a prospective study. The study was conducted at a single IVF center. Embryo transfer result is based on implantation rate, while the live birth data for all pregnancies are not yet available.

Wider implications of the findings: This is the first study to investigate the effects of resveratrol on the embryonic developmental competence and pregnancy potential of older women (over 40years).

Trial registration number: not applicable

Abstract citation ID: dead093.552
P-192 Blastocyst collapse during embryonic development is a marker of low pregnancy potential, independent of other blastocyst evaluation criteria

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Study question: Is evaluation of blastocyst collapse during embryonic development an independent marker for ongoing pregnancy in single frozen-thawed blastocyst transfer cycles?

Summary answer: Collapse of blastocyst more than 20.4% of the area may be a marker of low ongoing pregnancy potential, independent of other evaluation criteria.

What is known already: Time-lapse monitoring revealed that a section of the extended trophectoderm (TE) is ruptured during development. This TE rupture causes an efflux of blastocoel fluid and a reduction in embryonic volume. This phenomenon is described as collapse. Several studies have reported that the collapse results in a low clinical and ongoing pregnancy potential for the blastocyst. However, the collapsed blastocysts were associated with poor morphological quality. Previous studies have not evaluated whether collapse is an independent predictor of pregnancy based on other blastocyst evaluation criteria. Therefore, it is necessary to determine whether blastocyst collapse is an independent predictor of ongoing pregnancy.

Study design, size, duration: This retrospective, single-center study was conducted at the TAKAHASHI WOMEN'S CLINIC from January, 2018 to December, 2020. All blastocysts were derived from the ICSI cycles. Time-lapse monitoring using Embryoscope + (Vitrolife) was used to observe the presence and degree of collapse. The degree of collapse was calculated from the embryonic volume at the maximum expansion and the lowest volume after collapse. We analyzed the relationship between 904 cycles of single vitrified-warmed blastocyst transfer and ongoing pregnancy.

Participants/materials, setting, methods: The ongoing pregnancy rates of collapsed and non collapsed blastocysts were compared using chi-squared test. The cut-off value of the degree of collapse for ongoing pregnancy was calculated using receiver operating characteristic (ROC) curve analysis. The effect of collapse above the cut-off value on ongoing pregnancy was examined using multivariate logistic regression analysis, including confounding factors (patient age, body mass index, inner cell mass (ICM) grade, TE grade, day of blastocyst formation, and expansion stage).

Main results and the role of chance: During the study period, 329 of the 904 transferred blastocysts exhibited collapse during time-lapse monitoring, and the degree of collapse ranged from 2.10% to 75.90%, with a median of 30.70%. In total, 575 transferred blastocysts were determined to be non collapsed. The mean patient age (± standard division) was 36.89 ± 4.77 years in the collapsed blastocyst group and 36.52 ± 4.42 years in the non collapsed blastocyst group (p = 0.151). The proportion of blastocysts with poor morphological grade (<BB) was higher in the collapsed blastocysts group than in the non collapsed blastocysts group (32.5% vs. 17.91%, p < 0.0001). The ongoing pregnancy rate was significantly lower in the collapsed blastocysts group than in the non collapsed blastocysts group (45.4% vs. 34.0%, p = 0.0009). ROC curve analysis showed that the cut-off value for the degree of collapse for low pregnancy potential was 20.4%. Multivariate logistic analysis with confounding factors revealed that blastocyst collapse above the cut-off value (≥20.4%) had an effect on ongoing pregnancy (adjusted odds ratio: 0.53, 95% confidence interval: 0.34–0.82, p = 0.005) independent of ICM and TE grades, day of blastocyst formation, and expansion stage.

Limitations, reasons for caution: This retrospective study was conducted at a single fertility center. In addition, the analyzed blastocysts were derived only from the ICSI cycles. Therefore, it is necessary to analyze the effects of collapse in blastocysts derived from C-IVF cycles.

Wider implications of the findings: Our results demonstrate that ≥20.4% collapse may be an additional marker to consider when selecting blastocysts for transfer. If the blastocysts are of the same grade, it is better to select non collapsed embryos.

Trial registration number: not affect

Abstract citation ID: dead093.553
P-193 Comparison of ICSI outcomes and euploidy rates between AI and non-AI sperm selection

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Study question: Can artificial intelligence (AI) assisted sperm selection improve intracytoplasmic sperm injection (ICSI) outcomes and euploidy rates?

Summary answer: AI selected spermatozoa showed no statistical significance in various measures of ICSI outcomes but an increasing trend in euploidy rate can be observed.

What is known already: All ICSI practitioners would like to have an objective tool for the selection of sperm to be injected as it is one of the factors in producing successful ICSI. Sperm selection assistant (SiD: (IVF 2.0 Limited, UK) is a real-time AI spermatozoa identifier that assesses all spermatozoa in a visual field based on their motility patterns individually. Mendizabal-Ruiz et. al. (2022) have reported promising data showing SiD may reduce subjectivity in sperm selection, which can potentially be an alternative method to our current practice.

Study design, size, duration: From June 2022 to December 2022, 89 ICSI cycles (mean age: 35.3; age range: 26.0–47.0) were done in Alpha IVF & Women's Specialists. A sibling AI (Group A) and non-AI (Group B) assisted sperm selection study with ICSI was performed on the oocytes retrieved from these cycles. Of which, 76 cycles were planned for Preimplantation Genetic Testing for Aneuploidies (PGT-A) on utilizable blastocysts.

Participants/materials, setting, methods: All matured oocytes were injected using Piezo-ICSI method (Prime Tech Ltd, Japan). Half of the oocytes of each cycle were injected with SiD selected sperm whereas the other half by manual selection. All injected oocytes were cultured up to day-7 and trophectoderm biopsy for PGT-A screening (IonTorrent, USA) was done on utilizable blastocysts prior to vitrification (Cryotec, Japan). The fertilization, blastulation, utilization and euploidy rates were assessed in both groups.

Main results and the role of chance: In Group A, the fertilization rate (2PN), abnormal fertilization rate (>2PN), blastulation rate from 2PN, blastocyst utilization rate from 2PN and euploidy rate from utilizable blastocysts were 81.5% (419/514), 2.1% (11/514) 77.1 (323/419), 57.5% (241/419) and 45.2% (71/157) respectively. In Group B, the fertilization rate (2PN), abnormal fertilization (>2PN), blastulation rate from 2PN, blastocyst utilization rate from 2PN and euploidy rate from utilizable blastocysts were 79.7% (427/536), 2.8% (15/536), 75.9% (324/427), 59.0% (252/427) and 35.5% (60/169) respectively. No significant differences were observed in the fertilization rate ($p = 0.4828$), abnormal fertilization rate ($p = 0.5545$), blastulation rate from 2PN ($p = 0.6861$), blastocyst utilization rate ($p = 0.7278$) and euploidy rate ($p = 0.0898$). Although the ICSI outcomes are comparable between the two groups, AI assisted sperm selection showed an increasing trend in euploidy rates. SiD may be considered as a tool to assist embryologist in selecting better spermatozoa during ICSI procedures.

Limitations, reasons for caution: The patient sample size was small but further studies with a larger sample size certainly seem to be justified, particularly in association with PGTA on utilizable blastocysts. The value of SiD shall be greatly enhanced when spermatozoon morphology can also be measured.

Wider implications of the findings: This is more evidence suggesting that SiD may improve ICSI outcomes and euploidy rate when compared to manual selection by an embryologist. In addition, it could be used to train and assist embryologists learning ICSI procedures.

Trial registration number: not applicable

Abstract citation ID: dead093.554
P-194 Initial experience of using iDAScore as a tool to predict euploid blastocysts

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Study question: Can the annotationfree embryo scoring system (iDAScore) predict the likelihood of euploidy in embryos?

Summary answer: Our results have shown that there is a significant positive correlation between iDAScore and blastocyst euploidy.

What is known already: iDAScore (Vitrolife, Sweden) is an embryo ranking model that was developed by using artificial intelligence (AI) and deep learning. It does not require any user-dependent annotation which largely eliminates the subjectivity of manual annotation by embryologists. It has also been reported to be a robust predictor in likelihood of embryo implantation. However, the correlation between iDAScore and euploidy has not been extensively studied. Therefore, this study is to determine if iDAScore can be utilized as a predictor for embryo euploidy.

Study design, size, duration: A total of 860 blastocysts (mean age: 30.0; age range: 19.0-35.0) were assessed with iDAScore on day-5 and/or day-6 of embryo development from January 2022 to October 2022 at Alpha IVF & Women's Specialists. The euploidy rates were analyzed based on the stratified iDAScores (Group A:0.0-4.9; Group B: 5.0-7.4; Group C:7.5-8.9; Group D:9.0-10.0).

Participants/materials, setting, methods: Scores were computed by the iDAScore software at the Vitrolife Technology Hub (Vitrolife, Gothenburg, Sweden) prior to trophectoderm biopsy for Preimplantation Genetic Testing for Aneuploidies (PGT-A) (Ion Torrent, USA). The ploidy status (euploid or aneuploid) based on PGT-A results were recorded and assessed with the corresponding iDAScore.

Main results and the role of chance: The euploidy rates of Group A, B, C and D were 56.7% (17/30), 60.0% (105/175), 65.9% (178/270) and 74.8% (288/385) respectively. Group D shows significantly higher euploidy rate compared to Group B and Group C ($p = 0.0005$, $p = 0.0143$). A weak significance was observed between Group D and Group A ($p = 0.0505$). This is probably due to smaller sample size in Group A. No significant differences were displayed among Group A, Group B and Group C ($p = > 0.005$). Our results demonstrated an increasing trend between iDAScore and the proportion of euploid embryos.

Limitations, reasons for caution: Few of the blastocysts in Group A could be selected for biopsy so the sample size was only a fraction of the other groups. Further studies and larger sample sizes would allow the findings to be stated more confidently.

Wider implications of the findings: Although the iDAScore algorithm scores the chance of embryo implantation, we have demonstrated its potential to assist in predicting the embryo ploidy status in a non-invasive manner. This may be used to inform the decision of which and how many blastocysts to biopsy and transfer, thus reducing treatment costs.

Trial registration number: not applicable

Abstract citation ID: dead093.555
P-195 Can we predict blastulation, implantation and live birth in early stages of embryo development? Clinical validation of a classification algorithm based on automatic morphokinetic annotations

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Study question: Can a classification algorithm based on automatic annotations of early morphokinetic timings improve embryo selection in ICSI cycles?

Summary answer: The automatic scoring resulted significantly predictive of blastulation, implantation and live birth, especially when combined with morphological evaluation, but not of euploidy status.

What is known already: The Eeva Test® is an imaging system that automatically annotates morphokinetic parameters of the early embryo development. The latest version features the Xtend® algorithm, which classifies embryos on day 3 of development from highest (1) to lowest (5) potential to reach blastocyst stage, based on four parameters: the automatically recorded parameters P2 (t3-t2) and P3 (t4-t3), plus oocyte age and number of cells. Previous bibliography suggests that the Eeva scoring, in combination with morphologic evaluation, might also be predictive of implantation and live birth potential. However, the efficacy and reproducibility of this algorithm need to be validated with external data.

Study design, size, duration: Retrospective, observational cohort study, performed over 3736 embryos from oocyte donation cycles and 1291 embryos from autologous cycles with preimplantation genetic testing for aneuploidies (PGT-A) performed in a single IVF clinic over 3 years. All embryos were scored by the Eeva Test on day 3. The performance of the Eeva-Xtend scoring system as predictor of blastocyst development, euploidy, implantation and live birth was assessed.

Participants/materials, setting, methods: Embryo development was monitored by a time-lapse system. Embryo selection was performed by morphological (ASEBIR) criteria, resulting in the transfer of 959 embryos. The

correlation between the Eeva-Xtend scoring, individually and combined with morphological classification, and the study outcomes was quantified by generalized estimating equations (GEE) including main possible confounders, and expressed in terms of odds ratio (OR). The performance of the GEEs was quantified and compared through the Area Under the ROC Curves (AUCs).

Main results and the role of chance: A positive association ($P<0.001$) was confirmed between lower Eeva scores and higher odds of reaching blastocyst stage (OR (1 vs 5) = 15.849, 95%CI 12.510-20.078; OR (2 vs 5) = 11.592, 95%CI 9.229-14.560; AUC = 0.768 (95%CI 0.754−0.783)), and consistent in both oocyte donation and PGT-A cycles. Lower morphokinetic embryo scores also resulted significantly associated with higher chances of implantation (OR (1 vs 5) = 3.385, 95%CI 1.507-7.605; $P = 0.003$) and live birth (OR (1 vs 5) = 5.132, 95%CI 2.089-12.605; $P<0.001$) in oocyte donation cycles, but not in embryos subjected to PGT-A: OR (1 vs 5) = 2.089, 95%CI 0.321-13.614, $P = 0.441$ for implantation and OR (1 vs 5) = 0.916, 95%CI 0.161-5.205, $P = 0.921$ for live birth. In embryos subjected to PGT-A, the automatic scoring system did not result significantly associated with the euploidy status: OR (1 vs 5) = 0.755 (95%CI 0.255−0.981; $P = 0.489$). In global, the Eeva Test had similar performance than morphological evaluation for implantation (AUC = 0.610 (95%CI 0.572−0.648) vs 0.606 (95%CI 0.568−0.644), respectively) and live birth (AUC = 0.622 (95%CI0.584−0.660) vs 0.609 (95%CI 0.571−0.647)) prediction. However, the highest performance was achieved when combining both scoring systems: AUC for implantation = 0.629 (95%CI 0.592−0.666), AUC for live birth = 0.636 (95%CI 0.598−0.673).

Limitations, reasons for caution: The retrospective nature of the study introduces some variability in the characteristics of the sample. However, the large sample size partially overcomes said limitation. The automatic annotations of the morphokinetic parameters were not validated in this study.

Wider implications of the findings: Our results support the applicability and reproducibility of this automatic early embryo evaluation system. Furthermore, our findings support that the use of this automatic embryo evaluation system in combination with morphological evaluation can potentially reduce subjectivity, improve embryo evaluation and increase success rates in IVF treatments.

Trial registration number: not applicable

Abstract citation ID: dead093.556
P-196 PIEZO-intracytoplasmic sperm injection (P-ICSI) can improve the clinical outcomes of ICSI

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Study question: Can P-ICSI improve the clinical outcomes of ICSI, such as degeneration, fertilization and development to blastocyst, compared to conventional ICSI (C-ICSI)?

Summary answer: P-ICSI can improve the clinical outcomes of ICSI by increasing the number of fertilized oocytes and decreasing the number of degenerated oocytes after ICSI.

What is known already: In human IVF-ET, some of oocytes are damaged after ICSI when retrieved oocytes are inseminated by ICSI. The application of P-ICSI has been limited although it was developed over 30 years ago. Recently, it has been reported that P-ICSI can decrease damage after ICSI and improve fertilization and embryonic development compared to C-ICSI.

Study design, size, duration: This study included 50 subjects undergoing IVF and was performed between April and October 2022. Sibling oocytes were randomly assigned to C-ICSI and P-ICSI.

Participants/materials, setting, methods: A total of 1,207 oocytes were retrieved from 50 patients. Among them, 852 oocytes with visible spindle were inseminated by C-ICSI or P-ICSI in sibling oocytes. Four hundred twenty-five oocytes were inseminated by C-ICSI and 427 oocytes were by P-ICSI. Fertilization, degeneration after ICSI and blastocyst development on day 5 or day 6 were compared between the P-ICSI and C-ICSI. The differences between C-ICSI and P-ICSI were statistically analyzed using Mann-Whitney U test.

Main results and the role of chance: Normal fertilization rate of P-ICSI (78.2 ± 16.5%) were higher than that of C-ICSI (71.2 ± 18.9%). The difference was statistically significant ($P = 0.0447$). The oocyte degeneration rates after ICSI were 9.3 ± 11.8% (C-ICSI) and 4.8 ± 9.4% (P-ICSI). The degeneration rate of C-ICSI was significantly higher than that of P-ICSI ($P = 0.04338$). Blastocyst formation rate of C-ICSI was 58.6 ± 25.0% and that of P-ICSI was 64.3 ± 26.3%. The blastocyst formation rate of P-ICSI was higher than that of C-ICSI but the difference was not statistically significant ($P = 0.2113$). The rates of blastocysts that were available for embryo replacement or cryopreservation were higher in P-ICSI (56.0 ± 25.4%) than in C-ICSI (50.4 ± 24.7%) although the difference was not significant ($P = 0.26272$).

Limitations, reasons for caution: Pregnancy outcomes data were not available. The data of this study were obtained from single fertility center. More large-scale multicenter studies will be needed to confirm the effectiveness of P-ICSI.

Wider implications of the findings: Consistent with other studies, these results show that P-ICSI reduces the damage rate of oocytes after ICSI. The implementation of P-ICSI has the potential to improve clinical outcomes of human IVF although it is more complicated and takes longer than that of C-ICSI.

Trial registration number: not applicable

Abstract citation ID: dead093.557
P-197 Nuclear transfer with almost nonexistent mitochondrial carryover -a mouse model-

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Study question: Can the transfer of aggregated chromosomes (AC) as compared to spindle-chromosome transfer (SCT) reduce mitochondrial DNA (mtDNA) carryover and improve development rates?

Summary answer: The rate of mtDNA carryover after AC transfer was only 0.1%, which was much less than that of 2.0% after SCT.

What is known already: Nuclear transfer techniques such as SCT and pronuclear transfer have been applied to prevent mitochondrial diseases and to treat early embryonic arrest, however, the problem of mtDNA carryover has not yet been resolved. We previously reported on an AC transfer (ACT) method in humans which could overcome the above problem, however, consequent embryonic development rates have not been investigated in Japan due to legal restrictions and no animal study has been conducted, as the formation of ACs has not been found in any other species.

Study design, size, duration: Following our success in the creation of ACs in mouse oocytes using IBMX (3-Isobutyl 1-methylxanthine), a phosphodiesterase inhibitor, a mouse model of ACT was established. This was followed by a comparison of the rates of embryo development and mtDNA carryover in both ACT and SCT in over 100 oocytes. The distribution of mitochondria around SC and AC were also examined by confocal laser microscope (FV3000, Olympus, Japan).

Participants/materials, setting, methods: GV stage oocytes were collected from 8-12 week B6D2F1 mice. After GVBD, oocytes were incubated in KSOM medium, containing 3 mM IBMX, for 10-14 hours until AC is formed. ACs and SCs were injected into enucleated MII stage donor oocytes and then fertilized at the MII stage. Embryos were cultured for 5 days to compare the rates of embryo development. Enucleated ACs and SCs were subjected to real-time PCR, to analyze the carryover of mtDNA.

Main results and the role of chance: The rates of fertilization, embryo cleavage and blastocyst formation in AC oocytes without AC transfer (control), in ACT and in SCT were 60.7%, 55.9%, 13.2% (control), 82.5%, 80.8% and 30.8% (ACT), and 81.0%, 74.5% and 31.4% (SCT) respectively. The ACT group showed a significant increase in fertilization ($P = 0.007$), embryo cleavage ($p = 0.008$) and blastocyst formation ($p = 0.034$) rates, as compared to those in the control group. There were no significant differences between ACT and SCT regarding the rates of fertilization, embryo cleavage and blastocyst formation. The relative, real-time PCR showed that mtDNA carryover in the ACT group was 0.1% ± 0.07%, which was significantly lower than that in the SCT group of 2.0% ± 0.6% ($p = 0.027$). 3D confocal laser microscopy revealed that mitochondria were not localized around either SCs or ACs, as they were around pronuclei.

Limitations, reasons for caution: Although we succeeded in establishing a mouse model of ACT, validation in human oocytes is required to confirm the benefit of this method. Further experiments are required to confirm if healthy offspring can be obtained after ACT.

Wider implications of the findings: ACT could reduce the amount of mtDNA carryover and overcome heteroplasmy. As ACT improved the low embryo development rate caused by IBMX and had similar embryo development rates to SCT, the findings of this study may give rise to ACT being employed as a novel method for human nuclear transfer.

Trial registration number: not applicatable

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P-198 Evolution of artificial intelligence-based embryo selection models: a massive external validation on 70,456 embryos

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Study question: How do three artificial intelligence-based embryo selection models that have evolved over the years work?

Summary answer: The three automatic scores were positively associated with clinical outcomes, and the iDAScore v2 showed the best performance in conventional treatments with patient oocytes.

What is known already: Artificial intelligence (AI) models have been introduced in in vitro fertilization laboratories in recent years as an adjunct to clinical decision-making. The most initial function of the AI involved guiding embryologists in the annotations of embryonic events. This was followed by the development of embryo selection models based on machine learning, requiring annotations (i.e., KIDScore). Finally, the latest in this field are models based on deep learning, which analyze raw time-lapse video (i.e., iDAScore). In this study, the evolution of three AI-based models have been analyzed on the same large set of embryos.

Study design, size, duration: This single-center study includes 6,737 patients who underwent in vitro fertilization treatments for 6 consecutive years. A total of 7,722 cycles were analyzed, resulting in 70,456 embryos cultured in EmbryoScope® time-lapse systems. Embryos were routinely evaluated and selected according to conventional morphology (ASEBIR criteria) by senior embryologists. Retrospectively, the embryos were scored by three AI models from 1 to 9,9: KIDScore D5 v3 (n = 32,784), iDAScore v1 (n = 68,440) and iDAScore v2 (n = 68,471).

Participants/materials, setting, methods: Automatic embryo scores were compared with conventional morphology, ploidy, and clinical outcomes for single blastocyst transfers. Then, we performed multivariate logistic regression analysis (confounding factors: oocyte origin, donated-autologous; type of embryo transfer, fresh-frozen; oocyte age; patient body mass index; culture strategy, individual-group; day of embryo transfer, fifth-sixth day of embryo development) in different patient populations (PGT-A, oocyte donation program and conventional treatments with patient oocytes). Finally, the performance (AUC) was calculated for comparison.

Main results and the role of chance: The mean of the three scores increased as embryos had better morphological grade*. Regarding ploidy (euploid vs. aneuploid): 5,62 ± 1,78 vs. 5,00 ± 1,72 for KIDScore* (n = 6,580); 7.59 ± 1.61 vs. 6.92 ± 1.75 for iDAScorev1* (n = 7,089); and 6.31 ± 2.53 vs. 5.07 ± 2.55 for iDAScorev2* (n = 7,082). Regarding implantation (implanted vs. non-implanted): 6.24 ± 2.01 vs. 5.42 ± 2 for KIDScore* (n = 9,681); 8.36 ± 1.37 vs. 7.76 ± 1.70 for iDAScorev1* (n = 10,079); and 6.83 ± 2.18 vs. 5.75 ± 2.54 for iDAScorev2* (n = 10,068). Regarding live birth (positive vs. negative): 6.29 ± 2.01 vs. 5.51 ± 2.01 for KIDScore* (n = 9,668); 8,39 ± 1,34 vs.7,83 ± 1,68 for iDAScorev1* (n = 10,065); and 6.88 ± 2.15 vs. 5.87 ± 2.52 for iDAScorev2* (n = 10,054). In general, the multivariate analysis showed statistically significant odds ratio for the three models in predicting implantation and live birth (all patient subpopulations)*. Regarding oocyte donation program: the AUCs for predicting implantation were 0.636 [0.621−0.650] for KIDScore, 0.638 [0.624−0.652] for iDAScorev1, and 0.636 [0.622−0.650] for iDAScorev2; and the AUCs for live birth prediction were 0.630 [0.615−0.644] for KIDScore, 0.629 [0.615−0.643] for iDAScorev1, and 0.635 [0.621−0.649] for iDAScorev2. Regarding treatments with patient oocytes: the AUCs for predicting implantation were 0.667 [0.644−0.689] for KIDScore, 0.674 [0.652−0.696] for iDAScorev1, and 0.686 [0.664−0.708] for iDAScorev2; and the AUCs for live birth prediction were 0.664 [0.642−0.687] for KIDScore, 0.669 [0.647−0.692] for iDAScorev1, and 0.686 [0.664−0.708] for iDAScorev2.

*$p<0.001$

Limitations, reasons for caution: A major limitation of our study is its retrospective nature. Although ours is the largest external validation ever performed with an unselected ICSI population, its single-center design should be considered for the universal application of the models. Also, specific culture conditions should be addressed when considering the generalized application.

Wider implications of the findings: Our results showed a positive association between automatic scores and the success of IVF. Despite observing similar AUCs between the three embryo selection models, the use of the most advanced automatic algorithms should improve workflow, standardize the process between laboratories and allow embryologists to spend their time on other tasks.

Trial registration number: PI21/00283

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P-199 Ensuring no clinical risk: a cohort analysis on the agreement between the embryo selected by the embryologist and the embryo selected by artificial intelligence

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Study question: Is there any clinical risk in selecting the embryo to be transferred using a deep learning-based model?

Summary answer: Most embryos selected by senior embryologists match the automatically best scoring embryo, and clinical outcomes are better when this coincidence occurs.

What is known already: In vitro fertilization (IVF) techniques have changed over time with the aim of improving clinical results. Today, embryology is facing a change common to most areas of medicine, the introduction of automation. The use of automated systems in the IVF laboratory is already happening, for example, with electronic witnessing and the ranking of embryos according to their implantation potential. Relying on this type of system is not easy for operators, as they must ensure that the treatments will not be damaged.

Study design, size, duration: This is a single-center cohort study including 5,411 patients who underwent IVF treatments. Their embryos were cultured in EmbryoScope® time-lapse systems (Vitrolife, Denmark) and routinely evaluated by senior embryologists according to ASEBIR morphological criteria. Then, embryos were automatically scored using the iDAScore v2 algorithm.

Participants/materials, setting, methods: A total of 7,178 embryo transfers were analyzed. The transferred embryo was selected by the embryologists and retrospectively scored with the deep learning algorithm from 1 to 9.9. Finally, we performed a cohort analysis on the agreement between the embryo selected by the embryologist and the embryo selected by iDAScore v2. The relative risk (or incidence of implantation) (RR), relative risk reduction (RRR) and absolute risk reduction (ARR) were calculated.

Main results and the role of chance: In general, considering all transfers (fresh and frozen) with known clinical outcome (n = 7,178), the implantation rate was higher when the transferred embryo matched the highest-scoring embryo (58.37% vs. 55.49% p = 0.014). Embryos selected by senior embryologists for single embryo transfer in the fresh cycle (n = 2,783) matched the top-scoring embryo 63.50% of the time. An ongoing pregnancy was achieved in 57.96% of the patients. Out of the 36.54% of patients who had fresh transfer of an embryo that did not have the maximum iDAScore, 553 patients underwent transfer of a single frozen embryo. In this group, the first devitrified embryo coincided with the highest scoring embryo 44.67% of the time (n = 247). An ongoing pregnancy was achieved in 62.34% the patients. However, when the devitrified embryo did not correspond to the highest scoring embryo, the implantation rate was 55.27%. The RR showed that each patient was 1.13 times more likely to become pregnant if the transferred embryo matched the embryo with the highest score. The RRR stated that if there was this coincidence, the probability of implantation was increased by 12.79%. The ARR (0.07) suggested that for every 100 transfers with this match, 7 more embryos would implant than without this match.

Limitations, reasons for caution: This study is limited by its retrospective nature. Furthermore, the single-center design should be considered when generalizing the results, although our clinic was not involved in the development of this specific model.

Wider implications of the findings: The high coincidence between the embryologist's decision and the artificial intelligence's decision should comfort assisted reproduction professionals and patients. This coincidence also justifies that realistic models based on artificial intelligence perform the embryo selection procedure as good as the most experienced embryologist.

Trial registration number: Not applicable

Abstract citation ID: dead093.560

P-200 Tailoring IVF laboratory key performance indicators of the Vienna consensus in testicular sperm aspiration cases

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Study question: Are the IVF laboratory KPIs of the Vienna consensus equally applicable to ICSI cycles performed with ejaculated or testicular sperm aspiration (TESA) samples?

Summary answer: In addition to standard ICSI cycles, the Vienna KPIs are largely applicable to TESA cases, except for 2PN fertilization and day 3 development rates.

What is known already: The ESHRE SIG Embryology and Alpha Scientists in Reproductive Medicine produced the first international consensus on a systematic set of IVF laboratory KPIs. These efficiency measures can reveal the possible impact of extrinsic factors, such as operator skills or culture media, on the IVF process. However, to control for intrinsic factors – i.e., specific gamete characteristics – the Vienna Consensus focused on a "reference population" as defined by female age ≤39years, exclusion of PGT cases and use of own fresh oocytes and ejaculated sperm. This leaves the relevance of the Vienna KPIs to other patient populations, including TESA cases, uncertain.

Study design, size, duration: This was a retrospective, single-center cohort analysis of 1678 ART ICSI cycles carried out between 2010 and 2022. Treatments involving TESA and ejaculated sperm were 105 and 1573, respectively.

Participants/materials, setting, methods: Inclusion criteria were indication for IVF/ICSI with own ejaculated or TESA spermatozoa and blastocyst culture of all embryos formed in each cohort. Oocyte donation and PGT cycles were not included. Ovarian stimulation was performed with either recombinant-FSH or hMG, combined with GnRH to prevent spontaneous LH surge. Fertilization of collected oocytes was achieved by ICSI.

Main results and the role of chance: Both maternal and paternal age were comparable between the two groups. In the TESA cases, 2PN rate was lower (N = 593/915, 64.8% versus N = 7276/10350, 70.3%; p < 0.01), while 1PN rate was higher (N = 32/915, 3.5% versus N = 246/10350, 2.4%; p = 0.04). Other fertilization and developmental rates were comparable: 3PN (N = 24/915, 2.6% versus N = 341/10350, 3.3%); microinjection damage (N = 52/915, 5.7% versus 632/10350, 6.1%); failed fertilization (N = 4/105, 3.8% versus N = 61/1573, 3.9%); cleavage (N = 579/593, 97.6% versus N = 7105/7276, 97.6%); day 2 development (N = 318/593, 53.6% versus N = 3887/7276, 53.4%); day 3 development (N = 248/593, 41.8% versus N = 3279/7276, 45.1%); total good blastocyst development rate (N = 147/361, 40.7% versus N = 1728/3944, 43.8%). Compared with the Vienna Consensus, all ICSI outcomes were within normal ranges as defined by competency and benchmark values, while in TESA cycles 2PN fertilization and day 3 development rates were slightly below the competency thresholds.

Limitations, reasons for caution: The study, especially because retrospective and with small sample size, requires external independent validation

Wider implications of the findings: The study confirms the robustness of the Vienna Consensus recommendations for the monitoring of the IVF laboratory performance, while highlighting the need for fine-tuning individual indicators in specific patient populations.

Trial registration number: not applicable

Abstract citation ID: dead093.561

P-202 Using artificial intelligence platform coupled to an existing time system; external validation of an automatic embryo score to assist in selection

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Study question: How does a novel artificial intelligence-based embryo evaluation system work in Geri® time-lapse systems?

Summary answer: Automatic score platform provided externally for embryos cultured in Geri® was associated with conventional morphology, euploidy, implantation and live birth.

What is known already: Artificial intelligence has been making headway in assisted reproduction in recent years. Many companies have developed different models for automated embryo evaluation and selection, such as CHLOE™ software (Fairtility, Israel). According to the developers, it is an orchestration of cutting-edge morphological and morphokinetic AI algorithms, trained over 100,000 embryo videos and tens of millions of images. Different laboratories validated its use in specific time-lapse systems (Embryoscope, Vitrolife). However, this is the first time that an objective and independent review of CHLOE™ has been performed on Geri® (Genea Biomedx, Australia) time-lapse system.

Study design, size, duration: This retrospective analysis included 3568 embryos from 417 patients that underwent IVF treatments in a single center. Embryos were cultured in Geri® (Genea Biomedx, Australia) time-lapse systems by single step media (Geri Medium, Genea Biomedx, Australia) and routinely evaluated by senior embryologists by using Connect and Assess software according to the ASEBIR criteria (from A-high quality to D-low quality and excluded embryos). Then, embryos were automatically scored by CHLOE™ from 0 to 1.

Participants/materials, setting, methods: Automatic embryo score was compared with conventional morphology (n = 3568 embryos), ploidy (n = 467 embryos), and clinical outcomes for single blastocyst transfers (n = 461).

Main results and the role of chance: The comparison between the embryo score provided by CHLOE™ and the category assigned by embryologists showed a direct association*. The means were 0.97 ± 0.10 for A (n = 123); 0.89 ± 0.21 for B (n = 842); 0.74 ± 0.30 for C (n = 607), 0.24 ± 0.31 for D (n = 997) and 0.15 ± 0.25 for excluded embryos (n = 403). The automatic embryo score for oocytes that failed to fertilize was 0.06 ± 0.19 (n = 596). Regarding the chromosomal status, embryos with normal content had significantly higher score than abnormal ones. Following results are presented per quartiles of similar sample size: the euploidy rates were 35.9% for score < 0.81 (n = 117), 40.8% for score 0.81-0.96 (n = 120), 48% for score 0.96-0.99 (n = 125) and 58.1% for score >0.99 (n = 105)*. Implanted embryos achieved significantly higher marks than non-implanted embryos: 0.93 ± 0.15 (n = 251) vs. 0.85 ± 0.25 (n = 210)*. Also, automatic embryo score was higher for embryos that led to a live birth than those that did not: 0.94 ± 0.15 (n = 188) vs. 0.86 ± 0.24 (n = 243)*. Focusing on top quality embryos (A+B), the score means were 0.94 ± 0.16 for implanted good quality embryos 0.88 ± 0.22 for non-implanted ones* (*p<0.05).

Limitations, reasons for caution: This project is limited by its retrospective and single-center nature. Multicenter validation would be necessary to ensure it is a safe and effective method of embryo assessment.

Wider implications of the findings: In addition to verifying that automatic scoring agrees with embryologists, this study demonstrated its ability to be applied in Geri Time-lapse incubator to distinguish between potential embryos with similar morphological characteristics helping embryologist to make decisions.

Trial registration number: NOT APPLICABLE

Abstract citation ID: dead093.562
P-203 The Effect of c-Abl Tyrosine Kinase Inhibition in Cell Fate Specification and Morphogenesis During Preimplantation Mouse Embryo

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Study question: What are the mechanisms governing the regulation of c-Abl tyrosine kinase in preimplantation embryo development?

Summary answer: Inhibition of c-Abl impairs the fate determination of TE and ICM cells during mouse preimplantation embryo development, preventing cell specilization after compaction.

What is known already: The successful completion of polarization, compaction and lineage specification stages is required for the embryo to reach the blastocyst stage from the zygote stage during preimplantation embryonic development. c-Abl is a tyrosine kinase localized in cell nucleus and cytoplasm and also capable of nuclear-cytoplasmic shuttling. c-Abl is activated when DNA damage occurs and plays a role in the DNA repairment mechanism. The small-molecule inhibitor imatinib is classified as type II inhibitors which targets the inactive conformation of the kinase domain and specifically inhibit c-Abl.

Study design, size, duration: C57BL6 female mice at 6-8-wk old were superovulated with 7,5 IU *PMSG* and after 48 hr 7,5 IU *hCG, then* mated. 20 hours after hCG enjection, zygotes were collected and cultured for 96h at 5% CO_2, 5% O_2 at 37°C. We designed 5 experimental groups: control, DMSO, 1 μM, 5 μM and 10 μM (in KSOM+AA) imatinib group. To determine how altering the imatinib might affect embryonic growth and development, morphokinetic parameters were evaluated.

Participants/materials, setting, methods: Immunofluorescence staining for embryos was performed after 4% paraformaldehyde fixation. Then embryos incubated c-Abl, YAP, TEAD4, PARD6, E-cadherin and CDX2. DAPI mounting medium used for nucleus staining. Imaging was performed under a confocal microscope. Fluorescent intensity analysis was performed with ImageJ. We detected *c-Abl, Yap, Tead, Cdx2, Oct4 and Nanog* mRNA expression levels using the qRT-PCR. For the statistical analysis we used GraphPad Prism (2-Way ANOVA and and One-way ANOVA (used Geisser-Greenhouse correction)).

Main results and the role of chance: Treatment of zygotes with imatinib resulted in embryonic developmental arrest (control, 81%; 1 μM imatinib, 58.5%; 5 μM imatinib, 54%, 10 μM imatinib 0%) and led to a significant decrease in the rate of blastocyst formation. We detected that the expression level of c-Abl was decreased in the imatinib groups compared to the control group. We determined that YAP and TEAD, which should be localized in the nuclei of TE cells, lost their nuclear expression patterns and were expressed in the cytoplasm and their expression levels decreased. We detected the PARD6 pattern, which was evident at the cell borders in the control group, distributed throughout the cytoplasm in the imatinib groups. While cytoplasmic and decreased localization of CDX2, which should be nuclear in TE cells, was determined in imatinib groups, we also defined that E-cadherin distribution was impaired at cell borders compared to the control group. *c-Abl* mRNA level was decreased in the 1 and 5 μm imatinib groups, *Yap* mRNA level was decreased in the 5 μm imatinib group. *Cdx2* and *Nanog* mRNA levels were decreased in the 1 and 5 μm imatinib group (*p<0.1, **p <0.01, ***p <0.001, ****p <0.0001).

Limitations, reasons for caution: All animals used in this study were obtained from Yeditepe University Faculty of Medicine Experimental Research Center (YUDETAM) and all the experimental procedures have been approved by Yeditepe University Ethical Committee.

Wider implications of the findings: Imatinib significantly inhibited the development of eight-cell embryos to the blastocyst stage compared to controls a concentration-dependent manner, furthermore, c-Abl inhibition affected linegae specification by changing the localization of YAP/TEAD, markers of lineage differentiation. Therefore, c-Abl can regulate cell specification and cell fate determination during mouse preimplantation embryonic development.

Trial registration number: YAP-AP-SAB-21019

Abstract citation ID: dead093.563
P-204 The pattern of the zygote cleavage to three cells must be considered before embryo transfer

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Study question: What is the clinical outcome of instant irregular cleavage (IDC) from zygote to three cells?

Summary answer: IDC embryos, did not develop to blastocysts and did not acieve pregnancy; FC embryos that reached blastocyst stage exerted similar pregnancy rates as control embryos

What is known already: Evaluation of the zygote morphology is one of the earliest embryonic stages investigated. Several zygote grading systems were proposed based on pronuclei size and NPB (nucleolus precursor bodies) distribution, thus is an effective indicator of the embryo's potential to result in a life birth. The first cleavage of a zygote is a highly coordinated event. Disruptive timing of the first cleavage, named direct cleavage (DC, less than 5 hours), is associated with formation of poor embryo quality. Early identification of pathological embryos is challenging and impacts the timing of embryo fate decision in ART.

Study design, size, duration: A total of 1978 fresh IVF cycles from single unit were analyzed and a total of 4012 embryos were studied. Only 2pn embryos, cultured exclusively in time-lapse system for 5 days were included. The morphokinetics of embryos and their outcome were recorded, from z-score analysis through day 5. We discriminated between IDC, FC and normal cleavage pattern of embryos and compared clinical outcome of various morphokinetic parameters. We also evaluated possible early predictors for IDC.

Participants/materials, setting, methods: We generated three study groups according to embryo cleavage pattern: (I) Control, normal cleavage (n = 551); (II) FC, zygote to three cells within 5 hours (n = 1587); (III) IDC, instant cleavage from zygote to three cells (n = 922). The association between z-score of 138 IDC embryos and their sibling random controls was thoroughly investigated. All time lapse annotations were performed by senior embryologists. Statistical analysis was performed by SPSS software.

Main results and the role of chance: IDC embryos were mainly arrested at day 3 and the number of usable embryos (reached blastocyst stage and were suitable for embryo transfer or cryopreservation) was negligible; 4/922 (0.4%). In comparison, the amount of usable FC embryos was 108/1587 (6.6%) and control embryos usage reached 180/551 (32.7%). While the pregnancy rate of control and FC embryos, which reached embryo transfer were similar (40.35% and 42.55%, respectively); transfer of IDC embryos did not result in pregnancy. Additionally, the timetable as measured by time of PN fading and time from fading to first cleavage, differed significantly between the three groups. Therefore, we performed a thorough analysis of zygote morphology data of IDC embryos compared to control sibling embryos in search of early possible markers for these findings. We have not detected significant differences in z-score analysis, reflected by number and size of nucleoli, as well as pronuclear symmetry.

Limitations, reasons for caution: IDC embryos number used for z-score analysis was limited since many didn't meet the inclusion criteria (lack of overlapping of the pronuclei, presence of random of control embryo and good quality image).

Wider implications of the findings: The decision regarding the fate of IDC and FC embryos should include the pattern of first cycle cleavage. Culture IDC and FC embryos for 5 days up to the blastocyst will spare transfer of embryos that are fated to arrest even when their morphological grade on day 3 is acceptable.

Trial registration number: 0043-22-MMC

Abstract citation ID: dead093.564
P-205 comparison of GnRH antagonist protocol versus progestin-primed protocol in high ovarian responders: a propensity score-matched study

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Study question: To compare the clinical outcomes and cost-efficiency between gonadotropin-releasing hormone (GnRH) antagonist and progestin-primed ovarian stimulation (PPOS) protocol in high ovarian responders.

Summary answer: Comparing with PPOS protocol, GnRH antagonist protocol was associated with shorter time to live birth, higher OHSS rate and less cost in high responders.

What is known already: For high ovarian responders prone to develop ovarian hyperstimulation syndrome (OHSS), the best option should be the GnRH antagonist, as it has been shown to significantly reduce the incidence of OHSS. By applying ovarian stimulation using the combination of antagonist with agonist trigger and embryos freezing, OHSS can be erased. PPOS protocol in combination with a freeze-all strategy has proved to be a valid alternative to the conventional stimulation protocols. Recent studies showed that comparing with antagonist protocol, a lower cumulative live birth rate (CLBR) was associated with PPOS in general population, with no data available in high responders.

Study design, size, duration: This was a propensity score-matched retrospective cohort study. From Jan. 2016 to Jan. 2023, 5810 patients with AMH > 3.67ng/ml undergoing IVF treatment with antagonist or PPOS protocol at the Sixth Affiliated Hospital of Sun Yat-sen University were screened.

Participants/materials, setting, methods: Two-to-one propensity score matching was performed with a caliper of 0.2. After matching, 540 patients in GnRH antagonist group and 270 patients in PPOS group were included. Generalized estimated equation regression was applied to evaluate the impact of independent variables on the CLBR.

Main results and the role of chance: No significant difference in baseline characteristics was found in two groups. The OHSS (moderate to severe) rate was significantly higher in the GnRH antagonist group than in the PPOS group (7% vs. 3%). The clinical pregnancy rate and live birth rate per transfer were comparable between the two groups. After excluding patients who didn't achieve live birth but had surplus embryos, the CLBR was comparable between the antagonist and PPOS group (67.4% vs. 61.0%; Risk ratio = 1.106; 95% confidence interval [CI], 0.973-1.256, P = 0.109), and the generalized estimated equation regression analysis also showed that protocol selection had no significant impact on CLBR. The average time to live birth (TTLB) in the antagonist group was significantly shorter than the PPOS group (11.45 months vs. 12.13 months, P < 0.05). Besides, the average cost in the antagonist group was about $4933, while it was about $5465 in the PPOS group.

Limitations, reasons for caution: Although we recruited a large sample size and the sophisticate statistical analysis method, this was a retrospective study with an intrinsic limitation, besides more patients in the PPOS group had surplus embryos than in the antagonist group (24.1% vs. 19.8%). Further prospective RCTs are required to confirm our findings.

Wider implications of the findings: For infertile patients with high ovarian reserve, the clinical outcomes were comparable between GnRH antagonist and PPOS group. The antagonist group was associated with a shorter TTLB and more cost-effective, while the PPOS was associated with a lower OHSS rate.

Trial registration number: Not Applicable

Abstract citation ID: dead093.565
P-206 An evaluation of the incidence of total failed fertilization (TFF) following conventional IVF (cIVF) and the outcome of subsequent intracytoplasmic sperm injection (ICSI) cycles

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Study question: What is the incidence of TFF following conventional IVF (cIVF) and the chance of live birth in subsequent ICSI treatment cycles after TFF?

Summary answer: The incidence of TFF might be higher than previously reported and establishment of in-house benchmarks are recommended. Treatment continuation results in acceptable live birth rates.

What is known already: TFF following a cIVF is a devastating outcome for patients and physicians. The fear of this unfortunate event is assumable one of the main drivers for overutilizing ICSI. The incidence of TFF after cIVF

should be less than 5 % of stimulated cycles based on ESHREs recommended performance indicators. Data on TFF of an unselected population is indeed however only available from an era when ICSI was not in use and showed a higher rate. We investigated the incidence of TFF and evaluated the chance of live birth following of such event at the first cycle.

Study design, size, duration: A single center, retrospective chart review for the time period of 2010-2021 was done to identify all cycles where cIVF did not result in at least one oocyte with $\geq$ 2 pronuclei. Also, patients who had more than 2 oocytes retrieved in their first cycle and continued their treatment using ICSI as a fertilization method in their upcoming cycles were selected.

Participants/materials, setting, methods: A total of 5772 initiated IVF cycles were identified in the electronic database for the examined time period. Criterion for cIVF was normozoospermia and applied as fertilization method in 1001 (17.3%) cycles. All the cases where TFF was noted following cIVF were retrieved and the number of oocytes, the rank of the cycle, female age and the result of upcoming cycles if any were recorded. Data was analyzed using Chi-Square test, $p < 0.05$ was considered significant.

Main results and the role of chance: A total of 125 TFF cycles were identified out of the 1001 cIVF cycles (12.49% failed fertilization rate per cIVF). A significantly higher frequency of TFF was seen in cycles where less than 3 oocytes were retrieved compared to those with more than two oocytes (38/99 [38.38%] vs. 87/902 [9.65%], $p < 0.001$). No statistical difference was detected between the risk of TFF occurring at the first, second or the third rank cIVF cycle (87/664 [13.10%], 30/224 [13.39%], 5/75 [6.67%] respectively; $p > 0.05$). The differences remained non-significant when only cycles with more than 2 oocytes were analyzed (67/608 [11.02%], 16/199 [8.04%], 4/68 [5.88%] respectively; $p > 0.05$). Also, different age groups showed similar incidence of TFF (16/157 [10.19%], 41/362 [11.33%], 54/381 [14.17%] and 14/101 [13.86%] in $<$ 30, 31-35, 36-40 and $>$40 years of age respectively; $p > 0.05$). A total number of 56 patients were identified who continued their treatment using ICSI as fertilization method in their upcoming cycles. These patients completed 101 cycles resulting in 3 TFF cases (3%) and a fertilization rate of 75.1% (524/698) which was significantly higher to our baseline ICSI fertilization rate (70.9% [25231/35568]; $p < 0.05$). Nineteen of these 56 patients (33.9%) ended up giving at least one live birth following treatment continuation.

Limitations, reasons for caution: Selection criteria, e.g. patient characteristics and treatment type such as PGT-A, for choice of fertilization method are probably a source of variation in TFF incidence between centers. It is recommended that laboratories establish their own benchmark values based on their own data for the incidence of TFF.

Wider implications of the findings: Previous studies identified serum luteinizing hormone and progesterone levels at trigger and the number of oocytes as critical predictors for TFF. The similar chance of TFF after previous successful fertilization suggests more involvement of female factors in TFF. Acceptable live birth rates can be achieved following treatment continuation.

Trial registration number: not applicable

Abstract citation ID: dead093.566
P-207 Are the time of expansion and morphology of the blastocyst after thawing, assessed by time-lapse technology, decisive for implantation?

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Study question: Can blastocyst first expansion time after thawing, complete expansion, and morphology evaluated by time-lapse technology predict implantation?

Summary answer: Lower blastocyst expansion time after thawing and higher blastocyst quality assessed at the time of transfer are associated with a higher probability of clinical pregnancy

What is known already: Conflicting results have been published concerning the time of first and complete re-expansion of blastocyst after thawing or its quality and the attainment of pregnancy. Some studies reported no differences in pregnancy rate (PR) with different re-expansion duration, whereas other retrospective studies identified the degree of re-expansion, assessed within 6 hours after thawing, as the best predictor. Similarly, both studies reporting associations and non-associations between blastocyst morphology and PR have been published.

Study design, size, duration: We prospectively evaluated the time of first and complete expansion and quality grade of 141 homologous single blastocysts after thawing and relate the results to PR. The enrolled patients had a mean age of 38.7 $\pm$ 5.4 years. Morphokinetic parameters were evaluated using the Embryoscope time-lapse system. The study is currently ongoing.

Participants/materials, setting, methods: The study was conducted on 141 patients iundergoing homologous PMA cicles. Time lapse Embryoscope was used to assess post-thawing morphokinetic parameters as possible early markers of successful implantation. Time to first trophectoderm expansion, time and morphology two hours after thawing, and time and morphology of the blastocyst at the time of transfer were evaluated and recorded. Statistical analysis was performed using SPSS statistical package 28.0.

Main results and the role of chance: Both time of first (TfE) and maximum (TmE) blastocyst expansion were associated with a higher probability of pregnancy. Median TfE in women achieving pregnancy was 0.97 hours (range: 0.29-2.2) vs 1,4 (range 0.2-3.3) in those not achieving pregnancy ($p < 0.001$). Similarly, TmE was lower in pregnant women ($p < 0.05$). When blastocyst expansion occurred within 2 h, a PR of 71% was obtained vs 16% in women where expansion was post-poned ($p < 0.001$). ROC analysis showed that a TfE of 1,19h predicted attainment of pregnancy with an accuracy of 75%, a sensitivity of 75% and a specificity of 70% ($p < 0.001$). Blastocyst quality was found as another independent predictor of attainment of pregnancy. PR resulted of 21, 31,5 and 63,3% respectively with 0, 1 and 2 grade of blastocyst quality ($p < 0.001$, OR = 7.7).

Limitations, reasons for caution: Our study is preliminary and to draw firm conclusions, a higher number of blastocysts should be evaluated. The study is ongoing. Statistical correction for partners semen quality should be also performed.

Wider implications of the findings: Our preliminary results showed that TfE, TmE after thawing, and blastocyst quality are predictors of pregnancy achievement. Our study suggests that time lapse technology is useful for predicting pregnancy with good accuracy when transfer is performed after blastocyst freezing.

Trial registration number: not applicable

Abstract citation ID: dead093.567
P-208 Culture media affect gender after IVF treatment: A detailed analysis of explanatory variables

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Study question: Does embryo culture media affect gender at birth and why?

Summary answer: More male children were born after culture in the Sage 1-Step media compared to the G-TL media.

What is known already: ART increases the proportion of male children at birth. Laboratory techniques and culture conditions is known to impact secondary sex ratio (SSR). Physiological development differs in male and female embryos, leading to different metabolic requirements at the preimplantation stage. The composition of commercial culture medias varies and thus, the different medias may have different impact on embryo development and quality

according to gender. This, in turn, may influence SSR, as embryos are selected based on morphological scoring systems and developmental speed.

Study design, size, duration: The study was designed as a retrospective registry-based study with all data collected at a single clinic. All embryos resulting in live birth from frozen and fresh single blastocyst transfers, in the period from 1. January 2017 to 31. December 2020 were included. Donor embryos and embryos cultured in more than one culture media were excluded. A total of 1371 SET embryos were included.

Participants/materials, setting, methods: Embryos were cultured in either the G-TL media, Vitrolife (n = 686) or the Sage 1-Step media, Origio (n = 685). All embryos were monitored for 5-6 days in a time-lapse incubator, Vitrolife, before single blastocyst transfer. Multivariate logistic analysis of cycle and embryo characteristics was performed to identify all factors associated to sex. The association between culture media and embryo morphokinetic according to sex was evaluated using a mixed model analysis.

Main results and the role of chance: Significantly more male singletons were born after culture in 1-Step media compared to G-TL media (risk ratio (RR) 1,1 95% CI [1,0, 1,3], P = 0,01). The female/male ratio was 42,3/57,7% in 1-Step media and 48,5/51,5% in G-TL media. The multivariate regression analysis displayed a higher chance of a male child with higher expansion grade (grade 5) (RR, 1,2 95% CI [1,0, 1,4] P = 0,04), lower inner cell mass grade (grade B) (RR 1,13 95% CI [1,0, 1,3] P = 0,02) and second embryo transfer (RR 1,2 95% CI [1,0, 1,3] P = 0,03). Trophectoderm (TE) grade B reduced the probability of male child compared with TE grade A (RR 0,8 95% CI [0,8, 0,9] P > 0,05).

Male embryos developed significantly faster in the 1-Step media compared to the G-TL media for the stages of; blastocyst (tB) (-1,1 hours 95% CI [-2,1, -0,1]), expanded blastocyst (tEB) (-1,3 hours 95% CI [-2,3, -0,3]) and hatched blastocyst (tH) (-1,7 hours 95% CI [-3,0, -0,5]). Timing of development were the same for female embryos in the G-TL media compared to the 1-Step media. There was no significant difference in timing of development between female and male embryos in identical culture media.

Limitations, reasons for caution: This study was a retrospective study with data collection from only a single clinic, and as such in risk of confounding. Apart from culture media, culture conditions were similar during the two time periods. Paternal factors and type of infertility was not included.

Wider implications of the findings: Our observations suggest that culture media impact male embryo quality selectively, thus favoring selection of male embryos. As the impact appears to vary between different culture media, and has a measurable impact on gender after birth, the influence of culture media on embryo quality deserves more attention.

Trial registration number: not applicable

Abstract citation ID: dead093.568

P-209 Implication of delta opioid receptor in the in vitro maturation of oocytes and its effects on subsequent fertilization and embryo development in mice

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Study question: Could opiods modulate in vitro maturation of oocyte and improve fertilization rates?

Summary answer: Delta opioid receptor was present in mice oocytes, changing its expression pattern depending on the maturation stage and helping oocytes to reach blastocyst stage.

What is known already: The molecular mechanisms responsible for oocyte maturation are not completely understood and different molecules have been reported to be implicated as modulators of this process. The endogenous opioid peptides could be interesting candidates because they are involved in the regulation of reproductive physiology at multiple sites. When those receptors are activated, the adenylyl cyclase is inhibited, thus reducing cyclic AMP and blocking PKA. Moreover, opioids are also able to activate proteins involved in maturation. There is evidence suggesting a role for opioids during oocyte maturation because the signaling exerted by opioids is similar to that necessary for the meiosis resumption.

Study design, size, duration: Immature cumulus–oocyte complexes (COCs) from female 8 to 10 week old WT (C57BL/6xCBA) were retrieved after EGF stimulation. Mouse in vitro maturation culture was performed. In vitro grown oocytes, their corresponding cumulus (CC) and granulosa cells (GC) were classified at germinal vesicle stage (GV), at metaphase I (MI) and at metaphase 2 (MII).

Participants/materials, setting, methods: The presence of DOR was analysed in granulosa cells and oocytes at each stage of maturation by qRT-PCR and immunocytochemistry. AKT and MAPK signalling pathway (ERK 1/2) was studied by immunocytochemistry. Similarly, COCs were matured in vitro in the absence and presence of DPDPE and naltrindole at various concentrations. After IVM, in vitro fertilisation (IVF) was performed and development was observed until the blastocyst stage was reached, analysing fertilisation and embryo production percentages ($p < 0.05$).

Main results and the role of chance: Our findings showed that OPRD1 was present in mice oocytes and granulosa cells, changing its expression pattern depending on the maturation stage. The evaluation of RT-PCR examination and immunohistochemical (IHC) staining revealed the presence of the transcript for Oprd1 gene in the different stages of the mouse oocyte's maturation, as well as in the granulosa cells at the moment of the extraction from the ovary. The obtained transcript was compared with the transcript for Oprd1 of mouse cerebral cortex as a positive control. The expected 482 bp fragment was detected in the germinal vesicle stage and in granulosa cells, whereas in MI and MII stage the signal was lower. Immunofluorescence analysis revealed that OPRD1 protein was present in mouse oocytes but, its localization differed at the different stages of the maturation process. Furthermore, the selective delta opioid agonist DPDPE, modulating PI3K/Akt and MAPK pathways, helped oocytes to reach blastocyst stage. Finally, we examined that the observed effect of DPDPE during oocyte maturation in the in vitro fertilization and subsequent embryo development could be blocked by the opioid antagonist naltrindole.

Limitations, reasons for caution: Although we have obtained higher rates of blastocysts, the quality of these embryos still needs to be analysed and transferred to recipient females to evaluate the offspring.

Wider implications of the findings: Our study has clinical implications for the improvement of oocyte in vitro maturation techniques (IVM). OPRD1 could be a possible therapeutic target for in vitro maturation culture medium, as it could improve the blastocyst rates obtained in the actual reproduction assisted techniques.

Trial registration number: not applicable

Abstract citation ID: dead093.569

P-210 Utilization of coconut water as an additive to improve the human sperm quality following freezing-thawing Process

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Study question: Is the supplementation of the cryosolution with coconut water (CW) improve the motility and DNA integrity of spermatozoa after cryopreservation?

Summary answer: Coconut water as an additive to the cryosolution found to decrease the sperm DNA fragmentation and improve the sperm motility post-thawing.

What is known already: Despite the benefits of cryopreservation, it has adverse effects on sperm cell in different scenarios. Decrease in motility, viability and DNA fragmentation are the most common effects of cryopreservation on sperm function. The freezing/thawing shock produces physical and chemical stresses on the spermatozoa that could change the composition of the lipids in their plasma membrane resulting in increased production of reactive oxygen species (ROS) and increases the oxidative stress. Thus, supplementing the cryosolution with antioxidants may improve the recovery rate of

cryopreserved spermatozoa by reducing the oxidative damage. Coconut water is characterized by its high contents of proven antioxidant properties.

Study design, size, duration: The study was conducted on 70 semen samples from patients attended the fertility center in Al-Sadder medical city, Najaf, Iraq, for routine semen analysis over a period of ten months from March to December 2022. All the patients gave their consent for participation in this study. The samples had normal semen parameters according to World Health Organization (WHO) 2010 standard criteria.

Participants/materials, setting, methods: Sperm motility and DNA fragmentation index (SDF) test were done for each sample, then, each semen sample divided to three parts: P1: cryopreserved with glycerol-containing cryosolution only (SpermFreez™), P2: cryopreserved with glycerol-containing cryosolution and 5%coconut water (CW), and P3: cryopreserved with glycerol-containing cryosolution and 10% CW. After 1-month vapor-dependent cryopreservation, all samples were thawed and the sperm motility assed using computer-assisted sperm analyzer (CASA) and DFI was evaluated using Acridine orang stain method.

Main results and the role of chance: The percentage of total sperm motility and progressive motility in fresh samples were (39% and 35% respectively) and the level of sperm DNA fragmentation was (21%). The total and progressive sperm motility decreased significantly ($P < 0.05$) post-thawing, while the SDF increased. After cryopreservation the percentage of total and progressive sperm motility exhibited a significantly elevated levels in P2 (10.1% and 7.9% respectively) and P3 (14.6% and 11.3% respectively) than in P1 (6.8% and 4.2% respectively), however, the level of DFI significantly ($P < 0.05$) decreased in both P2 (27.4%) and in P3 (24.5%) than in P1 (30.1%). In comparison between the two concentrations of CW, P3 recorded a significant increase in the recovery of sperm total and progressive motility (14.6% and 11.3% respectively) than P2 (10.1% and 7.9% respectively). Furthermore, the level of DFI in P3 (24.5%) decreased significantly than its level in P2 (27.4%).

Limitations, reasons for caution: This study was to evaluate the efficiency of CW in the improvement of human sperm motility and DNA integrity post-thawing. Further studies required to evaluate the effect of using CW in sperm cryopreservation on the success rate of the conventional in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI).

Wider implications of the findings: In this study, we found that the additive of CW to the glycerol-containing cryosolution (SpermFreez™) can improve the human sperm motility and DNA integrity post-cryopreservation. The best recovery rate of sperm motility and DNA integrity was with the supplementation of 10% of CW to the cryosolution.

Trial registration number: Not applicable

Abstract citation ID: dead093.570
P-211 "Survival rates after vitrification of oocytes obtained after luteal phase vs follicular phase ovarian stimulation"

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Study question: Do the oocytes retrieved after luteal phase ovarian stimulation (OS) have the same survival rate as those retrieved after follicular phase (OS)?

Summary answer: Oocyte survival rate after vitrification is similar in oocytes retrieved in a follicular phase OS compared to those in luteal phase.

What is known already: Oocytes retrieved in luteal phase have recently captured scientific interest as dual stimulation is gaining popularity. Oocytes obtained in luteal phase OS added to those obtained in follicular phase shorten time to pregnancy per intention to treat in poor responders. Embryos obtained with these oocytes show similar ploidy rates, as well as clinical outcomes.

Study design, size, duration: We analyzed data from a randomized, non-inferiority Duo Stim Preimplantation Genetic Testing for aneuploidies (PGT-A) trial performed from 2017 to 2021 in women >38 years old. Only cycles with at least one oocyte retrieved in both follicular and luteal were included.

We compared survival rates from oocytes obtained in after follicular phase OS (n = 154 oocytes) versus luteal phase OS (n = 201 oocytes) within the same patient.

Participants/materials, setting, methods: A subtotal of 38 patients were included who underwent 2, 3, or 4 OS cycles to accumulate oocytes for subsequent ICSI with PGT-A. After retrieval and decumulation, oocyte vitrification was performed with Kitazato vitrification protocol (Kitazato, Japan) and Cryotop device, same as subsequent thawing.

Main results and the role of chance: Mean of age in the selected patients was 39,7 years. In total, 195 cumulus-oocyte complexes were retrieved after follicular phase OS vs 245 in luteal phase. From this group of oocytes, 145/195 were mature (78.97%) vs 201/245 (82.04%), respectively.

No statistically significant differences were observed in survival rate whether oocytes came from a follicular phase OS 81.16 % (125/154) versus luteal phase 87.06% (175/201) ($p = 0.3312$).

Our results confirm that survival rate of oocyte obtained in a luteal phase OS is comparable to conventional follicular phase OS, validating oocyte accumulation strategy in a Duo Stim protocol.

Limitations, reasons for caution: The limited sample size was due to the nature of the randomized, controlled trial. Our results need to be replicated in larger series for validation.

Wider implications of the findings: Luteal phase OS oocytes showed similar survival rates after thawing than conventional OS cycles, opening a new venue for oocyte vitrification in oncological patients or poor responders accumulating oocytes. This approach might be of interest as well in patients or countries where ethical/legal limitations may limit embryo accumulation.

Trial registration number: not applicable

Abstract citation ID: dead093.571
P-212 βhCG concentration in peripherical maternal blood after single embryo transfer, ongoing pregnancy rates and morphokinetics

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Study question: Is there a relationship between the concentration of the βhCG hormone in maternal blood, embryo morphokinetics and clinical outcome?

Summary answer: βhCG concentration is related to trophoectoderm quality, abortion rate and live birth rate, but not to embryo morphokinetics

What is known already: Measurement of βhCG has been widely used for early pregnancy detection after assisted reproduction treatments.

Shortly after embryo implantation, trophoblastic βhCG is detectable in maternal blood, However, βhCG concentration in several women showed high variability, and embryo implantation also depends on endometrial receptivity, what means that in early stages, everything affecting endometrial receptivity, may affect βhCG concentration.

Early high βhCG concentration has been related with higher pregnancy rate, but there are scarce information concerning relationship between βhCG early value and ongoing pregnancy rates

Study design, size, duration: Retrospective biomedical research of 508 patients having single embryo transfer of fresh embryos at IVIRMA VIGO clinic between 2017 and 2021 to analyse the relation between βhCG concentration, and embryo quality, embryo morphokinetics and ongoing pregnancy rate.

Patients with recurrent miscarriage, advanced maternal age, uterine pathologies and severe male factor were excluded of the study.

βhCG was measured in the peripherical maternal blood 13 days after embryo transfer

Participants/materials, setting, methods: All the embryos were generated by ICSI and were cultured in a time-lapse incubator under a 37°C, 6% CO_2 and 5% O_2 atmosphere.

T-Student and Youden test between embryo kinetics and βhCG values, Pearson correlation test to compare gestational successes with kinetic embryo data, and a linear regression model with βhCG like response variable and morphokinetic data like explanation variables were employed

Main results and the role of chance: βhCG concentration was greater in trophoectoderm A embryos compared to B and C (ASEBIR classification), 619.53 UI/ml, IC95% (483.16-755.90), 442.24 UI/ml, IC95% (350.18-534.31), 269.95 UI/ml, IC95%(88.88-451.01) ($p = 0.038$) respectively and there was a trend with Inner cell mass quality, being greater also in quality A vs B and C ($p = 0.053$)

Patients who had a clinic abortion showed lower βhCG concentration compared to those with ongoing pregnancy and live birth, 385.37 UI/ml, IC95% (268.45-502.28) vs 987.53 IU/ml, IC95% (845.03-1130.03).

T-student test linear regression analysis and Youden index, with βhCG cut-off points of 10 IU/ml and 100 IU/ml were employed, to analyze relationship between βhCG concentration and embryo morphokinetics, but it was not significant in any of the parameters analyzed

Limitations, reasons for caution: There is a limited sample size and data were obtained from a retrospective study. Patients and donor oocytes were analyzed in this study, although no differences were found when they compared

Wider implications of the findings: This study shows that a top quality trophoectoderm produces more βhCG, and that could be a predictive value for ongoing pregnancy

Trial registration number: 1712-VGO-122-EM

Abstract citation ID: dead093.572
P-213 The effects of vitrified embryos transported on IVF clinical outcomes: one center experience

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Study question: Does the transportation of vitrified embryos affect the survival rate and in vitro fertilization (IVF) outcomes compared to those produced on-site?

Summary answer: The regulated transport of cryopreserved embryos has shown no detrimental effects on embryo survival rate or pregnancy outcomes.

What is known already: Reproductive medicine has substantially evolved in the last decades. A growing number of patients have stored gametes or embryos following IVF treatments; contemporary innovations in cryopreserving techniques revolutionized the IVF laboratory. However, the IVF industry is changing; clinics worldwide are merging with larger international companies, and patients choose to travel abroad for fertility treatments, which requires the movement of cryopreserved embryos over long distances. So far, no exhaustive evidence has been provided regarding the possible detrimental effects on cryopreserved embryos due to their transportation.

Study design, size, duration: This retrospective observational study assessed the outcomes of 608 single frozen embryo transfers at Instituto Valenciano de Infertilidad (IVI) Rome (Italy) between February 2021 and March 2022, assessing survival and IVF outcomes.

Participants/materials, setting, methods: Data from N = 608 patients undergoing frozen embryo transfer (FET) from autologous or donated oocytes were analysed. Single blastocysts transferred at IVI Rome (Group A, n = 440), were compared to those generated and vitrified at IVI Spain clinics, and subsequently transported to IVI Rome (Group B, n = 168). The transport system was managed by the same truck company. Frozen embryos from abroad were rigorously checked and later thawed by a team of expert embryologists.

Main results and the role of chance: Analysis of baseline population characteristics showed a significant difference in female patients' age between Group A and Group B (39.78 ± 5.16 vs 42.47 ± 4.70; $p < 0.01$). The highest incidence of heterologous cycles was in Group B (45.4% vs 82.14%; $p < 0.01$). No significant differences were found between the age of the oocyte donors, male partners, or the patients' BMI. Considering IVF outcomes, we found no statistically significant difference comparing Group A and B respectively for embryo survival rates after thawing (N = 430/440, 97.7% vs. N = 165/168, 98.21%, $p = 0.71$), pregnancy rate (N = 221/440, 50.23% vs. N = 77/168, 45.83%, $p = 0.33$), clinical pregnancy rate (N = 200/440, 45.45% vs. N = 62/168, 36.90%, $p = 0.06$), and cumulative miscarriage rate (N = 42/221, 19,00% vs. 22/77, 28.57%, $p = 0.07$). The sub-analysis considering embryos screened for aneuploidies using preimplantation genetic testing for aneuploidies (PGT-A) showed no statistical differences across the different groups. The logistic regression analysis showed that PGT, semen parameters, endometrial thickness, type of endometrial preparation and age, do not affect embryo survival and IVF outcomes.

Limitations, reasons for caution: The retrospective nature was the principal limitation. One single fertility Company (IVI-RMA) and its network have been considered, which could bias the results, although we employed different clinics in two different countries.

Wider implications of the findings: Regulated transport of vitrified human embryos between clinics in different countries are safe and reliable, with no adverse effects on embryo survival after thawing and IVF outcomes following FET. Embryo transportation did not affect embryo implantation potential, offering opportunities for patients to transfer biological samples within different laboratories.

Trial registration number: not applicable

Abstract citation ID: dead093.573
P-214 The size of the embryo can be used as a single predictor for selecting embryo for transfer

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Study question: What is the performance of the blastocyst area feature compared to the state-of-art AI algorithms in the embryo ranking problem?

Summary answer: We study a simple embryo selection rule: the larger the better. This rule gives the same quality answers as other methods including AI.

What is known already: There are many existing methods for scoring embryos starting from the human executed rules, and ending with AI grading systems. Previous studies indicate systematic problems with comparing these methods. This is caused by the lack of a gold standard algorithm, i.e., the repeatable, easy and objective test that would not be susceptible to human interpretation. Common benchmarks typically lead to speeds-up in development of ML methods. We show that the embryo area, a single, interpretable feature, can be used as such a gold standard. It delivers the same quality results (aucroc = 0.66) as existing state-of-art AI algorithms.

Study design, size, duration: The data-set for retrospective study was collected from a single-center - Kriobank, Bialystok. The data-set includes 1550 time-lapse videos of embryos cultured in an incubator for up to 140 hours post-fertilization with no hatching. All these embryos were transferred with known implantation outcome (beta-hCG). All the videos have been recorded using the same optical magnification setting.

Participants/materials, setting, methods: Two methods of embryo ranking are compared:

- In the first method, the score was equal to the embryo area on the last frame of time-lapse. The area was computed using an AI segmentation algorithm that was trained on hundreds of manually annotated images/frames.
- In the second method, state-of-art AI algorithms were designed and trained to grade embryos directly from image data.

AUC ROC was used to compare the ranking performance.

Main results and the role of chance: The proposed gold standard, i.e., the embryo area, requires no training process. It induces the total ranking of all embryos, which should correlate with the treatment outcome, and the way to test it is ROC AUC score. For the whole data-set available (1550 cases) ROC AUC = 0.659 (CI 0.630-0.688), which is relatively high value and compares to the one reported for state-of-art AI algorithms based on deep-learning.

We have compared the area-based algorithms with state-of-art machine learning methods used in commercial solutions. In this test we have split the data-set into training (1236 cases) and test subsets (314 cases). The area-based algorithm resulted in ROC AUC = 0.657 (CI 0.597-0.717). For the deep-learning algorithm we have obtained ROC AUC = 0.659 (CI 0.599-0.719).

We note that several previous studies have proven relatively high disagreement between embryologists and AI-algorithms. Thus revealing a need for standardization in this area of study, and the need of development of common grounds for tests. The above study strongly suggests that embryo area can serve as a gold standard for further comparison of the developed algorithms, and clear improvement against such gold standards shall be expected.

Limitations, reasons for caution:

- Currently, the application of the embryo area rule is limited to devices with fixed optical magnification.
- Further work is needed to compare the segmentation algorithm with manually annotated images.
- Despite initial confirmation of the viability the algorithm should be verified and tested on a large, multi-centric data-set.

Wider implications of the findings:

- While AI based tools have the highest potential of increasing the efficacy of embryo selection, there are simple methods that can support embryologists without or with very simple software.
- There is a strong need for gold standards that will be further improved.

Trial registration number: not applicable

Abstract citation ID: dead093.574
P-215 Comparison of embryo utilisation and live birth rate between fresh and frozen donor oocytes, with assessment of the impact of paternal age on these parameters

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Study question: Does paternal age differentially affect the embryo utilisation and live birth rate of fresh and frozen donor oocytes?

Summary answer: The impact of paternal age on embryo utilisation and live birth for frozen donor oocytes is no different from fresh donor oocytes.

What is known already: Frozen donor oocytes are increasingly offered as an alternative to fresh oocytes as it is deemed a more accessible option for patients. There is conflicting data on the impact of paternal age on donor oocyte success rates, with some studies suggesting a declining live birth rate with advancing paternal age and others indicating no difference. However, the current literature appears primarily based on fresh donor oocytes. There is no available information on whether paternal age impacts frozen oocytes (and, if so, whether it differs from the impact on fresh donor oocytes).

Study design, size, duration: This retrospective cohort study analysed 169 616 ART (assisted reproductive technique) cycles from the Human Fertilisation Embryology Authority (HFEA) anonymised database from 2017 to 2018. To reduce bias resulting from age-related oocyte quality and intracytoplasmic sperm injection (ICSI) in frozen oocytes, only fresh and frozen donor oocytes with ICSI were analysed to assess whether paternal age plays a role. Frozen embryo transfer cycles were not included.

Participants/materials, setting, methods: We included 2287 ICSI oocyte donation cycles from the anonymised HFEA database, of which 1551 utilised fresh oocytes and 736 utilised frozen oocytes. Only two years' worth of data was analysed, as the technology behind oocyte freezing is evolving rapidly with concomitant improvement in success rates. The paternal age group is divided into >40 years old, and <40 years old for analysis to assess for differences.

Main results and the role of chance: Predictably, about 65% of our oocyte recipients in this cohort were more than 40 years of age at the time of treatment. 55% of oocyte donors were under 30 years, and about 64% of the male partners were over 40 years. Paternal age has not been shown to influence the outcome with either fresh or frozen oocytes. When restricting to cycles with paternal age <40 years, the fertilisation rate was higher with fresh oocytes (13.3% with over ten embryos), as compared to 3.0% with frozen oocyte ($P < 0.001$). In the >40 years paternal age group, the fertilisation rate was also higher using the fresh oocytes, with 13.8% of the group having more than ten embryos formed as compared to 3.2% in the frozen group ($P < 0.001$). More embryos were also suitable for storing in the >40 years old paternal age group, with 7% of the fresh cohort having more than five embryos suitable for freezing versus 3.6% in the frozen group ($P = 0.005$). No significant difference was noted in the clinical pregnancy and live birth rate.

Limitations, reasons for caution: Variations in laboratory protocols (such as vitrification methods) between different clinics might lead to the non-uniformity of the dataset, reducing its reliability. Data on cumulative live birth rate was unavailable, and a further project is planned, which would involve requesting the HFEA for this information from linked cycles.

Wider implications of the findings: With the increasing trend towards using frozen donor oocyte banks in the United Kingdom, understanding the implications would allow better patient counselling.

Trial registration number: not applicable

Abstract citation ID: dead093.575
P-216 Evaluation of embryo quality and morphokinetics following short exposure of oocytes to spermatozoa

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Study question: Does embryo quality and morphokinetics following short exposure of oocytes to spermatozoa differ from conventional long exposure?

Summary answer: No statistical differences were found in embryo quality and morphokinetics following short exposure. Short exposure technique enables better monitoring of the embryos and easier handling.

What is known already: Overnight exposure of oocytes to spermatozoa may lead to toxic effects associated with reactive oxygen species. In short exposure, the duration of oocyte incubation with the sperm is reduced to 2-4 hours. Few studies showed that embryo morphology as well as pregnancy rates improved by short exposure, however, this remains controversial. Embryo morphokinetics in a time-lapse incubator yielding more accurate information on embryo quality, has not been reported for embryos derived by the short exposure technique.

Study design, size, duration: A retrospective study (2013-2019) 536 IVF cycles were included: 110 short exposure and 426 conventional IVF (long exposure). All embryos were cultured in the EmbryoScope. Short and long exposure cycles were compared for the average embryo scores of general and adapted laboratory in-house models. Groups were analyzed according to 2, 3 and 5 days of culture and maternal age. Moreover, differences of KID (Known implanted data) embryos morphokinetics were compared for cell division timings.

Participants/materials, setting, methods: IVF patients with mechanical factor, normal sperm counts. In conventional IVF, the oocytes were inseminated 6-7 hours post retrieval and mechanically denuded after 18-20 hours of incubation. In short exposure, the oocytes were inseminated and denuded

2-3 hours post retrieval and immediately placed in the EmbryoScope, for accurate annotation. In both methods oocytes were washed, incubated in EmbryoScope and cultured till the day of transfer. Women were divided by age groups: <35, 35-40 and >40.

Main results and the role of chance: No statistical difference was found in embryo cleavage rates and number of transferred embryos per cycle between long and short exposure in all three maternal age groups. The clinical pregnancy rate was slightly higher in the short exposure compared to the long exposure group but did not reach statistical significance (37.5% vs 32.5, p = NS). In women younger than 35y, elective single embryo transfer resulted in a statistically significant higher clinical pregnancy rate in the short exposure group compared to the long (p = 0.037). No significant differences were found in the average embryo morphokinetic score according to the general model and the adapted in-house model between the two groups. Moreover, no difference was found in embryo morphokinetics between short and long exposure in 272 KID negative and 116 KID positive embryos.

Limitations, reasons for caution: Since the study was retrospective and the short exposure group was smaller than the conventional IVF group, additional studies are needed to support our results.

Wider implications of the findings: Short exposure is more convenient and easier to perform. It is as good as long exposure in terms of clinical pregnancy rates and may be beneficial in patients younger than 35 years old.

Trial registration number: 0015-20-CMC

Abstract citation ID: dead093.576
P-218 Does Artificial Oocyte Activation (AOA) improve the outcome beyond fertilization rate in ICSI cycles?

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Study question: Does CultActive© improve reproductive outcomes of ICSI cycles in cases of low fertilization rate and normal fertilization rate?

Summary answer: The application of CultActive© after ICSI improves fertilization rates only in clinical indication of fertilization failure.

What is known already: Oocyte activation deficiency is attributed in the majority of cases to fertilization failure in ICSI cycles, and these can be corrected by increasing initial levels of calcium (Ca2+) using assisted oocyte activation techniques (AOA), such as the use of Ca2+ ionophores. These agents enhance intracellular calcium release and increase the membrane permeability facilitating the influx of extracellular Ca2+, which initiates the activation cascade. Previous results suggest that Ca2+ ionophore treatment can increase the live birth rate after failed ICSI cycles in couples with poor fertilization rates, embryo developmental arrest, and also increase the number of good quality embryos.

Study design, size, duration: This was a retrospective observational study. There were 30 patients included and the first group consisted of 30 ICSI cycles, 231 oocytes and 56 embryos (without AOA). The second group consisted of the same patients (n = 30) with 30 ICSI cycles using AOA, 219 oocytes and 47 embryos. Subgroups were defined according to clinical indications (normal fertilization rate group: ≥ 65 % and low fertilization rate group: <65%). All data was collected from January-December 2022.

Participants/materials, setting, methods: 450 oocytes were assessed in the study. 231 oocytes belonged to the pre-AOA and 219 belonged to the AOA. The oocytes were exposed for 15 minutes after ICSI in a solution containing the Ca2+ ionophore A23187, CultActive© (Gynemed, Germany). Fertilization, good quality blastocyst formation and pregnancy rate were analyzed.Good quality blastocyst were defined as ≥ 3BB. Statistical significance was analyzed using the Paired Student T test (p < 0.05 was considered statistically significant).

Main results and the role of chance: The cohort of patients with normal fertilization rate (without clinical indications),was not significantly different. The AOA treatment gave a fertilization rate of 61% compared to 71% of the control cycles (p = 0.1398). There was no increase in the number of good quality blastocysts formation when the study group was compared to the control (p = 0.1660). In the cohort of patients with an indication of low fertilization, a significantly higher fertilization rate was recorded compared to the control (51% and 37%, respectively p = 0.0238). There were no significant difference in the quantity of good quality blastocysts compared with the control (p = 0.3328). Regardless of normal or low fertilization rates there was no significant difference in pregnancy rate when CultActive© was used in treatment (p > 0.05).

Limitations, reasons for caution: There are limitations in our study. First, the retrospective design and limited study population are major limitations. The data should be interpreted cautiously in the subgroup analyses due to potential bias, specifically the small population in the group without clinical indication.

Wider implications of the findings: AOA was only beneficial for couples with poor fertilization. Embryo quality and pregnancy rate did not improve with CultActiveive©. External variation was minimized by using the same patients in each cohort. This suggests AOA should not be used without clinical indication or for poor embryo quality/blastulation rate.

Trial registration number: not applicable

Abstract citation ID: dead093.577
P-219 Single-follicle spatial proteomics of the whole ovary by MALDI mass spectrometry imaging: towards the identification of protein-markers of the follicle's quality

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Study question: Can we improve our knowledge of the single-follicle proteome signature by MALDI mass spectrometry imaging?

Summary answer: We identified follicle-type proteins that function during follicle growth (NUMA1, TPM2), GV-to-MII transition (SFPQ, ACTBL, MARCS, NUCL), ovulation (GELS, CO1A2) and preimplantation development (TIF1B, KHDC3).

What is known already: The acquisition of oocytes developmental competence occurs through a bidirectional exchange of molecular information between the oocyte, the companion follicle cells, the stroma, and the vascular network. This reciprocal relationship is still poorly understood and particularly scarce is our knowledge of the proteins that are playing a regulative role during folliculogenesis.

Matrix-assisted laser desorption/ionisation mass spectrometry imaging (MALDI-MSI) is a powerful tool that allows the study of the proteomic landscape of small morphological structures inside a tissue, but in the field of reproduction has been only used to analyse rodent spermatogenesis.

Study design, size, duration: Nano-scale liquid chromatography-electrospray ionisation-tandem mass spectrometry (nLC-ESI-MS/MS) was combined with MALDI-MSI to identify the proteomic landscape and to map the changes occurring throughout folliculogenesis. We performed single-follicle spatial proteomics on all the follicles, from the secondary to the fully-grown preovulatory, present in histological serial sections of an entire prepubertal 25-day-old mouse ovary. Cross-referencing our results with those obtained by previous proteomics and transcriptomics analyses allowed to strengthen their significance.

Participants/materials, setting, methods: A single 25-day-old mouse ovary was fixed in 10% formalin, dehydrated, embedded in paraffin, and sectioned to obtain 133 6-μm serial sections. After paraffin removal, trypsin

digestion and matrix deposition, mass spectra were acquired using a rapifleX MALDI Tissuetyper™ (Bruker) with a raster sampling of 20 µm in both x and y axes. Then, tryptic peptides were extracted from sections and analysed with nLC-ESI-MS/MS using a Dionex-UltiMate-3000 LC-nano-system coupled with an Impact HD™ UHR-QqToF (Bruker).

Main results and the role of chance: This study proposes a spatial proteomics workflow to investigate the proteome of a whole prepubertal 25-day-old mouse ovary, preserving the spatial relationship of the peptides in this complex histological context. A total of 401 proteins were identified by nLC-ESI-MS/MS, 69 with a known function in ovary biology. Enrichment analysis highlighted significant KEGG and Reactome pathways, with apoptosis, developmental biology, PI3K-Akt, epigenetic regulation of gene expression, and extracellular matrix organisation being well represented. Then, correlating these data with the spatial information provided by MALDI-MSI on 276 follicles highlighted 94 proteins that were detected throughout the secondary to the pre-ovulatory transition. Of these, 37 proteins showed a gradual quantitative change during follicle differentiation, comprising 10 with a known role in follicle growth (NUMA1, TPM2), oocyte GV-to-MII transition (SFPQ, ACTBL, MARCS, NUCL), ovulation (GELS, CO1A2) and preimplantation development (TIF1B, KHDC3).

The proteome landscape identified includes molecules of known function in the ovary, but also those whose specific role is emerging. Altogether, this work demonstrates the utility of performing spatial proteomics in the context of the ovary and offers sound bases for more in-depth investigations that aim to further unravel its spatial proteome.

Limitations, reasons for caution: A limitation of MALDI-MSI is its 20 µm/pixel resolution which 1) leads to the acquisition of spectra comprising follicular and extra-follicular tissue, particularly with small follicles (15-30µm in diameter); and 2) does not distinguish among the different follicular cell types, thus precluding its application for single-cell *in situ* proteomics.

Wider implications of the findings: MALDI-MSI is a potent technology to study the proteome of ovary specimens, but it could also be used for the analysis of their lipidic or metabolic profiles. Our pipelline aim to identify protein-markers of the follicle's quality and, in turn, of the oocyte's developmental competence.

Trial registration number: not appplicable

Abstract citation ID: dead093.578

P-220 "Blame it on my youth": when very young age appears to be associated with poorer embryo development in oocyte donors

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Study question: Is a very young donor's age associated with different blastocyst usable rate and embryo quality?

Summary answer: Donors <20 years old have a significantly lower blastocyst usable rate but comparable average embryo quality than donors >25.

What is known already: Embryo development and quality in IVF cycles are affected by increasing female age. However, some authors also described a lower fertilization rate and reduced number of top-quality embryos in very young infertile patients <25 years old as compared to patients aged 25-35. It has even been reported that live birth rates are significantly lower in oocyte recipients when the donors' age is < 25. Still, the mechanisms underlying these apparently poorer outcomes in very young women remain speculative given that data on embryo development and quality in vitro are scarce.

Study design, size, duration: Retrospective-observational study of 1274 oocyte donor's and 1738 oocyte recipient's cycles with blastocyst transfer carried out in 2016-2022 in the Fertility Unit of a tertiary University Hospital. Cycles with vitrified oocytes, severe male factor or cleavage-stage embryo transfer were excluded.

The main parameters analyzed were blastocyst usable rate (% fertilized oocytes that became a blastocyst suitable for transfer/freezing) and blastocyst quality based on 2015 ASEBIR-scoring system (considering day of development and morphology):D5A, D5B, D5C, D6B, D6C.

Participants/materials, setting, methods: Cycles were categorized according to donor's age (Group A: <20, Group B: 20-25, Group C: ≥26). Oocyte donors were aged 18-34. Recipients were aged 18-50. Blastocyst usable rate and embryo quality were compared across groups according to donor's age. For multivariable analysis, a generalized logistic linear mixed model was applied to estimate the odds for every endpoint, taking Group C as a reference group. Donor and recipient were treated as random factors to avoid the repeated-observation effect.

Main results and the role of chance: A total of 1274 oocyte donor's cycles and 1738 oocyte recipient's cycles were analyzed.

Mean age was 26.1 ± 4.3 years old for donors, 42.6 ± 3.8 for recipients, and 43.3 ± 5.7 for male partners. Mean donors' AMH was 3.8 ± 2.1ng/ml, mean number of mature oocytes (MII) retrieved was 15.8 ± 7.7, and mean total dose of gonadotropins was 1413.1 ± 885.4 units. ICSI technique was used in 75.6% of the cycles.

The distribution of cycles by donor age was Group A: n = 55 cycles (4.3%), Group B: n = 532 cycles (41.8%) and Group C: n = 687 cycles (53.9%).

Blastocyst usable rate was significantly different according to donors' age: 33.2% (176/530) for Group A, 42.9% (2580/6016) for Group B and 43.5% (3109/7147) for Group C. Regression analysis, adjusting for confounding factors (AMH, MII, gonadotropins total dose, male age, insemination technique), has shown a significantly lower blastocyst usable rate in very young donors Group A (OR: 0.63; CI 95%; 0.48-0.83) as compared to Group C. Although similar differences were observed in the proportion of top-quality blastocysts (D5A+D5B) in the bivariate analysis A: 19.2% (102/530), B: 24.6% (1480/6016) and C: 24.4% (1742/7147), no significant differences were found after adjustment for confounding factors.

Limitations, reasons for caution: The main limitation of this study is its retrospective design; still multiple adjustments for confounding factors have been performed in order to minimize the risk of confounding bias.

Wider implications of the findings: Our finding of a lower blastocyst usable rate in very young donors may be associated with a higher aneuploidy embryo rate in this group, previously reported, advocating for further studies with PGT-A and basic research to decipher the underlying molecular mechanisms.

Trial registration number: not applicable

Abstract citation ID: dead093.579

P-221 Single-cell transcriptomic profiles reveal the characteristics of oocytes and CCs at GV, MI, and MII in oocytes before ovulation

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Study question: what is the mechanism of the interaction between oocytes and CCs supporting oocyte development during oocyte maturation?

Summary answer: Cumulus cells are responsible for the accumulation of lipids and fatty acids to ensure oocytes have enough energy available for later embryogenesis.

What is known already: The oocyte and its surrounding cumulus cells (CCs) exist as an inseparable entity. The maturation of the oocyte is reliant on the materials and energy provided by CCs

Although several reports have addressed the transcriptome of oocytes and granulosa cells during human folliculogenesis, many questions remain unanswered regarding the interaction between oocytes and CCs during the final stages of folliculogenesis before ovulation. Therefore, the mechanism of the interaction between oocytes and CCs supporting oocyte development during oocyte maturation is needed to be studied.

Study design, size, duration: We collected a total of 139 samples from 47 preimplantation genetic testing (PGT) patients for RNA sequencing , including 97 CCs (GV: 35; MI: 30; MII: 32) and 42 oocytes (GV: 22; MI: 11; MII: 9).10 cells were randomly selected from individually cumulus-oocyte complex per

CC sample to avoid bias due to the different number of CCs in each follicle. The samples were collected within a time frame of 3 months.

Participants/materials, setting, methods: The oocytes removed from CGCs were quickly transferred to the operating dish for marking, the maturation stage was assessed and recorded by observing the nucleus of oocytes, the CCs were mechanically isolated. Isolated RNA from oocytes and CCs underwent library preparation using an oligo deoxy-thymidine(dT) priming approach followed by deep sequencing. Data processing and bioinformatics analysis were performed using software, mainly including FASTP, STAR, String Tie, ARCANE and DESeq2 along with functional annotation analysis.

Main results and the role of chance: We found that oocyte maturation is a dynamic process and MI oocytes can be subdivided into GV-like-MI oocytes and MII-like-MI oocytes. we revealed unique transcriptional machinery, transcription factor networks, and crosstalk, displaying developmental stage-specific expression patterns at the three stages of oocyte maturation. We also identified that both lipid and cholesterol metabolism in cumulus cells are active during the late stage of oocyte maturation, and lipids may serve as a more efficient energy source for oocytes and even embryogenesis. Moreover, we also found that the metabolic profile of MII CCs, particularly those associated with the synthesis pathway of heparan sulfate synthesis, predicts the quality of DAY 3 blastomeres($P = 0.0001$).

Limitations, reasons for caution: The transcriptional profile of mural granulosa cells during the final stages of follicle maturation has not been fully explored.

Wider implications of the findings: This study provides a relatively systematic and comprehensive overview of the transcriptional features and interactions between oocytes and CCs at different stages of maturation before ovulation and may provide insights into developing a non-invasive method for the clinical assessment of oocyte quality and prediction of DAY3 blastomere quality in ART.

Trial registration number: not applicable

Abstract citation ID: dead093.580
P-222 Early blastulation on day 4 predicts ongoing pregnancy in fresh transfer cycles

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Study question: Do embryos with early blastulation (EB) on D4 show higher implantation potential compared to top D5 blastocysts in fresh elective single embryo transfer (eSET) cycles?

Summary answer: Fresh EB transfer with time start to blastulation (tSB)<96.6h and time of expanded blastocyst (tEB)<100h post-insemination is predictive of ongoing pregnancy for patients <35.

What is known already: Expansion and hatching of blastocysts are correlated to implantation potential in fresh single transfers.

Thanks to time-lapse morphokinetics, several time-points can be analyzed during embryonic growth. Embryos with faster growing speed are correlated to euploid status. The tSB of < 96.6h, day 4 post-insemination, is reported to have significant predictive value of euploid embryos, which are found to be associated not only with higher expansion scores, but also with shorter tSB, full expansion, and hatching. A study reported that significantly more embryos that reached the tSB by 100h implanted compared to those that did not.

Study design, size, duration: Prospective observational study in a single private hospital during 2022. In 160 cycles (n = 81 cIVF and n = 79 ICSI) an EB on D4 was observed. 48 patients (n = 28 ICSI and n = 20 cIVF) underwent a fresh eSET with an embryo showing EB on day 4.

Participants/materials, setting, methods: Patients with EB on D4 (tSB<96.6h, tEB<100h post-insemination) were enrolled regardless of indication, female age, attempt's rank or technique. Those with a fresh eSET on D4 were included in this study.

Gardner's classification was used to rate the blastocysts in Embryoscope + (Vitrolife). The outcome data of transfer for D4 blastocysts (B3/B4 AA-AB-BA) were compared to eSET with top D5 (B4/B5 AA) of reference group (female age ≤35).

The statistical analyses were performed by SPSS software.

Main results and the role of chance: In the study group, all the transferred embryos were graded according to Gardner's classification as B3AA, or B3AB, or B3BA, or B4AA, or B4AB, or B4BA.

In the reference group, we chose only the fresh transfer cycles with ultra-top grade D5 embryos classified as B4AA or B5AA.

The results obtained in the overall population (n = 48) were compared to those obtained in the reference group (n = 92): age 34.0 ± 3.8 *vs* 31.8 ± 2.3, oocyte maturity (OM) 79.8% *vs* 80.3%, fertilization rate (FR) 80.0% *vs* 77.0%, overall blastulation rate (OBR) 78.1% *vs* 74.3%, top blastulation rate (TBR) 59.1% *vs* 54.4%, total pregnancy rate (PR) 75.5% *vs* 61.4%, implantation rate (IR) 58.3 vs 51.2% and ongoing pregnancy rate (OPR) 58.6% *vs* 47.1%, and showed a statistical difference only in PR ($p<0.05$).

The results obtained in younger patients with age 32.1 ± 3.2 (n = 34) were also compared to reference group: OM 75.1% *vs* 80.3%, FR 85.0% *vs* 77.0%, OBR 78.1% *vs* 74.3%; TBR 58.7% *vs* 54.4% were comparable between the groups. A statistical difference was observed for PR (88.0% *vs* 61.4%, $p<0.01$) IR (70.6% vs. 51.2%, $p<0.05$) and OPR (70.6% *vs* 47.1%, $p<0.05$).

Limitations, reasons for caution: The small size of the sample needs to be expanded in order to confirm the encouraging findings in a larger population, especially in older patients yielding more aneuploid embryos. The study will continue during 2023 and will compel the live birth rate.

Wider implications of the findings: When a D4 embryo with EB is available, the best option is the fresh transfer on D4. The increased implantation with EB seems to correlate with embryo euploidy and the advanced endometrial receptivity in stimulated cycles. Higher IR and OPR are reported even in patients with lower rate of aneuploidy.

Trial registration number: not applicable

Abstract citation ID: dead093.581
P-223 Comparison of blastocysts' iDAScore® (intelligent Data Analysis-Score) if in vitro fertilization is performed using ICSI instead of IMSI

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Study question: Does blastocysts' iDAScore® differs when ICSI is performed instead of IMSI in case of teratozoospermia on the day of *in vitro* fertilization (D0)?

Summary answer: Blastocysts' iDAScore® is unchanged when in ICSI or IMSI is performed in case of teratozoospermia at the day of fertilization (D0).

What is known already: In routine practice, IMSI for D0-teratozoospermia is sometimes not performed because of its higher cost and/or laboratory workload. Time-Lapse studies have shown that *in vitro* fertilization (IVF) using ICSI or IMSI in case of suboptimal sperm parameters lead to morphokinetically similar embryos. However, in patients with oligoasthenoteratozoospermia/teratozoospermia, evidence indicate that IMSI may improve embryo morphokinetics and further clinical outcomes. Since these studies rely on morphokinetical features assessed by an embryologist, the use of artificial intelligence may limit the human subjectivity for choosing the optimal blastocyst for transfer. Whether ICSI or IMSI for D0-teratozoospermia impact blastocysts' iDAScore® has never been investigated.

Study design, size, duration: This retrospective monocentric study was performed between September 2021 and December 2022 in our reproductive medicine unit. A total of 605 oocytes (386 ICSI, 219 IMSI) from 58 patients (37 ICSI, 21 IMSI) were fertilized with fresh sperm using either ICSI or IMSI and then incubated for 5 days in Embryoscope8 (Vitrolife®) combined with iDAScore®. iDAScores® of 325 blastocysts (228 ICSI, 97 IMSI) were compared between IMSI and ICSI arms.

Participants/materials, setting, methods: For each couple 3 iDAScores® were available: i) mean iDAScore® of all blastocysts, ii) iDAScore® max, and iii) mean iDAScore® of useful blastocysts. These scores were compared according to the technique of IVF used (ICSI or IMSI). In addition, clinical pregnancy rates following blastocyst transfer were analyzed.

In all, 53 blastocyst transfers (34 ICSI, 19 IMSI) have been realized during fresh or frozen cycles. Clinical pregnancy rates were defined by presence of fetal heartbeat on ultrasound.

Main results and the role of chance: Patients in the ICSI and IMSI groups were comparable in terms of women age (32.3 ± 5.6 and 32.2 ± 5.6 years), BMI (24.9 ± 4.3 and 25 ± 4.3 Kg/m^2, respectively). In addition, mean IVF rank was 1.4 ± 1.5 and 2.8 ± 1.5 in ICSI and IMSI couples. The number of mature oocytes inseminated per patient was similar in ICSI and IMSI groups (10.9 ± 4.7 and 10.4 ± 4.6, respectively), as well as the mean number of blastocysts per couple (6.4 ± 3.5 and 4.6 ± 3.5) and the blastoformation rate (0.74 ± 0.2 and 0.66 ± 0.2, respectively). The mean iDAScore® of all blastocysts, iDAScore® max and mean iDAScore® of useful blastocysts in women having undergone ICSI and IMSI were comparable (7.60 ± 1.2 *vs.* 7.63 ± 1.1 ($p = 0.92$); 8.80 ± 1.1 *vs.* 8.78 ± 1.0 ($p = 0.67$) and 8.1 ± 1.0 *vs.*7.96 ± 1 ($p = 0.77$) respectively). Finally, cumulated pregnancy rates (CPR) were not different between ICSI and IMSI groups (22/34 and 10/18 respectively), (0.65 ± 0.49 *vs.* 0.55 ± 0.46, respectively ($p = 0.53$)).

Limitations, reasons for caution: These results should be interpreted with caution due to the retrospective design of the study and small population.

Wider implications of the findings: We observed no difference in iDAScores whether ICSI or IMSI is performed in case of D0-teratozoospermia. Since IMSI is more time-consuming and more expensive, if these results are confirmed by further studies, we may withdraw this indication of IMSI to avoid patients bear additional fees, especially in private clinics.

Trial registration number: not applicable

Abstract citation ID: dead093.582
P-224 Basonuclin 1 prevents intrauterine growth restriction via inhibiting necroptosis

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Study question: Is basonuclin 1 (BNC1) involved in intrauterine growth restriction (IUGR), and what's the underlying mechanism?

Summary answer: BNC1 is significantly decreased in the placenta of IUGR patients. BNC1 deficiency engenders IUGR in *Bnc1* truncation mutation ($Bnc1^{tr/tr}$) mice model by inducing placental necroptosis.

What is known already: IUGR is among the leading causes of perinatal morbidity and mortality. IUGR occurs due to multiple factors, including genetic, placental, fetal, and maternal factors. Among which genetic factors remain largely unknown. Our previous exploration found that *Bnc1* mutation-mice exhibit low birth weight and survival rates, indicating BNC1 may play a potential role in fetal and placental development. Necroptosis is a recently recognized cell death pathway that may contribute to placental pathophysiology in IUGR patients. While the underlying mechanism remains largely unknown.

Study design, size, duration: A clinical retrospective cohort study of 40 IUGR patients (diagnosed by Hadlock ultrasound measurements) and 40 controls were included (2017–2022) for placenta collection. *Bnc1* targeted mutation mouse was on a C57BL/6 J background. Male and female mice of the $Bnc1^{+/tr}$ genotype were mated to produce $Bnc1^{+/+}$, $Bnc1^{+/tr}$, and $Bnc1^{tr/tr}$ mice. Placenta and fetuses were obtained on the 15th and 18th days of pregnancy.

Participants/materials, setting, methods: The following analyses were performed: (i) Immunohistochemistry for detecting the location and expression of BNC1 in the placenta of humans and mice, (ii) placental and fetal weights for evaluation of IUGR development, (iii) morphometric evaluation of fetal utilizing Alcian blue and alizarin red staining, (iv) morphometric evaluation of placental compartments utilizing H&E staining, TUNEL staining, and TEM analysis, (v) RNA sequencing (RNA-seq) of placental tissues.

Main results and the role of chance: BNC1 was specifically highly expressed in trophoblasts and endothelial cells in the early and mature placental development stages of humans and mice. The expression of BNC1 was significantly decreased in the placenta of IUGR patients compared to the control group ($P < 0.0001$). In the mice model, the number of $Bnc1^{tr/tr}$ fetuses was significantly decreased compared with wild-type $Bnc1^{+/+}$ fetuses on the 18th day of pregnancy. While the number of $Bnc1^{tr/tr}$ fetuses and $Bnc1^{+/+}$ fetuses was similar on the 15th day of pregnancy, indicating the loss of $Bnc1^{tr/tr}$ fetuses between the two stages. Besides, both the $Bnc1^{tr/tr}$ fetal and placental weights were significantly decreased on the 15th day of pregnancy compared with $Bnc1^{+/+}$ fetuses and placenta ($P < 0.01$). The *Bnc1* truncation mutation caused poor development of the placental labyrinthine layer with an increased number of TUNEL-positive foci. Transmission electron microscopy also revealed a disorganized labyrinthine layer with defects in cytoplasm translucence, loss of plasma membrane integrity, swollen mitochondria, and decondensed chromatin. Finally, RNA sequencing of placental tissues indicated BNC1 deficiency-induced IUGR may be modulated by necroptosis. In conclusion, we found a relationship between the disturbed BNC1 expression and the occurrence of IUGR through necroptosis.

Limitations, reasons for caution: This study used only *Bnc1* truncation mutation mice model, the other animal model with placenta-conditional knockout mice was needed to consolidate the results. Besides, further mechanism exploration should be conducted in both human and mouse cell lines.

Wider implications of the findings: Our work suggests a new gene related to the development of IUGR, which provides early diagnosis and treatment for managing IUGR, thus improving outcomes for both fetal and gravida.

Trial registration number: not applicable

Abstract citation ID: dead093.583
P-225 Trustworthy AI algorithm for embryo ranking

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Study question: Deep-learning algorithms are known to be non-robust: can the variability and inconsistency of AI algorithms be reduced in embryo selection?

Summary answer: We reduced the variability of algorithms (measured on different tasks like rotations and brightness changes) by 86% while preserving their quality.

What is known already: Deep-learning methods are generally known to be non-robust, i.e., decisions change with even slight modification of input data. Current solutions for embryo scoring are not robust - for example rotating the input image results in a different score in most solutions on the market. Despite this fact and expressed concerns of embryologists, there are no other publications focusing on the problem of variance in AI solutions used in IVF. Most of the publications measure accuracy, sensitivity, specificity, and ROC AUC; there are no variance metrics.

Study design, size, duration: The data-set was collected within multiple clinics using various devices. It contains 34,821 embryos (4,510 were transferred with known pregnancy results), represented by time-lapse videos or images. This gives 3,290,481 frames of embryos at various maturity levels.

From the data-set 925 randomly selected embryos were chosen as a test set.

The frames were modified by methods that are not supposed to change the results of the algorithm.

We measured the variability of the scores given by our algorithm.

Participants/materials, setting, methods: We have considered seven different modifications of images that should not influence embryo scoring:

- Rotations (10 different angles);
- Brightness and Contrast modifications;
- Substitutions of Frames (from time-lapse monitoring taken from a 2 hours interval);
- Blur (Generalised Normal filter);
- Gaussian Noise;
- Gaussian Blur;
- Sharpening.

We used several techniques to reduce variance of our deep neural network model (architecture commonly used for embryo selection):

- Ensemble (of different models in cross validation);
- Test time augmentation (TTA);
- Robust training.

Main results and the role of chance: In order to measure the variance we have used the following method. First, the scores are stretched to the standard uniform distribution. In other words we look in which percentile the score lies. This way the range of the scores are normalised thus the variance can be compared.

Second, we train the EMBROAID model on the augmented data that includes all the above modifications.

Third, we compute the variance of the normalised scores on the test set.

The mean variance dropped by 86% (0.0055 to 0.0008) across all measured input modifications.

The individual drops in the variance on measured input modifications: Rotations: 77% (0.009 -> 0.002), Brightness and Contrast: 81% (0.0036 -> 0.0007), Substitution of Frames: 76% (0.0076 -> 0.0019), Blur 94% (0.012 -> 0.0008), Gaussian Noise: 96% (0.0049 -> 0.0002), Gaussian Blur: 95% (0.0052 -> 0.0003), Sharpening: 77% (0.0015 -> 0.0003). The significance was tested with Wilcoxon Rank Sum Test giving the p-value < 0.01 on all input modifications.

Finally, we stress that these results were obtained without any loss in the ROC AUC metric. We have tested the algorithm both on the original testset. Both models achieved an ROC AUC of 0.66 (CI 0.63-0.69) on both testsets.

Limitations, reasons for caution: Further work needs to be done to extend the set of possible augmentations of data.

Wider implications of the findings: Increased reliability of AI scoring algorithms for embryo selection.

It is possible to obtain consistent results over a wide range of data modifications.

Trial registration number: not applicable

Abstract citation ID: dead093.584

P-226 Conventional IVF rather than ICSI when only on oocyte is retrieved: time to overcome irrational fears

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Study question: Is the use of conventional-IVF (c-IVF) a viable option in single oocyte retrieved cycles?

Summary answer: The use of c-IVF on single oocyte seems to be a successful strategy in terms of laboratory performance indicators outlined in the Vienna Consensus.

What is known already: Intracytoplasmic Sperm Injection (ICSI) has greatly improved the chances of reproductive success in cases of male factor infertility, but it is often used without strong evidence, such as in cases of low numbers of retrieved oocytes, assuming it will avoid unexpected total fertilization failure (TFF). On these bases, several ART centers irrationally opt for ICSI when few oocytes are retrieved. This approach is due to the fear of facing TFF the following day. This situation is boosted when only one oocyte is available. Available studies on c-IVF in these circumstances are generally reassuring, but evidence is limited and inconclusive.

Study design, size, duration: This is a monocentric retrospective observational study performed at the Infertility Unit of the Fondazione Ca' Granda Ospedale Maggiore Policlinico between 2014 and 2021. The primary outcome of the study is normal fertilization rate, which is expected to be $\geq 60\%$ according to the Vienna Consensus. The study sample (about 300 cycles) was calculated to obtain a 95% CI within the range of $\pm$ 5%. A binomial distribution model was used to determine the 95%CI of proportions.

Participants/materials, setting, methods: Only women who had recovered one oocyte at the oocyte retrieval were included in the study. Collected data included age of both partners, previous pregnancies, female BMI, antral follicle count, total motile sperm count. A multivariate analysis was performed to identify predictive factors for fertilization. Based on the policy of our Center, c-IVF was performed only in case of normal sperm variables according to WHO 2010 manual and in the absence of history of TFF.

Main results and the role of chance: Out of 700 cycles with single oocyte retrievals, 304 were treated with c-IVF, which resulted in normal fertilization (2PN) in 209 cases, corresponding to a fertilization rate of 69% (95%CI: 63-74%). In 13 cycles, oocytes were shown to be immature (germinal vesicle or metaphase I) at the time of fertilization check. In these cases, fertilization could not occur. If we exclude them, the fertilization rate raised to 72% (209/291) (95%CI: 66-77%). This fertilization rate was within the Vienna Consensus KPI for competency range and was no more than 5% lower compared to the benchmark value. Clinical pregnancy and live birth rates per cycle were 14% and 11%, respectively. Finally, we compared baseline characteristics of cycles with normal fertilization to those with failed fertilization. Both univariate and multivariate analyses failed to identify predictive factors of fertilization.

Limitations, reasons for caution: The retrospective nature and the absence of a control group do not allow us to draw robust and definitive conclusions. However, the large sample sized allowed us a precise estimation of the fertilization rate.

Wider implications of the findings: Fear about TFF is not a valid reason to opt for ICSI in women retrieving only one oocyte. The use of ICSI increases costs without providing benefits. We also failed di identify predictive factors of fertilization, hampering the possibility to select a subgroup of women who may benefit from ICSI.

Trial registration number: Not applicable

Abstract citation ID: dead093.585

P-227 ICSI outcomes after using in-situ microfluidics of fluidic walls versus DGC: a prospective non-inferiority comparative pilot study in sibling oocytes

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Study question: Does the novel strategy *in-situ* microfluidics (isM) yield comparable ICSI outcomes to the control sperm selection methodology density gradient centrifugation (DGC)?

Summary answer: ICSI outcomes show that handmade *in-situ* microfluidics of fluidic walls are as effective as DGC to achieve fertilization and usable day 5 blastocyst formation rate.

What is known already: Microfluidics technologies are proving their capability to select suitable sperm for ICSI. Increasingly, publications show novel and ingenious microfluidics strategies aimed to integrate sperm biomimicry during *in vitro* sperm selection while simplifying the IVF-workflow. We recently designed a microfluidics system which allows selecting sperm for ICSI in the same ICSI-dish, only using microfluidics and disregarding centrifugation, washing and plasticware. A previous proof-of-concept study showed that this methodology was efficient to select suitable sperm for ICSI as it could separate at least 20 progressive spermatozoa in less than 15 minutes in a clean microdroplet free of any remaining seminal plasma.

Study design, size, duration: The present pilot study included a total of 280 fresh MII-oocytes allocated in a 1:1 ratio to the study (isM-ICSI) and control (DGC-ICSI) groups. The statistical power was established at 80% at a CI 95%. For comparison we relied in the KPIs: ICSI normal fertilization rate (INFR) and Day 5 usable blastocyst rate (D5UBR). The non-inferiority margin (Δ) was established using 2-sigma warning limit and our historical control mean values (ΔINFR = 7% and ΔD5UBR = 10%).

Participants/materials, setting, methods: An informed consent was signed by all participants. The comparative study included 29 consecutive ICSI cases performed in fresh oocytes from women under 40 years old and with an oocyte yield ≥6 MII. All semen samples had $\geq 1 \times 10^6$/ml progressive spermatozoa and were split into three aliquots: 1-seminogram (100 µl), 2-isM (10 µl), 3-DGC (surplus). Embryos were cultured in bench-top Time-lapse incubators, using GTL-media under 6,4% CO2 and 5% O2 conditions up to blastocyst stage.

Main results and the role of chance: Sperm characteristics showed the following total mean values for total concentration ($42,83 \pm 31,1 \times 10^6$/ml), total motility (51,28 ± 14,16 %) and progressive sperm concentration ($17 \pm 12 \times 10^6$/ml). The mean number of MII per patient was 9,66. From the total number of oocytes (n = 280), 139 were allocated to isM (study group) and 141 to DGC (control group) groups. INFR was 72,0 % vs 76,6 %, respectively. Non-significant differences were observed (p = 0,54). We observed that the mean total number of usable blastocysts (day 5/6) per patient was 1,83 (53/29) in isM vs 1,66 (48/29) in DGC. Moreover, D5UBR was 53,0% vs 44,4 % (p = 0,26) with a difference between the study and the control of within the non-inferiority margin. We observed a lower rate of arrested embryos (up to day 6) in the study group (19,0% vs 30,0%), although results were not statistically significant (p = 0,08).

Limitations, reasons for caution: This pilot study includes a limited number of oocytes. The present isM protocol depends on a free-hand preparation. Although the use of templates and the training reduces inter and intra-operator variability, a ready-to-use surface would benefit the operability. Further investigation is required to evaluate clinical pregnancy and live birth outcomes.

Wider implications of the findings: Sperm DNA fragmentation is cited among the causes of embryo arrest. As the use of isM resulted in a lower proportion of arrested embryos, exploring its capacity to select non-fragmented sperm warrants further interest. The isM protocol streamlines sperm selection for ICSI, reduces costs and risks along the procedure.

Trial registration number: na

Abstract citation ID: dead093.586

P-228 Reliability of blastomere versus trophectoderm biopsy in preimplantation genetic testing for mitochondrial DNA disorders

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Study question: Can a single blastomere or trophectoderm (TE) biopsy accurately reflect the heteroplasmy levels of the whole embryo for mitochondrial DNA (mtDNA) disorders?

Summary answer: Heteroplasmy levels of single blastomere and TE are comparable to those of the rest of the embryo, including the inner cell mass (ICM).

What is known already: Oocytes of women carrying mtDNA mutations can display varying mutation loads – percentage of mutated mtDNA copies. Preimplantation genetic testing (PGT) aims at reducing the risk of having affected children by screening for embryos with minimal mtDNA heteroplasmy. The technique involves either a blastomere biopsy on cleavage stage embryos or TE biopsy from blastocysts, followed by genetic analysis to select embryos suitable for transfer. However, it remains unclear whether there is a uniform segregation of heteroplasmic mtDNA during the early stages of embryogenesis, and therefore whether the mutation load from blastomere or TE biopsies are representative of the whole embryo.

Study design, size, duration: We investigated the suitability of PGT for mtDNA disorders by comparing the mutation load between a single blastomere recovered at the eight-cell stage, TE cells and rest of the same blastocyst (n = 15) in a mouse model heteroplasmic for the m.5024C>T mutation. We also compared the levels of heteroplasmy between TE and ICM of heteroplasmic human blastocysts (n = 5) and between blastomeres of arrested cleavage stage embryos (n = 4) donated by two mtDNA disease patients.

Participants/materials, setting, methods: To obtain heteroplasmic mouse embryos, we mated female mice carrying a mitochondrial $tRNA^{Ala}$ mutation (m.5024C>T) with wild-type males. Whereas in human experiments, we analysed preimplantation embryos from two different carriers of the m.3243A>G mutation, associated with MELAS syndrome, who showed 16% and 32% mutation load in their peripheral blood. After embryo biopsy, NGS was performed in three independent technical replicates and profiles were compared between samples from the same embryo to assess heteroplasmy levels.

Main results and the role of chance: Analysis of heteroplasmic mouse embryos showed a strong correlation in mtDNA heteroplasmy between blastomere and the corresponding embryo ($r^2 = 0.76$), and between TE and the rest of the blastocyst ($r^2 = 0.94$) (n = 15). Moreover, a strong correlation was observed between the biopsied blastomere and TE from the same embryo ($r^2 = 0.90$). Heteroplasmy levels in the embryos analysed ranged from 55.3% to 88.3%, and the mean overall mutation load was 73.7 ± 7.9%. Development of biopsied embryos was comparable to those of non-manipulated mutant (n = 10) and wild-type embryos (n = 10) (blastocyst rate = 85.7%).

For human blastocysts (n = 5), statistical analysis showed a strong correlation in heteroplasmy levels between ICM and TE portions ($r^2 = 0.98$). Moreover, a modest correlation was observed among blastomeres of cleavage stage human embryos ($r^2 = 0.60$) (n = 24 from 4 embryos). The mutation load in the embryos analysed ranged from 10.3% to 57.4%, and the mean overall heteroplasmy was 42.8 ± 12.2% in cleavage stage embryos and 38.6 ± 17.3% in blastocysts.

Overall, concordance in heteroplasmy levels between single blastomeres, TE portions and corresponding blastocyst was established in human and mouse embryos.

Limitations, reasons for caution: These results should be further validated by increasing the sample size and by the inclusion of different mtDNA mutations. Furthermore, current data are suggestive, but not definitive, to guarantee that the mtDNA mutation load in the embryo will remain constant throughout life.

Wider implications of the findings: Taken together, results suggest that TE biopsy may be better than blastomere biopsy for mtDNA disorders. Moreover, biopsy at the blastocyst stage is likely less harmful for embryo development. Following, we will also investigate how heteroplasmic mtDNA segregates at the peri-implantation stages after the resumption of mtDNA replication.

Trial registration number: Not applicable

Abstract citation ID: dead093.587

P-229 Prospective comparative study between two commercial heavy oils for culture using sibling donor oocytes collected

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Study question: To compare embryo development and clinical outcomes between two commercial heavy oils using sibling donor oocytes collected.

Summary answer: Our study suggests that both commercial heavy oils achieve similar embryo development and clinical outcomes rates.

What is known already: Current tendencies in IVF laboratories, such as extending the embryo culture uninterruptedly until day 6/7 or the use of dry time-lapse incubators, have enhanced the importance to use good quality oils supporting human embryo culture in vitro. The coating of the culture dishes with oil is highly important to maintain the ideal conditions that embryos need for an optimal development. Specifically, oil plays an essential role in maintaining a stable temperature, provides a barrier against external agents and contributes preventing the media evaporation, and thus, to the maintenance of an optimal pH and osmolality for the correct embryo development.

Study design, size, duration: This is a single-centre prospective study performed between February and November 2022 that included 180 donors and 213 recipients. Donors were randomized using a computer-generated randomization list. Each case was processed and cultured with a commercial single medium coated with a layer of the commercial heavy oil assigned, a mineral oil (A) or a paraffin oil (B).

Participants/materials, setting, methods: Oocytes were injected by ICSI and then cultured in 16-well dishes (EmbryoSlide+®, EmbryoScope+™,Vitrolife) prepared with each heavy oil (1700µl oil/dish). These were cultured in a time-lapse incubator (EmbryoScope+™,Vitrolife) at 37.29 ± 0.05 °C in an atmosphere of 6.5% CO_2 and 5% O_2. These parameters were controlled periodically (T+Button,BrightSentinel and G100,Geotech). Laboratory conditions, such as temperature, humidity and volatile organic compounds levels were monitored continuously (Octax Log&Guard™,Vitrolife) during the study period, and pH was measured in a weekly basis.

Main results and the role of chance: A total of 2554 MII oocytes were injected by ICSI (oil A, n = 1304 and oil B, n = 1250). The proportion of fertilized oocytes was identical between the two oils (A:80.00% vs B:80.53%), as well as, abnormal fertilized oocyte rate (A:6.85% vs B:5.62%) and oocyte degeneration rate post-ICSI (A:6.14% vs B:6.00%). The mean number of embryos that reached the blastocyst stage and the proportion of blastocysts suitable for clinical use (transferred or cryopreserved) was almost the same independently of the oil used (A:69.84% vs B:67.15% and A:62.39% vs B:60.71%, respectively). Statistical data analysis was performed without referring to statistical significance ($p > 0,05$).

205 patients had an embryo transfer on day 5/6 with either fresh or cryopreserved blastocysts cultured coated with a layer of oil A (n = 101) or B (n = 104), with a mean number of 1.42 ± 0,55 and 1.33 ± 0,53 blastocysts transferred/patient in each group, respectively. No differences were found in terms of clinical pregnancy (A:69.30% vs B:67.31%) or implantation rates (A:62.37% vs B:61.53%) between both groups. Miscarriage rates were similar between group A (11.88%) and group B (12.62%).

The pH average value during the study was 7.26 ± 0.06. The mean values of the room temperature, humidity and VOCs were stable at 21.7 ± 0.4 °C, 66.7 ± 6.9% and 0.098 ± 0.01ppm, respectively.

Limitations, reasons for caution: Although heavy oils are the most competent in keeping optimal culture conditions over time, there are several culture oils with different features available in IVF market. Thus, further studies should be performed comparing among them. Future research is also needed to compare peroxidation rates of our culture oils studied.

Wider implications of the findings: The present study suggests that both commercial heavy oils used in a continuous approach may provide similar in vitro fertilization rates regarding fertilization, blastocysts suitable for clinical use or clinical pregnancy. Heavy oil features, laboratory conditions and the culture environment should be properly validated independently on each IVF center.

Trial registration number: not applicable

Abstract citation ID: dead093.588

P-230 Does laser-assisted hatching improve pregnancy rates : a retrospective analysis

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Study question: Is there any effect of laser-assisted zona drilling in improving pregnancy outcomes in women undergoing vitrified-warmed embryo transfers?

Summary answer: Our data suggests that there is evidence of possible improvement in pregnancy rates with laser-assisted hatching of embryos among women >40 years of age.

What is known already: Mammalian embryo is surrounded by a glycoprotein shell called zona pellucida. The embryo must hatch out of this shell for it to implant in the uterus. However, certain factors such as sub-optimal in vitro culture conditions, advanced maternal age, zona-hardening due to vitrification and warming, among others, negatively impact the ability of the embryo to escape from the matrix surrounding it. Laser-assisted hatching is the safest and easiest technique available to overcome the problem of hatching by artificially disrupting the zona, though there isn't sufficient data available on whether this has any actual benefit or is just a fancy add-on.

Study design, size, duration: Retrospective cohort study comparing embryo transfer results of 88 women whose embryos underwent LAH prior to transfer with that of a control group of women. Data was collected from embryo transfers that took place between March 2021 to December 2022.

Participants/materials, setting, methods: Beta-hCG results of women (N = 88) whose embryos underwent LAH prior to transfer was compared with the results of transfer without LAH in women (N = 88) of similar age and clinical history. Patient age, clinical indications and embryo grades were comparable across both groups. LAH was performed immediately post-warming of blastocysts using a 1.48 µm diode to drill the zona at a point opposite the ICM. Data was analyzed using independent t test and chi square test.

Main results and the role of chance: Women from both test and control groups were further stratified into the following sub-groups based on age – A (25-29 years), B (30-34 years), C (35-39) and D (>40 years). Beta-hCG results were assessed and was found to be lower in sub-groups A (57.1% vs. 78.5%) and B (46.4% vs. 67.7%) of the test group as compared to control. It was marginally higher in test group C as compared to that of control (50% vs. 47.3%). However, we found an increase in pregnancy outcomes of test sub-group D in comparison to control (83.3% vs. 20%).

Limitations, reasons for caution: This is a retrospective, preliminary study to assess initial results of effect of LAH on vitrified-warmed embryo transfer outcomes. A prospective study with a larger sample size is needed to confirm our results in both frozen and fresh embryos.

Wider implications of the findings: Our results indicate that laser-assisted hatching does not provide any advantage except possibly in older women above the age of 40 years. Thus we can select the exact group of patients who would benefit with this add-on and offer it to them routinely to improve their pregnancy rates.

Trial registration number: Not applicable

Abstract citation ID: dead093.589

P-231 The effect of warming blastocysts in Vitrolife media previously vitrified in Cook media on reproductive potential and clinical outcome

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Study question: Does the use of a different manufacturer's media for vitrification and warming of blastocysts affect pregnancy, miscarriage and live birth rates.

Summary answer: Warming blastocysts in Vitrolife media, previously vitrified in Cook media, does not significantly affect the pregnancy or live birth rate.

What is known already: Current good practice is to vitrify and warm blastocysts in media manufactured by the same company. This is due to the optimisation of the protocols and media composition intending to maintain high survival rates and associated pregnancy rates. However, circumstances can arise, such as supply and production issues, whereby it is not possible to source the same media for warming vitrified blastocysts as the media they were vitrified in.

Study design, size, duration: This retrospective cohort study was conducted at TFP-Oxford Fertility. Frozen embryo transfer (FET) cycles included in the study were performed between 2018 and 2022. Embryos that had undergone genetic testing were excluded from the study. The mean women's age was 35 in the control group (Cook warming media) and 36 in the study group (Vitrolife warming media). The average number of embryos transferred was 1.06 vs 1.17 in control vs study group.

Participants/materials, setting, methods: For the present study 817 patients underwent FET between 2016 and 2018 whereby blastocysts were vitrified in cook media (Sydney IVF Blastocyst Vitrification Kit, K-SIBV-5000) and warmed in cook media (Sydney IVF Blastocyst Warming Kit, K-SIBW-5000). In comparison 704 patients underwent a FET between 2018 and 2022 whereby blastocysts were vitrified in cook media and warmed in vitrolife warming media (Rapidwarm™ Blast, 10120). The pregnancy, clinical pregnancy and live birth rates were compared.

Main results and the role of chance: Fisher's exact test was used to calculate the p value and a level of 0.05 was used for significance. The results showed that there was no significant difference in the pregnancy rate, clinical pregnancy rate or live birth rate for blastocysts warmed in Vitrolife media vs Cook media (59.0% vs 57.0% p = 0.56, 47.2% vs 43.9% p = 0.44, 39.3% vs 37.0% p = 0.72)

Limitations, reasons for caution: This is a retrospective study at a single centre. The number of previous treatment cycles per couple was not included in the study. The study only looked at a comparison of Cook and Vitrolife warming media, no other commercially available media was tested.

Wider implications of the findings: Although good practice to vitrify and warm blastocysts in the same manufacturer media, our results show that the reproductive potential and clinical outcome may not be adversely affected by using a different manufacturer's media. However, composition of the media and protocol should be considered and further studies should be conducted.

Trial registration number: not applicable

Abstract citation ID: dead093.590

P-232 Beyond 12 months, blastocyst cryostorage duration in close system affects live birth rate: a monocentric retrospective study

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Study question: In order to evaluate the effect of blastocyst cryopreservation duration on live birth rate (LBR).

Summary answer: Blastocyst cryopreservation duration negatively affects live birth rate when blastocysts are vitrified in complete close system for more than 12 months.

What is known already: With the development of embryo vitrification, frozen-thawed program embryo transfer has been widely used in the past decade. However, the association between cryopreservation duration and frozen blastocyst transfer outcomes are limited. So far, very few studies have provided evidence on the relationship between blastocyst storage duration and LBR.

Study design, size, duration: This monocentric retrospective study included 1267 women (n = 1462 cycles) who underwent single frozen blastocyst transfer. Duration of vitrification in close system was categorized into five groups included **2144** frozen blastocysts (Day 5/6) : <3 months (n = 417; mean female age = 32.6 ± 4.74y), 3-6 months (n = 596; mean female age = 33.2 ± 4.96y), 6-12 months (n = 440; mean female age = 33.4 ± 4.83y), 12-36 months (n = 512; mean female age = 32.6 ± 4.75y), and >36 months (n = 179; mean female age = 31.1 ± 3.90y).

Participants/materials, setting, methods: Clinical pregnancy and live birth outcomes were compared between the cohorts stratified according to blastocyst cryostorage duration. A binary multivariate logistic regression was performed, adjusting for confounders: female age at vitrification, male age, ART technique (IVF or ICSI) and endometrial preparation protocol (spontaneous, substituted and stimulated FET cycle).

Multivariable analysis was used to assess the association between crystorage duration of vitrified blastocyst and LBR. All frozen blastocyst transfers were realized in spontaneous, substituted or stimulated cycles.

Main results and the role of chance: After multivariable analysis, live birth rate was significantly different depending on blastocyst cryostorage duration (all groups combined) ($p = 0.033$): live birth rate was significantly lower when blastocyst cryostorage duration was between 12-36 months (OR 0.740 IC95%[0.558; 0.981] $p = 0.036$) and beyond 36 months (OR 0.674 IC95%[0.456; 0.986] $p = 0.044$) compared to less than 3 months of blastocyst cryostorage duration. However, no significant impact of blastocyst cryostorage duration was observed on clinical pregnancy rate ($p = 0.52$).

Limitations, reasons for caution: Our study was limited by its retrospective design with restricted samples.

Wider implications of the findings: This study provides new findings on the effect of blastocyst cryostorage duration with a significant impact on LBR beyond 12 months of vitrification in close system. However, it's crucial to confirm that cryostorage duration doesn't lead to adverse obstetric and perinatal outcomes after vitrified blastocyst transfer.

Trial registration number: not applicable

Abstract citation ID: dead093.591

P-233 Novel, non-invasive imaging techniques in quality assessment of mammalian eggs

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Study question: Can Optical Coherence Microscopy (OCM) imaging and Cytoplasmic Movement Velocity (CMV) analysis be useful in quality assessment of mature mammalian oocytes?

Summary answer: OCM allows for assessment of oocytes' metaphase spindles, and CMV – the actomyosin cytoskeleton functionality, therefore they are potentially useful in oocyte selection for IVF.

What is known already: The success rate of IVF has significantly improved over the recent years, yet its efficiency plummets with the increasing age of female patients. Therefore, novel approaches to oocyte/embryo quality assessment are in high demand. Among the most promising new methods are OCM, a label-free, 3D tool for subcellular structure visualization, such as the meiotic spindle in mature oocytes or nuclear structure in immature oocytes, and the analysis of CMV, a time-lapse imaging-based method for studying properties of the actomyosin cytoskeleton, crucial for a plethora of cellular

process. Both these methods provide unique insights into oocyte competence.

Study design, size, duration: MII spindle dimensions were measured in OCM-imaged freshly ovulated oocytes collected from young mice (FO-MII, n = 108) and correlated with the outcome of preimplantation development achieved after the oocytes' activation. Spindles of FO-MII (n = 45) were also compared to spindles of oocytes collected from mice in advanced maternal age (AMA-MII, n = 44). CMV was compared in time-lapse imaged FO-MII (n = 24), postovulatory aged oocytes collected from young mice (PA-MII, n = 16), and AMA-MII (n = 19).

Participants/materials, setting, methods: Oocytes were collected from hormonally primed mice and imaged using a customized OCM protocol. They were then activated parthenogenetically and cultured for 5 days, followed by immunofluorescence staining to assess the total number of cells and number of cells in the first embryonic cell lineages. FO-MII, PA-MII, and AMA-MII were time-lapse imaged (6 frames per minute; 15 min). The images were subjected to particle image velocimetry analysis and the mean CMV was calculated.

Main results and the role of chance: Oocytes that achieved the blastocyst stage had smaller spindle volume ($p < 0.001$) and width ($p < 0.05$) as compared to those failing to do so. Univariate logistic regression analysis indicated that a 1 µm rise in the spindle width decreases the odds of blastocyst formation by 28% and the number of cells on average by 8, whereas a 100 µm3 rise in the volume – by almost 10% and 2.7 cells respectively. Moreover, the spindle volume is also related to the number of trophectodermal cells, and the spindle length - to the number of primitive endoderm cells in the blastocysts. Noteworthily, young oocytes' spindles had significantly smaller volume ($p < 0.0001$), as well as were narrower ($p < 0.0001$) compared to their counterparts from aged females. A multivariate linear regression model combining spindle length with three morphokinetic parameters (t3, cc2b, tSB) explained 60% of the variability in the total number of cells in 5-day-old embryos ($R2 = 0.604$) while models based solely on a single morphokinetic parameters explained only up to approx. 54% ($R2 = 0.537$, for tSB). On the other hand, CMV analysis revealed that freshly ovulated eggs display faster cytoplasmic movements than those postovulatory-aged ($p < 0.0001$). Reduced cytoplasmic speed was also observed in oocytes collected from aged mice ($p < 0.0001$).

Limitations, reasons for caution: All studies presented here were performed on a mouse model, so the obtained data needs to be verified in the target species for validation of these methods as novel tools in oocyte selection protocols.

Wider implications of the findings: Our results suggest that OCM-based assessment of the MII spindle and short, 15-minute CMV imaging allow for the selection of higher quality oocytes and could potentially find application in assisted reproductive technologies, as they are non-invasive and may enhance the universally acclaimed oocyte/embryo quality assessment protocols.

Trial registration number: not applicable

Abstract citation ID: dead093.592
P-234 Increased live birth rates follow increased normal fertilization and usable blastocyst rates with use of low lactate culture medium

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Study question: Does an increase in usable blastocyst rates with low lactate culture medium lead to increased live birth rates?

Summary answer: Increased overall usable blastocyst rate correlates with an increased live birth rate following low lactate embryo culture.

What is known already: Lactate is essential to the oocyte and early embryo and while it is produced naturally through the normal glycolytic pathways of the same, it is nonetheless added to available embryo culture media. In embryo culture using a continuous culture medium containing only 1mM lactate, an increase in day 5 and overall usable blastocyst rates has been documented as compared to higher lactate (6-10mM) in a sequential media system. There are now also studies showing an increase in normal fertilization with a decrease in no fertilization and abnormal fertilization with oocytes placed in low lactate at oocyte retrieval.

Study design, size, duration: In a prospective interventional study of IVF cases between October 2020 and April 2021, patient oocytes were divided randomly amongst a control (Vitrolife G1/G2) and treatment (Continuous Single Culture Medium-NX Complete) group. Oocytes were split following oocyte retrieval and embryo culture proceeded in one of the two groups through day 6. Total oocytes in the control group, 258, and in the treatment group, 273. Patients of all ages and diagnosis' were included.

Participants/materials, setting, methods: All cases were inseminated via intra-cytoplasmic sperm injection, blastocyst biopsy performed for next generation sequencing, and blastocysts vitrified with Vit Kit or Vit Kit–Freeze NX for frozen embryo transfer. All blastocysts were warmed with Vit Kit–Warm NX and transferred using SG/G2-Plus. Primary analysis included initial beta-HCG, clinical pregnancy rate (fetal cardiac activity) and live birth. Secondarily, fertilization rates were examined for comparison of normal (2PN), abnormal (1PN and 3PN), and no fertilization (0PN).

Main results and the role of chance: Chi-square analysis was applied on all considerations to assess statistical significance and $p < 0.05$ considered significant. In the analysis of fertilization parameters for the control versus treatment group, normal fertilization (69.0% vs 74.0%), abnormal fertilization (6.2% vs 8.0%) and no fertilization (19.8% vs 15.8%) rates were not significant. The initial beta HCG rates following frozen embryo transfer in the control and treatment groups were significant at 65.3% and 90.9%, respectively ($p = 0.036$). The rate of clinical pregnancy in the control was 38.5% and in the treatment group was 68.2% ($p = 0.040$). Live birth rate in the control versus treatment group was 30.8% and 59.1%, respectively ($p = 0.049$). There was no difference in the implantation rate between groups (control = 56.7%, treatment = 80.0%) ($p = 0.065$). In patients where the first embryo transfer did not result in clinical pregnancy, a subsequent transfer with an embryo from the opposite culture medium yielded a live birth rate of 11.1% (control) and 33.3% (treatment).

Limitations, reasons for caution: The pregnancy data in this particular study all result from frozen embryo transfers, with no fresh embryo transfers, and the possible effect or interference of the vitrification media, recovery medium and/or transfer medium cannot be accounted for, therefore, more study is needed.

Wider implications of the findings: The pregnancy and live birth rates in this analysis support previous findings that low lactate culture medium increases the instance of usable blastocysts and demonstrates that those increases can ultimately correlate with, and result in, a higher live birth rate for patients.

Trial registration number: Not Applicable

Abstract citation ID: dead093.593
P-235 Influence of female karyotype polymorphic variants on oocyte quality and embryo development

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Study question: Do polymorphic variants in the karyotype of women undergoing an IVF cycle directly affect oocyte and embryo laboratory parameters?

Summary answer: The presence of certain polymorphic variants negatively affects the oocyte survival rate and the blastocyst quality in an IVF cycle.

What is known already: In the recent years, there has been a growing interest in the study of polymorphic variants in infertile patients because their incidence, compared to the fertile population, is increased. However, most research has focused on the male patient study. Several studies have reported information about clinical outcomes, but the influence they may have on IVF

laboratory procedures and embryo development has rarely been studied up to the blastocyst stage. In addition, it is very rare to find publications that show the study of polymorphisms according to their type or combination.

Study design, size, duration: Retrospective evaluation of a cohort of women who underwent autologous IVF cycles and karyotyping. The sample included 424 IVF cycles performed between July 2017-December 2021: control group (CG) with normal karyotype (211) and study group (SG) with polymorphisms (213). We studied the correlation between karyotype polymorphisms and laboratory outcomes in terms of: Number of Oocytes Retrieved, Fresh Oocyte Maturity (MII), Oocyte Survival after Thawing (TS), Fertilization (FZ), Oocyte Degeneration (OD) and Non-viable embryo rates.

Participants/materials, setting, methods: Analysis of the women karyotype, prior to the IVF cycle, was performed using the guidelines of the International System for Human Cytogenetic Nomenclature (ISCN).

Differences between the different study groups (presence or absence of different chromosomal polymorphisms) were assessed with the appropriate statistical test according to the normality or non-normality of the variable distribution.

Statistical analysis was performed using R statistical software (v.4.2.0) and SPSS (v.23.0, Chicago, IL, USA).

Main results and the role of chance: Average female age was 37.72 ± 3.98 (CG) and 36.73 ± 3.59 (SG). Average number of mature oocytes (MII) was: 6.45 ± 4.96(CG) and 7.59 ± 5.10 (SG).

Statistically significant differences were found regarding the number of oocytes retrieved between the CG (8.14 ± 5.90) and the SG (9.53 ± 6.63) ($p = 0.036$), increasing when the ps+ variant was present (9.75 ± 7.21) ($p = 0.045$). However, no statistically significant differences were found between the presence of polymorphism (SG) and the CG in terms of: number of MII: 79.85% vs. 80.48% ($p = 0.447$), FZ: 73.76% vs. 70.49% ($p = 0.352$) and OD: 8.76% vs. 8.36% ($p = 0.563$) rates.

In addition, the TS rate was statistically significant when there was the ps+ variant (82.95%) ($p = 0.010$) and/or combinations of more than one polymorphic variant (87.80%) ($p = 0.044$), compared to the CG (93.51%).

Finally, according to embryo quality there was an increase in the non-viable embryos rate, on day 5 and/or day 6 of development, between the CG (34.17%) and the SG (43.02%) ($p < 0.001$), increasing when the ps+ variable was present (45.57%) ($p < 0.001$) and/or combinations of more than one polymorphic variant ($p = 0.045$).

The results were corrected for confounding variables such as maternal age, oocyte origin and male factor variables, including polymorphic variants in the male karyotype.

Limitations, reasons for caution: Other variables that have not been analyzed may influence the outcomes. Larger prospective studies including homogeneous cohorts are needed in order to corroborate our initial results.

Wider implications of the findings: The polymorphic variants in the female karyotype, especially the "ps+" variant and the combination of multiple variants, could influence certain parameters in the laboratory. Therefore, it is important to request a karyotype to all patients before starting IVF treatment.

Trial registration number: NOT APPLICABLE

Abstract citation ID: dead093.594

P-236 IL-6 levels in follicular-fluid and day5/6 spent culture drops indicate its role as a biomarker of oocyte maturation and an embryokine influencing embryo developmental competence

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Study question: To elucidate the role of IL-6 in folliculogenesis and embryo development during human in-vitro culture

Summary answer: High IL-6 levels in follicular-fluid positively correlate with oocyte maturity status whereas higher IL-6 secretome in day5/day6 spent culture media inversely correlates with blastocyst/ICM grades

What is known already: Although some studies have attempted to explore the correlation of FF IL-6 with fertilization and embryo development rates; the results have been inconclusive. Similarly, studies comparing FF IL-6 levels with endometrial receptivity and pregnancy outcomes have been ambiguous and contradictory. Recent research has reported improvement in bovine blastocyst ICM grades in culture media supplemented with IL-6. No study has yet evaluated the role of IL-6 as a biomarker of oocyte maturity nor investigated IL-6 as an embryokine that could regulate embryo developmental competence in humans. We have employed the FF metabolomic and Secretomic approach for IL-6 in human IVF cycles.

Study design, size, duration: Prospective study in women (25-35 years) undergoing IVF cycles ($n = 256$) for unexplained/tubal-factor infertility from 2018-2020. Only those women whose FF and day5/6 spent culture media sample could be obtained, were included. Women with endometriosis, PID, Genital Kochs were excluded. IL-6 levels were measured by diagnostic kit in spent micro-drops and FF (pooled per patient per cycle). Cycles were divided into Low and High IL-6 groups (FF: ≤ 47.5 and >47.5pg/ml; Spent Medium: <0.5 and ≥0.5pg/ml).

Participants/materials, setting, methods: Antagonist cycles involving COH with r-FSH and ICSI for all. Noted oocyte Nuclear maturity (GV/MI/MII). Oocyte Cytoplasmic maturity graded as Top and non-Top, based on presence of granulation, refractile bodies, sER, visco-elasticity. Post ICSI, oocytes cultured individually in 50µl single-step media micro-drops till day3 and shifted to fresh 50µl micro-drops for extended culture till day5/6. Blastocyst gradation was done using Gardner's classification for expansion, ICM and trophectodermal cells. Statistical analysis done using graph-pad prism VI

Main results and the role of chance: High FF/IL-6 group ($n = 103$) had significantly higher proportion of MII (92.08 vs. 76.33%; $p < 0.001$) and top-quality (88.10 vs. 70.0%; $p < 0.001$) oocytes and fertilization rates (89.45 vs. 77.30%; $p = 0.009$) compared to Low FF/IL-6 group ($n = 153$) although total number of days of stimulation/gonadotropin dose, mean oocytes retrieved did not differ significantly between the two groups. Cleavage (84.64 vs. 75.92%; $p = 0.061$) and blastocyst formation (53.86 vs. 42.37%; $p = 0.053$) rate, although higher, did not differ significantly between the two FF/IL-6 groups. FF/IL-6 level correlated strongly with overall oocyte maturity status (Pearson $r = 0.61$, 95% CI 0.52-0.8). Threshold FF/IL-6 level for obtaining top-quality MII oocytes was >39.2pg/ml ($ROC_{AUC} = 86\%$, Sensitivity = 78%, Specificity = 83%).

In D5/6 spent culture drops, likelihood of top-quality blastocyst formation (expansion grade ≥3 and ICM/TE grades AA/AB/BA) increased by 41% (odds ratio: 0.44, 95%CI: 0.4-0.5; χ^2 $p < 0.0001$) if IL-6 concentration was <0.5 pg/ml. Lower culture-drop IL-6 levels correlated strongly with top ICM grades (Pearson $r = -0.66$, 95% CI 0.6-0.7). Highest IL-6 levels were found in spent culture-drops containing grade-C blastocysts whether on day5 or day6. IL-6 levels were comparatively higher in day6 than in day5 micro-drops, correlating with higher percentage of top-quality blastocysts on day5 than on day6. Threshold micro-drop IL-6 level for top-grade blastocyst <0.42 pg/ml.

Limitations, reasons for caution: We estimated IL-6 levels in pooled FF (per patient, per cycle). Although cumbersome & time-consuming, it would be worthwhile to evaluate individual FF (per follicle, per patient, per cycle). Also, measuring IL-6 in spent media involving group embryo culture, may facilitate study of the probable paracrine effects of this 'embryokine'.

Wider implications of the findings: IL-6 is a pleiotropic cytokine and its role/s in folliculogenesis, embryo development and endometrial receptivity is not yet clearly etched out. This study attempts to elucidate the functions of IL-6 in a systematic manner by deriving threshold values for obtaining top quality oocytes and blastocysts in human in-vitro culture conditions.

Trial registration number: NOT APPLICABLE

Abstract citation ID: dead093.595

P-237 In vitro time to maturation of Metaphase I oocytes: a time-lapse study correlating blastocyst formation and ploidy status

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Study question: What is the impact of metaphase I (MI) time to mature period (TTM) regarding blastocyst development and ploidy status?

Summary answer: A short period of TTM (0.1 to 1.0) is related to an increase number of blastocyst formation when compared to longer TTM (1.1 to 3.0).

What is known already: To achieve meiotic competence, coordinated nuclear and cytoplasmic changes must occur in the oocyte to support fertilization and DNA replication and to ensure correct ploidy of the zygote and embryonic genome activation. Concomitant with chromosome condensation and migration, oocyte maturation goes by cytoplasm reorganization throughout the transitions of MI, polar body extrusion and MII. Despite the optimization of COH protocols, nearly 20% of the retrieved oocytes remain immature at the GV or MI stages. In vitro maturation of MI could increase the number of available embryos; however, longer culture time may affect oocyte quality, resulting in abnormal fertilization and cleavage.

Study design, size, duration: Prospective cohort study from 244 patients (n = 277 cycles) that had at least 1 MI oocyte after oocyte retrieval (OPU) following controlled ovarian hyperstimulation (COH) for in vitro fertilization (IVF) treatment according to medical referral in an single private ART center from January/2019 to November/2022. All MI oocytes were placed in a time-lapse incubator (TL, EmbryoScope, Vitrolife, Sweden) to check the exact time of first polar body extrusion (TTM to MII) before intracytoplasmic sperm injection (ICSI).

Participants/materials, setting, methods: Three hours after OPU, oocytes were denuded and assessed for maturation. Six-hundred and seven MI oocytes were followed in TL and those that reached the MII stage in a maximum of 6 hours post-OPU, were injected by ICSI (at 6h post-OPU) and stratified according to the TTM (0.1-1.0 hours and 1.1-3.0 hours). Fertilization rate, blastocyst formation, and ploidy were assessed. Kruskal-Wallis, chi-squared and Fisher tests were applied for statistical analysis. $p < 0,05$ was considered significant.

Main results and the role of chance: Patient mean age (n = 244) was 38,50 ± 3,40 years. From those, 25 patients (10.3%) had 2 cycles and 4 patients (1.6%) had 3 cycles with at least 1 MI oocyte. Age, anti-Müllerian hormone (AMH), #OPU, #MII, %MII (MII/OPU), #MI and MI% (MI/OPU) mean were not statistically different in patients with 1, 2 or 3 cycles in our cohort. One hundred forty-nine patients (61%) in 165 cycles (59.6%) had at least 1 oocyte that reached MII stage. In total, 248 oocytes were injected after 0.1-1.0h (group 1, n = 104, 41.9%) and 1.1-3.0h (group 2, n = 144 oocytes, 58.1%) of in vitro maturation. Age (38,99 ± 3,01 vs 38,85 ± 3,31), AMH (1,27 ± 1,29 vs 1,63 ± 1,87), #OPU (8,98 ± 4,32 vs 8,90 ± 4,34), #MII (4,41 ± 2,74 vs 4,44 ± 2,178), %MII (47.6% ± 18.8% vs 48.4% ± 17.1%) and %MI that followed to ICSI (59.2% ± 28.5% vs 54.8% ± 29.2%) were not different between groups 1 and 2 respectively. The mean number of MI that followed to ICSI were higher in group 1 (1,28 ± 0,48 vs 1,10 ± 0,32, p = 0.0008). Normal fertilization (2PN) and embryo cleavage were similar between groups (54.8% vs 45.1%, p = 0.157, 96.6% vs 93.3%, p = 0.467, respectively). However, blastocyst formation rate (#blastocyst/#fertilized) was higher in group 1 (n = 33, 55.9% vs n = 26, 34.7%, p = 0.015). Euploidy rates were similar between groups (9/28, 32% vs 4/24, 16%, p = 0.336).

Limitations, reasons for caution: Besides the retrospective nature of this study, male factor and infertility reason were not considered in this analysis. Due to the low number of blastocyst formation and consequentially ploidy assessment and number of embryo transfer, the results regarding euploidy rate may be underestimated and pregnancy rates were not included.

Wider implications of the findings: Oocytes that reached maturity up to 1 hour after denudation showed higher blastocyst formation rate, thus increasing embryo availability to transfer. In vitro-matured oocytes exhibit increased spindle and chromosomal abnormalities compared with oocytes matured in vivo, which may explain the lower rates of euploid embryos, especially in group 2.

Trial registration number: Not applicable.

Abstract citation ID: dead093.596

P-238 Non-interventional study comparing the effectiveness and safety of r-hFSH:r-hLH versus r-hFSH in women aged 35-42 years with normal ovarian reserve undergoing GnRH antagonist ART treatment

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Study question: Is ovarian stimulation (OS) with r-hFSH:r-hLH more effective than OS with r-hFSH alone in women aged 35-42 with normal ovarian reserve treated with ART?

Summary answer: Compared to r-hFSH alone, OS with r-hFSH:r-hLH for ART treatment was associated with a significant increase in clinical pregnancy and live birth per initiated cycle.

What is known already: Meta-analyses and secondary analyses of clinical data provide evidence that OS during ART treatment with r-hFSH:r-hLH is associated with improved reproductive outcomes when compared to r-hFSH alone in various patient groups with: a) advanced maternal age , b) low ovarian reserve or , c) hypo response to OS. Other subgroups of patients might also benefit of the use of r-hFSH:r-hLH during OS for ART treatment. We aimed to determine if, in a real-world setting, OS with r-hFSH:r-hLH is associated with improved clinical benefit when compared to r-hFSH alone in patients aged 35-42 years with normal ovarian reserve.

Study design, size, duration: A non-interventional study including women aged 35-42 years with normal ovarian reserve biomarkers (menstrual cycle day 2 or 3 FSH<12IU/L or 9<AFC<20 with a 2<diameter<10 mm, or AMH ≥1.2ng/ml) undergoing OS for ART treatment (fresh transfer only; GnRH antagonist protocol) in 12 French centers between 1/1/2008 and 31/12/2016, with a follow-up period up to 31/12/2017. The analysis included two cohorts: a) 4,323 OS cycles with r-hFSH alone, and b) 1,070 OS cycles with r-hFSH:r-hLH.

Participants/materials, setting, methods: Anonymized data were extracted from the Retrospective Multicenter Statistical database, including 12 French centers. A generalized linear model was used to address lack of randomization. The fixed effect part included age, an ovarian reserve estimator and stimulation; the random effect included age, AMH, AFC, FSH, cause of infertility and number of attempts, center and patient effect. Clusters (matched subsets) were built based on the propensity score by using a two-stage algorithm.

Main results and the role of chance: We included in our analysis 4,472 women receiving ART treatment after OS with r-hFSH (mean age: 37.8 years; 4,323 cycles) or with r-hFSH:r-hLH (mean age: 38.1 years; 1,070 cycles). In the r-hFSH cohort the mean AMH level was 2.81 ng/ml and mean AFC was 12.0, in the r-hFSH:r-hLH cohort they were 2.14 ng/ml and 10.0, respectively. The mean total r-hFSH dose were 2,275 IU (r-hFSH cohort) and 3,002 IU (r-hFSH:r-hLH cohort). Mean ± SD number of oocytes were 8.1 ± 5.7 (r-hFSH cohort) and 6.1 ± 4.3 (r-hFSH:r-hLH cohort); mean ± SD number of embryos were 4.0 ± 3.4 (r-hFSH cohort) and 3.3 ± 3.2 (r-hFSH:r-hLH cohort). The unadjusted ongoing pregnancy rate reached 17.1% in the r-hFSH cohort and 20.4% in the r-hFSH:r-hLH cohort, whilst the unadjusted live birth rate reached 17.0% and 20.2%, respectively. Unadjusted pregnancy failure rate was 6.6% and 3.7%, respectively.

After adjusting for confounders, when compared to OS with r-hFSH, OS with rhFSH:rhLH was associated with a higher clinical pregnancy rate (Risk ratio,RR 1.232; 95% CI 1.058 to 1.436; p-value = 0.007) and a higher live

birth rate (RR 1.452; 95% CI 1.227-1.1719, p-value <0.001) per initiated cycle.

Limitations, reasons for caution: Since this was a real-world data study, no random assignment of stimulation was performed, and some residual confounding is possible due to unmeasured confounders. Live births resulting from further transfers of frozen embryos were not included in the analysis.

Wider implications of the findings: This study demonstrated that OS with r-hFSH:r-hLH improves live birth rate in women > 35 years old with a normal ovarian reserve, compared to r-hFSH only. Sound RWE studies can be combined with RCTs to guide clinical and payer's decision making, paving the way to precision medicine.

Trial registration number: Not applicable

Abstract citation ID: dead093.597
P-239 Comparison of reproductive outcomes for cleavage- and blastocyst-stage frozen embryo transfer: A retrospective study

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Study question: Does blastocyst-stage (day 5-6) embryo transfer improve pregnancy and live birth rates per frozen transfer compared to cleavage-stage (day 2-3) embryo transfer?

Summary answer: Pregnancy rate is significantly higher following frozen blastocyst-stage embryo transfer, while live birth rate does not differ.

What is known already: The extended culture of cleavage-stage (day 2-3) embryos to blastocyst-stage (day 5-6) embryos has been largely adopted in recent years. In fact, blastocyst culture may allow a better selection of the embryo to be transferred based on its developmental history and morphological criteria, and result in a lower number of exceeded embryos to be frozen for storage. However, the literature comparing pregnancy and live birth rates for cleavage- and blastocyst-stage transfer remains contradictory, and the benefits of frozen blastocyst transfer is still under discussion.

Study design, size, duration: This retrospective study investigated couples having frozen embryo transfer at the cleavage (days 2 or 3) or the blastocyst-stage (days 5 or 6) at our clinic between 2014–2021. Results from more cycles were analyzed individually. We excluded cycles based on fresh embryo transfer, oocyte or sperm donation. Cycles were further sub-classified based on the type of treatment, as natural or stimulated, and the pregnancy and live birth rates were compared.

Participants/materials, setting, methods: Data was obtained from patient records at our fertility clinic and sorted by two authors. Variables included female age, treatment plan, the stage of the embryo transferred, and the report of pregnancy and live birth. Statistical analysis was conducted by using MedCalc Software, and Chi-squared test applied to compare the outcomes' frequency based on the day of frozen embryo transfer. $P < 0.05$ was considered significant.

Main results and the role of chance: A total of 2,922 cycles were included for analysis, with age of female patients being 37.2 ± 5.0 years at the time of transfer. Most cycles were stimulated (n = 2,217, 75.9%) compared to natural cycles (n = 705, 24.1%). In all cycles, IVF was used for fertilization. Approximately one third of the cycles (n = 954, 32.6%) underwent frozen embryo transfer at the cleavage-stage (days 2 or 3), while the remaining had transfer at the blastocyst-stage (n = 1,968, 67.4%).

Pregnancy rate was significantly ($P < 0.0001$) higher for blastocyst-stage embryo transfer compared to cleavage-stage embryo transfer (36.2% vs 20.4%, respectively) when all cycles were analyzed. This significance was found when natural (23.3% vs 14.8%, $P = 0.0052$) or stimulated cycles (38.0% vs 25.8%, $P < 0.0001$) were sub-analyzed.

While we observed 19.0% of successful live birth per embryo transferred in the entire cohort, this did not significantly vary depending on the stage of frozen embryo transferred (cleavage or blastocyst-stage, 13.6% vs 21.5%, respectively) and when patients were sub-classified based on the type of treatment chosen in natural (9.0% vs 14.2% respectively) or stimulated (18.0% vs 22.6%, respectively) cycles.

With a large sample size and a very significant P-value for pregnancy rate, the role of chance is limited.

Limitations, reasons for caution: As a retrospective study, the non-random allocation to treatment groups might hide selection bias and limit the statistical power of the data analysis, especially for the live birth rate. Also, no adjustment for confounders was performed.

Wider implications of the findings: Frozen embryo transfer at blastocyst-stage may offer an improved chance of pregnancy in both natural and stimulated cycles, possibly resulting in less embryos stored and lower costs for patient treatment. Although no difference was observed, larger prospective studies are needed to clarify the impact on live birth rate.

Trial registration number: Not applicable

Abstract citation ID: dead093.598
P-240 High mitochondrial DNA content in Day6 blastocyst may impair ongoing pregnancy rate

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Study question: Is there an association between mitochondrial DNA (mtDNA) content and Ongoing Pregnancy Rate (OPR) in D5 or D6 euploid blastocyst?

Summary answer: Day6 OPR is decreased in high mtDNA content blastocyst. Day5 and Day6 mtDNA levels are independent of maternal age or standard morphology.

What is known already: Preimplantation development is an energy-demanding process and mitochondrial ATP production is crucial for cellular activity in fast replicating cells. Mitochondrial content in preimplantation embryo has been proposed as a marker of embryo potential in term of viability and implantation success though the dynamics and distribution of mitochondria in human embryo is still debated. In recent years, it has been suggested that low mtDNA levels were associated with euploid chromosomal complement and higher implantation rate while other studies failed to find a correlation between mtDNA content and reproductive outcome.

Study design, size, duration: This study is a retrospective cohort analysis from 2020 to 2022 including 343 Day5 and 187 D6 single euploid blastocyst transfer. Primary endpoint was OPR beyond pregnancy week 16. Day5 and Day6 frozen-thawed transfer were divided based on blastocyst mtDNA content in four groups: Day5-high, Day5-low, D6-high, D6-low. Secondary endpoints were relationship between mtDNA content, maternal age and standard morphology in all obtained blastocysts. Statistical differences were compared by Chi-Square test.

Participants/materials, setting, methods: 771 couples performed IVF cycles with next-generation sequencing (NGS)-preimplantation genetic testing of aneuploidy (PGT-A) (age 19-47yrs; Mean age 37.8yrs). Following culture in sequential media 700 euploid blastocyst were obtained and mitochondrial and chromosomal DNA copy number variation were examined simultaneously with next generation sequencing (NGS) methodology. mtDNA copy numbers was based on the observed ratios of sequence coverages between mtDNA and nuclear DNA.

Main results and the role of chance: In our clinical setting OPR is similar in Day5 vs. Day6 transfers (55% vs. 49% n.s.). However when mtDNA content is considered D6-low OPR is comparable to D5-high or low (57.3%; 53.3%; 57,3% respectively n.s.) while D6-high transfers yield significantly decreased OPR (31,2% $p < 0.05$). When stratified by age only Advanced Maternal Age (AMA) patients (>=38yrs) showed this decrease at a significant level (Day6 high OPR 20% $p < 0,05$).This result in transferred blastocyst is obtained despite high:low ratio of all biopsied blastocyst is constant in Day5 (1:1) (47%-53%) and markedly decreased in Day6 (1:4) (25%-75%) either overall or in younger (n.s.) and AMA patients (n.s.). Moreover when standard morphology distribution is evaluated according to Gardner classification criteria top, fair and poor quality blastocysts display the same 1:1 high:low blastocysts mtDNA ratio in Day5 and 1:4 in Day 6 overall and in younger and

AMA patients. Hence, the mtDNA blastocyst content is independent of patients' age and blastocyst quality. The origin of Day6 OPR decrease could therefore depend on the number of Day6-high blastocyst transferred in AMA patients and ultimately in the number of blastocyst available to transfer for each patient.

Limitations, reasons for caution: Due to the small numbers of Day6-high mtDNA blastocyst, a larger sample size is required to confirm these preliminary findings. Moreover, the retrospective nature of the study may introduce some bias mainly in patients' characteristics and the strategy of embryo selected for transfer.

Wider implications of the findings: While Day5-high blastocyst yield higher OPR the persistence of higher mtDNA levels in Day6 blastocyst may hamper their implantation potential. Our findings may help to select the embryo to transfer to maximize transfer success. D6 blastocysts with low mtDNA content should be preferred whenever a choice is possible.

Trial registration number: Not Applicable

Abstract citation ID: dead093.599
P-241 Cumulative live birth rates after embryo transfers at Day-2/3 versus Day-5/6: a comparative nationwide cohort study

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Study question: Are there significant differences between cumulative live birth rates (CLBR) after a short and an extended embryo culture when comparisons are performed *per* cycle?

Summary answer: The CLBR *per* cycle was significantly lower in the Day-5/6 group and higher *per* embryo transfer as compared with the Day-2/3 group.

What is known already: Previous evidence has shown the probability of live birth following a fresh embryo transfer in *In Vitro* Fertilization (IVF) is higher after extended embryo culture (E-EC: Day-5/6) than after a shorter embryo culture (S-EC: Day-2/3). A further controversy literature has arisen on cumulative pregnancy rates, related to the higher risks of embryo transfer cancellation after E-EC compared with S-EC. Moreover, all of these studies used slow freezing/thawing embryo protocols with a higher survival for Day-2/3 embryos than Day-5/6 embryos. Finally, a recent study combining vitrification highlighted CLBR improvements after E-EC but CLBR were expressed *per* embryo transfer, not *per* cycle.

Study design, size, duration: A French national register study including all IVF cycles with at least one cleaved embryo at day-2 performed in France from 01/2016 to 12/2018 was carried out (n = 165,808 cycles), after exclusion of gamete donation cycles, attempts with preimplantation genetic diagnosis, surgical spermatozoa, viral context, and for which the embryo freezing was performed by slow-freezing. We chose to include only cycles from IVF centers with standardized CLBR similar or above to the national mean (99%CI).

Participants/materials, setting, methods: From the biomedicine Agency's registry, two groups were identified: cycles with embryo(s) at Day-2/3 with/without embryo transfer (S-Group) and cycles with all embryos cultured to Day-5/6 with/without embryo transfer (E-Group). The CLBR defined as delivery of at least one live-born infant in fresh or in subsequent frozen-thawed cycles were compared. Only the first delivery was considered. Regression model adjusted for women age, parity, Day-2 embryos, IVF-method and attempt rank was used.

Main results and the role of chance: In total, 48,293 attempts fulfilled the inclusion criteria. Among them, 31,769 cycles were included in the S-Group and 16,524 in the E-Group. Overall, the CLBR *per* cycle was significantly lower in the E-Group compared with the S-G group (32.9% *versus* 33.5%; aOR: 0.89, 95%CI [0.86-0.93], $p<.0001$). A significant higher rate of fresh embryo transfer cancellation was observed in in the E-Group compared with the S-Group (19.8% versus 3.1%, $p<.0001$). However, when we performed further comparisons restricted to data from IVF centers with LBR strictly significantly above to the national mean LBR, the CLBR were similar between both groups (36.4% versus 38.5% in S-Group and E-Group, respectively; aOR: 0.97 [0.90-1.04], $p=0.402$).

Finally, the CLBR in E-group was significantly higher than in S-group when expressed *per* embryo transfer (aOR: 1.15 [1.10-1.21], $p<.0001$).

Limitations, reasons for caution: Main limitations are: the retrospective design and the 1 year follow-up that could not include all frozen embryo cycles performed.

Wider implications of the findings: Overall, the nationwide results *per* cycle suggest that extended embyo culture until blastocyst stage, even used in combination with vitrification, could not improve live birth rates.

Trial registration number: not applicable

Abstract citation ID: dead093.600
P-242 Genetic laboratories report significantly different aneuploidy rates for identical patient populations, a finding with important implications for clinics using PGT-A

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Study question: If a clinic changes the genetic laboratory it works with, should it expect its new PGT-A results to be equivalent to those it received previously?

Summary answer: Changing the genetic service provider can result in a significant difference in the proportion of embryos classified euploid, with important clinical implications.

What is known already: Preimplantation genetic testing for aneuploidy (PGT-A) aims to distinguish potentially viable euploid embryos from embryos harbouring lethal chromosome abnormalities. Typically, IVF clinics perform biopsy at the blastocyst stage and send the resulting trophectoderm specimens to specialist genetic laboratories for analysis. However, many commercially available genetic methods have not been subjected to rigorous validation, leading to uncertainty about their accuracy and predictive value. It would be concerning if the classification of embryos, derived from the same patient population, and from the same clinic, varied depending on the company that provides PGT-A services. Such differences would be unlikely to reflect biological reality.

Study design, size, duration: Our clinic recently began working with a new genetics company. Our previous PGT-A reference laboratory issued results for 1,136 blastocyst biopsy specimens over ~15 months. Subsequently, the second company tested 784 biopsy samples in 7 months. Patient populations during the two periods were essentially identical (average maternal age 38.0 years).

Participants/materials, setting, methods: The first company used whole genome amplification followed by next generation sequencing (NGS) to evaluate the relative amount of DNA from each chromosome. The second company's method was highly validated, involving targeted amplification of thousands of sites in the genome, again followed by NGS and analysis of relative DNA quantity from individual chromosomes. Additionally, they genotyped numerous polymorphisms, which assist in the detection of haploidy/triploidy, and also reveals problems that can compromise accuracy (e.g. contamination).

Main results and the role of chance: The first PGT-A company classified 44.4% of blastocysts euploid, 4.1% low-level mosaic, 51.5% aneuploid. In contrast, the second company reported significantly fewer embryos aneuploid (44.0%; P = 0.0019). Even if the mosaics reported by the first company are considered for transfer, the second company is still associated with more potentially transferable embryos (relative increase greater than one-sixth). If PGT-A results from the second company are correct, it implies that potentially viable embryos may have been misclassified by the first company, risking their exclusion and loss of the pregnancies they might have produced.

Conversely, if the first company is correct, the second company may be failing to detect some aneuploidies, leading to inadvertent transfer of abnormal embryos, lower pregnancy rates and a higher incidence of miscarriage. 299 transfers following PGT-A using the first company produced 171 pregnancies (74.7%), while 64 single embryo transfers have taken place after PGT-A with the second company, resulting in 53 pregnancies (82.8% per transfer). Losses (biochemical or miscarriage) affected 21.6% and 16.9% of pregnancies after PGT-A conducted by the first and second companies, respectively (ongoing pregnancy rates of 58.5% and 68.8% per transfer, respectively) Thus, there is no evidence that aneuploidies are being missed by the second company.

Limitations, reasons for caution: Although there were no obvious differences between the patients with embryos tested by the two companies, this study was not prospective nor randomized, so a possibility of undetected differences remains. The study was not sufficiently powered to test the significance of apparent differences in implantation, miscarriage and ongoing pregnancy rates.

Wider implications of the findings: It is important that IVF clinics appreciate that individual PGT-A methods differ significantly, and that some have undergone much more rigorous validation than others. It is recommended that clinics ask PGT providers to share their validation data, especially clinical studies confirming that embryos classified 'aneuploid' or 'abnormal' are truly nonviable.

Trial registration number: Not applicable

Abstract citation ID: dead093.601
P-243 A commonly used temperature probe could result in setting heated surfaces within the IVF lab too high

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Study question: Which temperature probe (thermocouple Type T or Pt100) is the most accurate and robust for measuring medium temperatures in dishes on a heated surface?

Summary answer: A Pt100 probe attached to the bottom of the dish provides a more accurate and repeatable measurement than the commonly used thermocouple Type T probe.

What is known already: The success of an IVF cycle is dependent on several variables. Ensuring the gametes and embryos are exposed to appropriate temperatures is a critical variable during incubation and manipulation. There are different temperature measurement probes available for this purpose. Their specific properties could make them more, or less suitable for the various temperature measurements required in the laboratory.

Study design, size, duration: The aim of the study was to determine the best method (thermocouple Type T probe placed in the medium or a Pt100 sensor attached to the bottom of a dish) to measure temperature in medium held in two types of dish placed on a heated surface. Measurements were made in triplicate and standard deviation determined.

Participants/materials, setting, methods: The temperature of the medium in two types of dish (5 well and 40mm direct surface contact dishes, Vitrolife) were measured with two types of probes. The commonly used thermocouple Type T, which is composed of two wires, a copper and copper nickel alloy, that are welded together, and a resistance sensor composed of a small platinum plate attached to the bottom of the dish (Pt100).

Main results and the role of chance: With a 100 µl droplet under 5.0 ml of oil in a 40 mm dish (with the lid on) the tip of the Type T probe measured a temperature 3.2 ± 0.26 °C lower than the Pt100 probe. The Type T probe also measured a lower temperature in the 5 well dish. With a well containing 500 µl (lid on) it measured a temperature 2.1 ± 0.15 °C lower than the Pt100 probe. When the entire length of the Type T probe was placed inside of an incubator set at 37.0 °C the measured temperature was 0.1 °C lower than the Pt100 probe. This suggests the length of the Type T probe within the environment to be measured is critical, i.e., the lower temperatures obtained with the Type T probe when measuring medium temperature on heated surfaces depends on the method used rather than the probe itself. The reason for the lower temperature is correlated to the high temperature conductivity of the copper in the Type T probe. This also makes the measurement less repeatable as different factors (e.g., length of probe submerged, ambient temperature, air flow, etc.) will affect the reading. The Pt100 is not influenced by such factors.

Limitations, reasons for caution: The dishes used in this investigation create a uniform direct contact with the heated surface. This means that the heated surface will have a temperature close to that measured by the Pt100. This will not be the case with dishes with an air gap design.

Wider implications of the findings: This investigation highlights the importance of using the correct type of thermometer probe for the purpose. It is likely that when using a thermocouple Type T probe, temperature in the medium can be 2-3 °C higher than measured, which could impact gametes/embryos and lead to deleterious results.

Trial registration number: Not applicable

Abstract citation ID: dead093.602
P-244 Nucleation errors at four-cell stage reduces chances of live births in single euploid-blastocyst transfers

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Study question: What is the impact of nucleation errors (NE) and morphokinetic parameters on live birth rates in single euploid blastocyst transfers?

Summary answer: Nucleation errors (NE) at the four-cell stage was the only parameter associated with decreased live birth (LB) rates.

What is known already: Morphokinetic studies using time-lapse monitoring (TLM) systems have proposed algorithms to select embryos with the highest implantation potential. However, no single biomarker can reliably predict live-birth so far. Morphokinetics can deselect embryos for Preimplantation-Genetic-Testing for Aneuploidies (PGT-A). However, it is unclear whether morphokinetic markers can be used for selecting embryos with highest chance of LB among the euploid blastocysts. Performing morphokinetics enable detection of features such as nuclear errors (NE) which could be underdiagnosed with intermittent evaluation. It remains to be determined to which extend NE, next to morphokinetic parameters, are associated with LB potential of euploid blastocysts.

Study design, size, duration: Retrospective observational study including 383 single euploid blastocyst transfers in FET cycles between October 2017 to June 2021. These blastocysts were graded ≥BL3CC (Gardner criteria) and underwent TE biopsy for PGT-A by Next Generation Sequencing (NGS). Only ICSI with fresh autologous cycles using ejaculated sperm were considered. Outcomes of euploid blastocyst transfers were recorded and as pregnant (P): biochemical pregnancy (BP) (n = 17), clinical miscarriage (CM) (n = 35), live birth (LB) (n = 205); or not pregnant (NP) (n = 126).

Participants/materials, setting, methods: Patient baseline characteristics (Age, AMH, BMI) and infertility duration were considered. Morphokinetics were annotated with time-lapse-imaging (KID-score) for time (t) of PB2 (tPB2), tPNa (PN-appearance), tPNf (PN-fading), t2-t9, CP (compaction), tM (Morula), tSB (start blastulation), tB (blastocyst) and timings between each developmental event. Nuclear errors (NE) such as micronucleation, binucleation, multinucleation were annotated when present in at least one blastomere at two- and four-cell stages between 19.4-51.4 hours and 22.1-63.1 hours, respectively, post-insemination.

Main results and the role of chance: Median (IQR) values of Age and AMH of female partner, and infertility duration were similar among the P and NP groups. BMI was significantly higher in patients who miscarried with

Median (IQR) values of 28.4kg/m2 (26.1-33.4) vs < 27.9kg/m2 in all other groups (P < 0.01).

Significantly more blastocysts with type A inner cell mass quality resulted in LB (24.9%) than to CM (14.3%), BP (5.9%) and NP (13.5%) (P < 0.001). Concerning TLM morphokinetics evaluation, none of the timed parameters evaluated, neither the timings between the different morphokinetics events resulted in significant differences among the LB and the other P and NP groups. However, LB group had significantly less euploid embryos presenting NE at four-cell stage (7.8%) compared to CM (17.0%), BP (23.5%) and NP (17.5%) (P = 0.02). In a logistic regression model, NE when present at four-cell stage but not at two-cell stage, had a negative impact in LB rates (OR:0.39, CI: 0.2-0.72; P = 0.003).

Limitations, reasons for caution: The retrospective nature of the study and the fact that NE evaluation was performed manually based on TLM videos by a single observer.

Wider implications of the findings: NE-constituted embryos can give rise to euploid blastocysts and have an implantation potential. Since these embryos are associated with lower LB, it is relevant to include NE as an evaluation parameter to deprioritize multinucleated four-cell embryos for transfer, even when they result in euploid blastocysts.

Trial registration number: not applicable

Abstract citation ID: dead093.603

P-245 Birth of twenty-seven healthy babies following transfer of fresh and frozen-thawed embryos derived from monopronuclear zygotes: a retrospective study

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Study question: What are the neonatal outcomes of fresh/frozen-thawed embryos derived from monopronuclear zygotes (1PN)? Is there any influence of the method of fertilization?

Summary answer: A total of 231 monoembryonic transfers (TF) of fresh or frozen/thawed embryos derived from 1PN zygotes allows to the birth of twenty-seven healthy babies.

What is known already: Zygotes were classified 16 to 18 hours post insemination according to the extrusion of the second polar body and the number of pronuclei (PN). The «normally fertilized» zygotes display 2 PN and those with 0, 1 or more than 2 PN (> 2PN) are considered as abnormal. If some zygotes are always discarded (>2PN) due to the increased risk of aneuploidy, the transfer of embryos derived from 1PN zygotes remains more controversial.

Study design, size, duration: Our retrospective study included all conventional in vitro fertilization (IVF), intracytoplasmic sperm injection (ICSI) and frozen embryo transfer (FET) cycles performed between january 2018 and december 2022.

Embryo and pregnancy outcomes were analyzed in a first group of 781 fresh cycles (731 patients) and in a second group of 167 FET (150 patients) with embryos derived from 1PN zygote. Only monoembryonic transfers were analyzed for live birth rate (LBR).

Participants/materials, setting, methods: Only embryos derived from 1PN zygotes were analyzed in our study. For fresh cycles (n = 2762), embryo outcome was analyzed for 1234 (1PN) zygotes (IVF = 648 and ICSI = 586). Fresh embryo TF was performed in 64 cycles. For frozen cycles, TF was achieved in 167 cases. For a total of 231 TF on day 2, 3 or 5, pregnancy and live birth rates were analyzed.

Main results and the role of chance: At first, 46% of 1PN zygotes from fresh IVF cycles gave rise to embryos of sufficient quality to be transferred or frozen (day 3 or 5/6). This rate decreased to 33% in the fresh ICSI cycles. Blastulation rate was also more important in IVF group (44%) in comparison to ICSI group (20%) without signification (p = 0.1).

Fresh TF of 64 patients (32 in each fertilization group) allowed 7 pregnancies in the IVF group (PR = 22%) as compared to 4 pregnancies in the ICSI group (PR = 12%). The 7 IVF pregnancies ended in 4 deliveries of healthy newborns, 2 miscarriages and one pregnancy still ongoing. In the ICSI group, 1 birth of a healthy newborn and 3 miscarriages were observed.

Secondary, thirty-six pregnancies were obtained in the 167 FET cycles. A non significative difference was observed between embryos derived from IVF cycles (PR = 26%) and ICSI cycles (PR = 16%) with respectively 15 and 6 healthy babies born. Two pregnancies in each group are still ongoing.

Limitations, reasons for caution: The main limitation of this study is that it was a retrospective, single-center study with a relatively small number of births.

Wider implications of the findings: In conclusion, we observed better outcomes in IVF cycles in comparison to ICSI cycles and our center policy to transfer a good quality embryo developed from 1PN zygotes allowed 26 deliveries of 27 healthy newborns during the period analyzed.

Trial registration number: not applicable

Abstract citation ID: dead093.604

P-246 Exposure to oral contraceptives alters human endometrial stem cells culture media metabolomics

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Study question: Can oral contraceptives (OCs) influence the metabolism of endometrial stem cells (EnMSC)?

Summary answer: Altered metabolomic profiling in culture media may be an effect of OC hormonal properties on EnMSC metabolism.

What is known already: The reconstitution of the endometrial tissue relies on the presence of EnMSC, which reside in the perivascular space in the endometrium. This process can be affected by OCs by decreasing the secretory potential of the glands. In case of high dose OCs, hyperplasia of endometrial vessels and stroma atrophy of the glands may occur. However, the precise effect of OCs on the production and metabolism of EnMSCs remains unknown.

Study design, size, duration: This was a prospective study that included samples of menstrual blood from 5 volunteers. The study was conducted for one year and received approval from Ethics in Research Committee. Written informed consent was obtained from all participants.

Participants/materials, setting, methods: Five women with regular menstrual cycles were included in two groups according to the use of OCs: OC (n = 2) and non-OC (n = 3). Samples were collected by the menstrual cup on the second day of the menstrual cycle. The culture medium of EnMSC was collected from each passage. Quantitative metabolomics was performed by multiple reaction monitoring, followed by liquid chromatography mass spectrometry. Data was analyzed by PLS-DA and T-test. Potential biomarkers were assessed by ROC curve.

Main results and the role of chance: The cells were characterized as mesenchymal stem cells with a range of 47,4% to 85% of positive markers. From 186 metabolites quantified in the culture media of OC and non-OC groups, 15 metabolites were proposed as of high discrimination between groups by the PLS-DA, which also demonstrated a total groups separation on the statistical model. The ROC curve showed that 4 out of 15 metabolites presented more than 80% of sensitivity. These metabolites are Alanine, Phosphatidylcholine (PC) aa C30:0, Glycine and PC aa C32:2, whose concentrations were higher in the OC group than in non-OC group. The Students' T-Test analysis confirmed that the metabolites with higher discrimination between groups presented significant p values. These metabolites have been associated with several metabolic processes, including energy production and alteration of molecular pathways related to thrombosis and cancer.

Limitations, reasons for caution: This study was conducted with a small number of participants and further studies are necessary to confirm the findings.

Wider implications of the findings: The use of OCs could affect endometrial characteristics that are crucial for reproductive success, such as endometrial receptivity. This study provides a preliminary insight into the EnMSCs response to OCs based on specific metabolite signatures, which may contribute to the development of future new reproductive therapies.

Trial registration number: Foundation for Coordination of Higher Education Personnel (CAPES – Brazil).

Abstract citation ID: dead093.605
P-247 Total Fertilization Failure after conventional IVF: it is not the end of the story

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Study question: Could Rescue IntraCytoplasmatic Sperm Injection (R-ICSI) coupled with frozen transfers be an effective treatment for Total Fertilization Failure (TFF) after conventional InVitro Fertilization (c-IVF) cycles?

Summary answer: Applying R-ICSI after TFF of c-IVF, a blastulation rate of 28% could be obtained, allowing the cycle rescue in a consistent proportion of couples.

What is known already: Conventional IVF is to date the most indicated technique in infertile couples in the absence of male factor. However, the incidence of TFF following c-IVF, in presence of normozoospermic samples, ranges from 5 to 20%. TFF leaves experts with limited alternatives, which include cancelling the cycle with subsequent indication to proceed with ICSI in the next cycle or attempting R-ICSI in the current cycle. R-ICSI coupled with frozen embryo transfer may overcome most of the technical and biological issues associated with fresh transfer after R-ICSI, possibly representing an efficient procedure for couples experiencing TFF following c-IVF.

Study design, size, duration: Our retrospective study included 33 couples who underwent assisted reproduction techniques (ART) at the Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico of Milan from October 2021 to December 2022 and that experienced TFF after c-IVF, regardless the number of oocytes retrieved. Overall, TFF was reported in 7% of c-IVF cycles (33/439). An indication for male factor infertility was an exclusion criterium. World Health Organization (WHO) reference values for human semen characteristics have been adopted.

Participants/materials, setting, methods: Fertilization failure was considered in terms of absence of the two pronuclei and the second polar body. Out of 158 unfertilized oocytes, 128 MII oocytes were microinjected after c-IVF in the same cycle. R-ICSI consists of performing ICSI 22-24 hours after insemination with an unsuccessful c-IVF, using the same semen sample maintained at room temperature overnight. Fertilized oocytes after R-ICSI were cultured to the blastocyst stage according to our standard protocol.

Main results and the role of chance: The fertilization rate was 42% (95%CI: 34-51%). Fertilization failure was still observed in 9 patients (27%, 95%CI: 13-46%) after R-ICSI, with a median value of 2 [2-5] oocytes available for insemination. In the 24 patients who obtained at least one zygote, the blastulation rate was 28% (95%CI: 16-42%). A total of 15 blastocysts were cryopreserved in 10 patients on day 5 or 6 after R-ICSI. Of these 10 patients, 6 have undergone frozen/thawed single embryo transfer, and the success rate (ongoing pregnancy) was 50% (95%CI: 12-88%).

Limitations, reasons for caution: One of the limitations is the small sample size, since this is an emergency procedure. However, the study is still ongoing. Women were not subjected to inclusion criteria, thus fertilization failure could be attributed to several factors different from semen quality.

Wider implications of the findings: This study turns out to be important in light of the interesting implantation rate obtained. With the advantage of reducing both costs and psychological burden of the couple, the technique can be proposed as a rescue strategy in cases of TFF allowing to confidently increase the number of c-IVF procedure.

Trial registration number: not applicable

Abstract citation ID: dead093.606
P-248 The meiotic spindle is preserved during freezing but post-thawing rehydration causes its destabilization in human eggs

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Study question: Is the human egg spindle integrity impaired by vitrification and thawing procedures?

Summary answer: Cryoprotective-caused dehydration stabilizes the MII spindle, but the re-entry of water during thawing induces depolymerization of microtubules, thus promoting instability of the division apparatus.

What is known already: Freezing sperm and preimplantation embryos have become routine IVF procedures with excellent clinical results, whereas cryopreservation of oocytes remains problematic. The major factor underlying the human oocytés notorious propensity to cryoinjury is the temperature sensitivity of the meiotic spindle, the fidelity of which is critical for faithful post-fertilization development. Most studies focus on evaluating vitrified-thawed oocytes' post-fertilization outcome, but behavior of the meiotic spindle during the freezing process has received only a little attention.

Study design, size, duration: The experimental study involved a total of 165 human oocytes donated for research. The presence and morphology of the meiotic spindle were examined by polarized light microscopy (PLM) and fluorescent confocal imaging to map out the MII spindle dynamics during the vitrification-thawing procedure.

Participants/materials, setting, methods: A total of 114 women gave informed consent for their surplus immature oocytes to be used in this project. Oocytes that completed maturation in vitro were subjected to spindle imaging (Oosight) and vitrified using a Kitazato/Cryotop open system. The PLM-positive/negative oocytes were fixed at 6 steps of the vitrification-thawing protocol and 2 hours after thawing (15 oocytes in each subgroup). Microtubules and DNA were fluorescently labeled to inspect chromosome-spindle configuration.

Main results and the role of chance: Time-course experiments showed that PLM-detectable bipolar spindle remained intact in all but one oocyte (44/45) fixed during pre-vitrification equilibration. Notably, the PLM signal became more prominent after adding a cryoprotectant that displaced intracellular water. In contrast, the MII spindle signal progressively disappeared during thawing. Specific tubulin labeling revealed that the microtubule mass was still present in most (41/45) oocytes from the vitrified-thawed sample group. However, the division apparatus tended to lose its bipolarity and disintegrate (8/45) during the washing steps. This trend was accented in the group of oocytes that lacked PLM-detectable spindles before freezing. Here, only a minority of cells (11/45) undergoing rehydration displayed characteristic chromosome-spindle configuration. The difference in eggś capability to ensure spindle bipolarity was apparent even 2 hours after thawing when 13/15 of initially PLM-positive but only 7/15 PLM-negative oocytes exhibited normal-shaped spindles. Based on these data, we hypothesize that the transient disappearance of the PLM spindle signal during warming may be attributed to the restoration of microtubule dynamics and spindle destabilization. The speed and efficiency of spindle reconstruction seem to depend on the overall eggś fitness.

Limitations, reasons for caution: This research study involved a limited number of hormonally-primed oocytes that extruded a polar body in vitro shortly after retrieval. The obtained imaging data represent snapshots of the process. A technically challenging live imaging study is needed to complement the description of the oocyte spindle dynamics during post-vitrification recovery.

Wider implications of the findings: Our results demonstrate that meiotic spindles in frozen-thawed eggs are not products of de novo spindle assembly

but are built on preexisting microtubule mass. Suboptimal handling and reducing the interval between thawing and ICSI may enhance unavoidable spindle instability and thus compromise the developmental potential of vitrified eggs.

Trial registration number: not-applicable

Abstract citation ID: dead093.607

P-249 Exploring the possible impact of single-step culture media refreshment on embryo ploidy: A prospective sibling oocyte study

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Study question: Does refreshing the media on day 3 of development improve rates of blastocyst utilization and euploidy rate?

Summary answer: Refreshing single-step culture medium on day 3 of embryo development does not improve blastocyst euploidy rate, neither does it improve embryo utilization rate.

What is known already: The impact of culture conditions on blastocyst development and aneuploidy is not fully understood. Single-step culture media provide embryos with all the nutrients required throughout the development. The debated practice till today is whether refreshing media during the seven days of culture is required to compensate for possible, even subtle, osmolarity changes, and to replenish nutrients and exchange metabolites, alleviating embryos from a possible toxic environment. Up to date, no study has investigated the effect of using the same type of culture media, refreshing it or not, in a sibling oocyte manner, on blastocyst developmental quality and embryo ploidy.

Study design, size, duration: A prospective sibling oocyte study, conducted between July 2021 and December 2022 in a tertiary IVF center. A total of 1549 metaphase II (MII) oocytes from 93 PGT-A cycles were randomized between the 2 groups. Outcomes were defined as euploid rate, mosaicism rate and blastocyst utilization rate.

Participants/materials, setting, methods: Patients undergoing ICSI and PGT-A with females aged 18-40yrs and BMI<35kg/m^2 who signed the informed consent. MII oocytes were randomized between Group 1(GI): embryos were moved into a newly calibrated culture media only on day 3, 67-69 hours post ICSI (hpi), and Group 2(GII): only on day 5, 114-118hpi. ICSI was performed by the same operator for both groups. Fertilization check was done 18-20hpi and no further assessment was done till day 5.

Main results and the role of chance: Patients and cycle characteristics presented as median (IQR) were female age 31yrs (28-34yrs), BMI 26.5 kg/m2 (23-28.6 kg/m2), AMH 3.4ng/ml (2.1-5.9ng/ml), cumulus-oocyte complex (COC) 21 (14-26) and oocyte maturation rate 0.8 (0.7-0.9). In GI, 775 MIIs and in GII, 774 MIIs were inseminated by ICSI. Fertilization rates were similar in both groups (77.2% vs 78.7%, mean difference: -1.5%, 95% CI: -5.9% to 2.9%, GI vs GII, respectively, P = 0.499). No significant differences were observed in day 5 blastulation rates between groups (66.3% vs 63.9%, mean difference: 2.4%, 95% CI: -3.7% to 8.5%, G1 vs G2, respectively, P = 0.436). Blastocyst utilization rate (blastocysts biopsied per 2PN) were similar between the groups (52.6% vs 50.7%, mean difference: 1.8%, 95% CI: -4.4% to 8.0%, G1 vs G2, respectively, P = 0.567). PGT-A outcomes were defined as euploid, aneuploid or mosaic aneuploid. Overall euploid rates were similar between GI and GII (57.86% vs 59.5%, p = 0.68, respectively), as well as mosaicism rates (7.7% vs 6.8%, p = 0.67, respectively). The rate of blastocysts biopsied on day 5,6 or 7 was comparable between both GI and GII (p = 0.62, 0.83 and 0.59, respectively).

Limitations, reasons for caution: While the outcome of this study did not show improvement in euploid rate or blastocyst utilization rate by refreshing the culture medium on day 3, the results may not be reproducible in other laboratories applying different protocols since culture conditions and protocols may differ among laboratories.

Wider implications of the findings: The fact that there are no significant differences in blastulation and euploid rates between the groups, supports the stability of culture conditions without the need for media refreshment and allows for reducing embryo manipulation, workload and costs adhered by culture medium refreshment on day 3.

Trial registration number: NCT04969575

Abstract citation ID: dead093.608

P-250 The exclusive role of sperm DNA fragmentation on the clinical outcome of a sibling donor oocyte cohort

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Study question: Can sperm chromatin fragmentation (SCF) overwhelm the oocyte repair mechanism of healthy, young oocytes and impair embryo development and clinical outcome?

Summary answer: Elevated SCF, with its double-stranded DNA (dsDNA) component, remarkably impairs clinical outcome, despite oocyte repair mechanisms present in a sibling donor oocyte cohort.

What is known already: Despite a normal semen analysis, approximately 10-20% of males of reproductive age still have a subtle infertility. Therefore, conventional semen analysis is insufficient to identify the specific issues related to sperm function and its embryo developmental competence. Indeed, SCF with its dsDNA break component, is known to be associated with poor clinical outcome such as poor embryo development and pregnancy loss, by possibly increasing structural chromosomal abnormalities. Although a healthy oocyte may address certain levels of SCF, these oocyte repair mechanisms are incapable of repairing dsDNA breaks.

Study design, size, duration: Thirty-one couples were divided according to the degree and type of SCF, whether normal or abnormal, and shared the same sibling donor oocytes. The male partners in both groups had comparable semen parameters. Total SCF and dsDNA fragmentation were assessed in all men. Fertilization, implantation, clinical pregnancy (CPR; +FHB), delivery, and pregnancy loss rates were compared between the two cohorts.

Participants/materials, setting, methods: A total of 31 infertile couples underwent ICSI cycles utilizing split sibling donor oocytes due to advanced maternal age or premature ovarian insufficiency. Oocytes from the same donor were equally allocated among 2 couples.

Semen analyses were performed according to WHO 6th edition. Overall SCF was assessed by TUNEL with a ≤ 15% normal threshold. Double-strand DNA (dsDNA) fragmentation was assessed by neutral comet assay with ≤3% considered normal.

Main results and the role of chance: A total of 18 male partners (44.3 ± 7yrs) with a normal SCF of 8.9 ± 2% had their spermatozoa injected into sibling donor oocytes, while 13 male partners with abnormal SCF at 25.4% ± 11 ($P<0.001$) had their spermatozoa injected into the remaining half. In the latter, the average dsDNA fragmentation rate was 3.6%, while in the former it was 0.3% ($P<0.001$). Sibling donor oocytes injected with normal SCF resulted in a fertilization of 87.4% (146/167), implantation of 56.4% (22/39), +bHCG of 69.0% (20/29), CPR of 62.1% (18/29), and delivery rate of 51.7% (15/29). The other half of the sibling oocytes, injected with spermatozoa with elevated SCF, resulted in a fertilization of 70.8% (97/137) ($P<0.001$), implantation of 15.4% (4/26) ($P<0.01$), +bHCG of 42.1% (8/19), CPR of 21.1% (4/19) ($P<0.01$), and delivery rate of 10.5% (2/19) ($P<0.01$). The pregnancy loss in cycles utilizing the oocyte cohort injected with spermatozoa with normal SCF was 8.7% (2/23), while the pregnancy loss in those injected with spermatozoa with elevated SCF was 50.0% (2/4).

Limitations, reasons for caution: This preliminary study indicates that SCF and dsDNA fragmentation negatively affects clinical outcome, overwhelming oocyte repair mechanisms. Nonetheless, the study has a limited number of observations and needs to be validated in a larger cohort.

Wider implications of the findings: SCF, with its dsDNA fragmentation component, seems to contribute a higher incidence of chromosomal abnormalities. The compounded aneuploidy of male and female gametes may

explain the compromised embryo development and higher pregnancy loss rate observed in infertile couples with apparently normal semen parameters and younger oocytes.

Trial registration number: not applicable

Abstract citation ID: dead093.609

P-251 Differences in fertilization, blastocyst, and ploidy rates in intracytoplasmic sperm injection (ICSI) versus conventional insemination for patients undergoing in vitro fertilization (IVF)

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Study question: Are there differences in fertilization, blastocyst development, or euploidy rates when comparing ICSI versus conventional insemination among patients with male and non-male factor infertility?

Summary answer: Fertilization rate was lower for male factor ICSI. There were no differences in blastocyst development. Euploidy rate was lower for non-male factor ICSI.

What is known already: ICSI was developed for male factor infertility due to its requirement for a very small number of viable sperm. Its use has expanded beyond male factor due to some studies suggesting enhanced fertilization, and lower risks of failed fertilization or inadvertent contamination of embryo biopsy specimens. There are potential risks associated with ICSI, including imprinting disorders, birth defects, and significantly increased cost. It is also unclear whether ICSI contributes to genetic abnormalities by bypassing the egg's natural sperm selection and potentially disrupting the meiosis apparatus.

Study design, size, duration: This retrospective cohort study of 576 patients was conducted at a single clinic from January 2021 to December 2021. Patients were grouped into (1) ICSI with male factor (n = 201), (2) ICSI with non-male factor (n = 160), and (3) conventional insemination with non-male factor (n = 215). Primary outcome was ploidy status, determined by calculating the percentage of euploid, aneuploid, and mosaic (both low and high mosaic) among the three groups. Secondary outcomes included fertilization and blastocyst development rates.

Participants/materials, setting, methods: Patients undergoing autologous IVF at a single clinic, with PGT-A performed at a single lab were included. Fertilization rate was defined as the percentage of 2PNs per number of mature eggs injected/inseminated. Blastocyst rate was defined as the percentage of blastocysts per 2PNs. Ploidy rate was defined as the percentage of euploid/aneuploid/mosaic blastocysts per total biopsied blastocysts for which a result was obtained. Percentage data was compared using N-1 Chi-squared test.

Main results and the role of chance: There were no significant differences in patient age, number of oocytes retrieved, or number of mature oocytes among the three groups.

Fertilization rate for male factor ICSI was significantly lower compared to both non-male factor ICSI (74.2% vs 77.8%, p = 0.005) and conventional insemination (74.2% vs 76.8%, p = 0.018). There was no significant difference in fertilization rate between non-male factor ICSI and conventional insemination.

There were no significant differences between blastocyst rates on Day 5, 6, or 7, or total blastocyst rate among the three groups.

The euploidy rate for conventional insemination was significantly higher than that for non-male factor ICSI (53.4% vs 46.8%; p = 0.008). Non-male factor ICSI yielded lower euploidy rate than male factor ICSI (46.8% vs 52.4%, p = 0.034). Aneuploidy rate for non-male factor ICSI was significantly higher than both male factor ICSI (36.1% vs 31.0%, p = 0.041) and conventional insemination (36.1% vs 31.0%, p = 0.030).

Mosaicism levels were similar between the three groups.

There were no significant differences in number of biopsied embryos with no results: 5 for ICSI male factor group (0.59%), 6 for ICSI non-male factor (0.96%), and 10 for conventional insemination (0.89%).

Limitations, reasons for caution: This was a retrospective analysis of patients from a single clinic, with a relatively small sample size so results may not be generalizable to all populations. Extensive demographic information and baseline characteristics (including reason for ICSI performed among non-male factor patients) were not analyzed so there may be confounding factors.

Wider implications of the findings: This is the first study that we are aware of to investigate the ploidy status of embryos created among non-male factor patients undergoing ICSI. Conservative use of ICSI can significantly decrease the cost of IVF. Larger studies are needed to further elucidate the role for ICSI among these patients.

Trial registration number: not applicable

Abstract citation ID: dead093.610

P-252 A roadmap to normal human embryo development – a timelapse study of embryos resulting in the live birth

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Study question: What are the morphokinetic parameters of preimplantation development in normal human embryos?

Summary answer: We provide a timeline of early developmental events and the incidence of embryo morphological features compatible with childbirth.

What is known already: Timelapse embryo monitoring provides unique insight into early human development. Implementation of this technique in clinical practice allows for assessing developmental dynamics for improved embryo selection. Several markers and ranking algorithms have been proposed to predict blastulation or implantation outcomes, but only a few studies assessed time-lapse data with live birth as the primary endpoint. Therefore, the comprehensive morphokinetic profile of embryos that gave rise to live birth has not been defined.

Study design, size, duration: Retrospective observational analysis of 300 time-lapse records of IVF embryos known to develop into healthy newborns. Out of total 73 quantitative/qualitative signs annotated, we focused on 9 developmental events and 14 morphological features and evaluated their timing, duration, and incidence. The influence of potential confounding factors (i.e., egg age, donor egg, donor sperm, sperm quality, and sex of the child) was evaluated by advanced statistical methods.

Participants/materials, setting, methods: Embryo monitoring was performed by a GERI-timelapse incubator. Image acquisition of individual embryo development lasted 5-6 days, starting 10 minutes after ICSI, which was used as reference time point. Transmitted light images were automatically captured in 5-minute intervals and 7-11 focal planes. The embryo selection for transfer was based solely on its morphology. The annotation data represent the consensus of minimum 3 experienced embryologists. Generalized linear models were used to explore relationships between dataset variables.

Main results and the role of chance: The average age of female participants was 27.5 years; 71.67% of embryos were derived from young donor eggs without fertility disorders. The severe andrological factor was present in 8% of male patients; donor sperm was used in 23% of cases; 16% of embryos were derived from dysmorphic oocytes. Both sexes were equally represented among analyzed embryos. Our analysis showed that early development was characterized by remarkably short first mitosis (2.68 ± 0.73h) and the interval between the second and third division (t4-t3 = 0.65 ± 0.66h), whereas the third cleavage was less synchronous (t8-t5 = 5.46 ± 4.9h) No association between quantitative signs and confounding factors was found. Interestingly, 2-cell stage embryos often contained multiple nuclei in at least one blastomere (51%), but multinucleation was reduced at the 4-cell stage (8%). Multipolar divisions were absent during zygotic division and rare (2.3%) during the second cleavage. Preterm and partial compaction was observed in 26.6%

and 34% of embryos, respectively. Importantly, we noticed that 11.6% of embryos in our dataset deviated from conventional scenario of cleavage dynamics. These "atypical" embryos were characterized by a significantly higher incidence of multipolar divisions during the second cleavage compared to "typical," regularly dividing embryos. Additionally, the odds of partial compaction increase 10 times in "atypical" embryos.

Limitations, reasons for caution: The presented analysis only involved embryos confirmed to result in a live birth after transfer. The comparison with data from embryos that did not produce pregnancy was not performed because it would be impossible to discriminate between the role of the embryo and endometrial factors in implantation failure.

Wider implications of the findings: Human embryos may develop into healthy children despite irregular cell division. A high incidence of partial compaction in "atypical" embryos suggests a self-correction mechanism rescuing their developmental potential. A larger-scale analysis of features compatible with live birth is needed to improve embryo selection criteria for evidence-based decision-making.

Trial registration number: not applicable

Abstract citation ID: dead093.611
P-253 Clinical efficacy of PGT-A according to maternal age and embryo quality in blastocyst stage

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Study question: To establish the usefulness of PGT-A as an embryo selection tool based on the quality of the blastocyst and the age of the patient.

Summary answer: The euploidy rate of good quality blastocysts decreases dramatically with increasing maternal age.

What is known already: One of the fundamental objectives to achieve an optimal gestation rate is the selection of the embryo with the highest implantation potential. For this purpose, morphokinetics and aneuploidy detection are used. Most aneuploidies arise in maternal meiosis, and increase exponentially in women over the age of 35 years, coinciding with the rapid decline in IVF success and live birth rates in patients of advanced maternal age.

Study design, size, duration: In the present retrospective study, embryo quality of 321 blastocysts was evaluated from June 2020 to January 2023. They were analyzed according to the ASEBIR (2015) classification and using preimplantation genetic testing for aneuploidy (PGT-A) with next-generation sequencing (NGS).

Participants/materials, setting, methods: A total of 321 blastocysts were analyzed using PGT-A from which 101 were euploid. This 101 blastocysts were classified according to the morphokinetic (A or B (good quality) and C (medium quality)) and the maternal age (<35, 35-36, 37-38, 39-40, 41-42, 43-44 and 45 -46 years). Out of the 101 euploid blastocysts, 78 embryos were classified as A or B (good quality) and 23 as C (medium quality) according to the ASEBIR classification.

Main results and the role of chance: The euploidy rate of the embryos decreases as the age of the patient increases, being 73% for good quality embryos in patients under 35, 54% in patients between 35-36, 37% in patients between 37- 38, 31% in patients between 39-40, 23% in patients between 41-42, 5% in patients between 43- 44 and 0 % in patients between 45-46 years. For medium quality embryos, the euploidy rate is 58% in patients under 35, 38% in patients between 35-36, 30% in patients between 37-38, 26% in patients between 39-40, 0% in patients between 41-42, 0% in patients between 43-44 and 0% in patients between 45- 46 years.

In patients under 35 years, PGT-A will improve pregnancy rates considering that it is an invasive and costly technique. In patients between 35-36 years, PGT-A is a very useful tool when embryos have C quality as well as, in patients between 37- 40, regardless of embryo quality. Furthermore, patients between 41-42 years with good quality embryos, PGT-A will decrease the abortion rates and increase the pregnancy rates. In contrast, patients with C-quality embryos should not start new cycles, as well as patients with over than 43 years due to the low probability of euploidy.

Limitations, reasons for caution: Studies of larger sample size and study period are required to validate our current findings. Furthermore, more information about fertilization, pregnancy, birth rate and perinatal outcomes could improve the current results.

Wider implications of the findings: Making the decision whether or not to use PGT-A on the day of blastocyst formation helps to choose the best strategy by individualizing each case according to the maternal age and the quality of the blastocysts. Decreasing cycle times will always be beneficial to patients and their outcomes.

Trial registration number: Does not apply

Abstract citation ID: dead093.612
P-254 Short-time or overnight co-incubation with sperm in conventional IVF delivers similar pre-implantation development outcomes: a sibling cumulus-oocyte complex study

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Study question: Does a short-time co-incubation in conventional IVF improve rates of fertilization, embryo development and euploidy, when compared to overnight co-incubation?

Summary answer: There is no significant difference in fertilization, embryo development and euploidy rates between sibling oocytes subjected to short co-incubation versus overnight co-incubation.

What is known already: Conventional IVF usually implies coincubation of cumulus-oocyte complex(es) with sperm for an overnight period. This overnight incubation might create an over exposure of COCs to reactive oxygen species (ROS), which might disturb oocyte homeostasis, and impact on embryonic developmental rate. To this extend, many early studies have compared a short co-incubation time (2-6 hours) with overnight co-incubation, with reported conflicting outcomes. None of the studies combined time-lapse monitoring with short co-incubation, which allows the identification of early morphokinetic events of the inseminated oocytes after conventional IVF, as already known for ICSI oocytes in time-lapse incubators.

Study design, size, duration: This single center prospective study included 604 COCs in 38 IVF/ICSI cycles with preimplantation genetic testing between December 2020 and November 2022. After oocyte retrieval a min of 6 COCs were assigned for insemination by IVF and equally divided between short(2hrs) and overnight (16-20hrs) co-incubation. Supernumerary oocytes were denuded and inseminated by ICSI. Primary endpoint was fertilization rate; secondary endpoints were maturation rate, ploidy rate after trophectoderm biopsy, embryo development and morphokinetics.

Participants/materials, setting, methods: Couples with a female age between 18-43 (average:34.4) years, BMI $\leq$30kg/m^2 and normal semen parameters (WHO) in the fresh ejaculate on the day of OR, were eligible for the study. After IVF, all embryos were cultured in a time-lapse imaging system in Global Total LP media. Blastocysts ($\geq$BI3BCC) were subjected to trophectoderm biopsy on day 5-7 and next generation sequencing (NGS) to determine blastocyst ploidy status.

Main results and the role of chance: In total, 288 oocytes (47.7%) were denuded for ICSI, whereas 316 (52.3%) oocytes were randomized 1:1 to short and overnight co-incubation groups. Maturation rates (M2/COC) and

normal fertilization rates (2PN/COC) in short and overnight co-incubation groups were similar (88.0% vs 83.5%, P = 0.074 and 60.8% vs 62.0%, P = 0.193, respectively). Day-3 embryo cell numbers (P = 0.383), fragmentation rates (P = 0.529), blastulation rates on day 5 (39.9 vs 38.0% P = 0.696), time to blastulation (median: 112.6 vs 111.1 hours after insemination by time lapse, P = 0.544) were similar between short and overnight co-incubation groups. In total, 48 embryos in short and 45 embryos in overnight co-incubation groups were of sufficient quality for biopsy resulting in similar euploidy rates between the groups (39.6 vs 44.4%, P = 0.791).

When compared to their siblings in ICSI, short (mean difference: -6.48%, 95% CI: -18.5 to 5.59%, P = 0.290) and overnight co-incubation (mean difference: -0.87%, 95% CI: -12.9 to 11.2%, P = 0.886) attained similar fertilization rates per cycle. No significant differences regarding blastulation and euploidy rates were observed in short or overnight co-incubation compared to ICSI in unadjusted and adjusted analyses.

Limitations, reasons for caution: This is a prospective study with a limited patient population with a normal ovarian response. Results might not be transferable to patients with high or low ovarian reserve.

Wider implications of the findings: The present study demonstrated that there is no difference between short and overnight co-incubation in terms of fertilization and euploidy rates. Therefore short co-incubation might allow us to benefit from early denudation, and to investigate early morphokinetics in time lapse monitoring, which are otherwise missed by overnight co-incubation.

Trial registration number: NCT04627545

Abstract citation ID: dead093.613
P-255 Increased time in thawing solution improves human oocyte warming outcomes

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Study question: Can increased time in thaw solution improve oocyte survival and outcomes following vitrification/warming?

Summary answer: Exposing vitrified oocytes to the first step thawing solution for 90 seconds improved oocyte survival compared to 60 second exposure.

What is known already: Oocytes are a sensitive cell type and careful handling of the cells is required to obtain high outcomes following vitrification and warming. Carefully timed exposure to various vitrification solutions is required to avoid toxicity and ensure proper vitification. Similarly, proper and carefully timed exposure to warming solutions is required to avoid oocyte damage upon warming. Recent reports suggest a modified and rapid warming protocol may be beneficial for vitrified blastocysts. However, oocytes are unique cells and may require unique considerations to optimize warming.

Study design, size, duration: Retrospective analysis of donor oocyte warming outcome data within a single IVF lab over a 12 month period comparing a 60 second first step during oocyte warming (control n = 637 oocytes) versus a very consistent 90 second exposure (n = 672 oocytes) to the first step thawing solution.

Participants/materials, setting, methods: Human donor oocytes were vitrified using the standard Kitazato protocol with ES drop merging and loading on CryoTop devices using the wicking method prior to plunging into liquid nitrogen. Warming was performed using the standard protocol of 60 seconds in thaw solution (TS) or 90 seconds exposure. All cycles utilized ICSI and excluded testicular sperm samples. Survival, fertilization and blastocyst development were compared between the two exposure groups. Data were analyzed utilizing Fisher's Exact Test.

Main results and the role of chance: Oocyte survival differed between the two timing treatments, with exposure to TS for 90 seconds yielding higher oocyte survival (96.1%) than 60 secondsTS exposure (92.0%), $p < 0.002$. No differences in fertilization following ICSI (78.3.0% vs. 79.0%) orin good quality blastocyst conversion (≥3BB) on day 5 (22.1% vs. 25.1%) or by day 7 (55.3% vs. 55.7%) were apparent. The aneuploidy rate of blastocysts were similar, with 28.5% in the 90 second TS treatment group and 27.3% in the 60 second TS exposure group.

Limitations, reasons for caution: Data were examined retrospectively and sibling oocyte splits were not utilized. Clinical outcomes following resulting embryo transfer were not examined.

Wider implications of the findings: These data may help improve oocyte warming protocols and resulting outcomes.

Trial registration number: not applicable

Abstract citation ID: dead093.614
P-256 Large for gestational age in singletons born following frozen embryo transfer: due to freezing technique or to endometrial preparation protocol? Lessons from the French registry

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Study question: Is large for gestational age (LGA) observed in singletons born following frozen embryo transfers (FET) either due to freezing technique or to endometrial preparation protocol?

Summary answer: Artificial cycles were associated with a higher rate of LGA when compared to ovulatory cycles, whereas no difference was observed between the freezing techniques.

What is known already: Several studies compared neonatal outcomes after fresh embryo transfer (fresh ET) and FET, and showed that FET was associated with improved neonatal outcomes, including reduced risks of preterm birth, low birth weight and small for gestational age (SGA) when compared to fresh ET. However, these studies also revealed an increased risk of LGA after FET. The underlying pathophysiology remains unclear; parental infertility, laboratory procedures including embryo culture conditions and freezing-thawing processes, as well as endometrial preparation treatments might be at play.

Study design, size, duration: A multicentric epidemiological data study was performed through a retrospective analysis of the standardized individual clinical records of the French national registry of in vitro fertilisation (IVF) from 2014 to 2018, including deliveries resulting from fresh ET and FET that were prospectively collected in fertility centres. Complementary data were collected from the participating fertility centres including the vitrification media and devices, as well as the endometrial preparation protocols.

Participants/materials, setting, methods: Data were collected from 35 French fertility centres, leading to the inclusion of a total of 72,789 fresh ET, 10,602 slow-freezing FET and 30,062 vitrification FET cycles. Fetal growth disorders were defined in liveborn singletons according to gestational age and sex-specific weight percentile distribution: SGA and LGA if $< 10^{th}$ and $> 90^{th}$ percentile, respectively. Analyses were performed using linear mixed models with the centres as random effect.

Main results and the role of chance: Among a total of 26,585 liveborn singletons, 16,413 babies were born from fresh ET, 1,644 from slow-freezing FET and 8,528 from vitrification FET. Birthweight was significantly higher in the FET groups compared to the fresh ET group, with no difference between the two freezing techniques. Likewise, LGA rates were higher and SGA rates were lower in the FET groups compared to the fresh ET group (12.0% *vs* 6.4%, $p < 0.001$, and 7.8% *vs* 13.4%, $p < 0.001$ respectively). In a multivariable analysis, the risk of LGA following FET was not different between stimulated and natural cycles (adjusted odds ratio: 1.06, 95% confidence interval: 0.83-1.35, p = 0.64) but was significantly increased in artificial compared to natural

cycles (aOR 1.36 [1.11-1.67], p = 0.003). On the contrary, the risk of LGA was not associated with either the freezing mode (slow-freezing vs vitrification) or the embryo stage (cleaved embryo vs blastocyst). When focusing on vitrification, the risk of LGA was not associated with either the freezing medium used or the embryo stage, and no difference was observed according to the vitrification device.

Limitations, reasons for caution: Most of the vitrification techniques were performed using the same device, and with two major vitrification media, limiting the interpretability of the comparison of the risk for LGA according to the device or the vitrification media.

Wider implications of the findings: Our results seem reassuring regarding potential fetal growth disorders following embryo vitrification in comparison to slow-freezing. Even if other factors may be involved, the impact of the endometrial preparation treatments seems to prevail for LGA risk following FET. FET during ovulatory cycles could minimize the risk for fetal growth disorders.

Trial registration number: N/A

Abstract citation ID: dead093.615

P-257 Transcriptomic signature of live birth revealed in cumulus cells of women undergoing an ICSI cycle for infertility treatment

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Study question: Is the transcriptome of cumulus cells surrounding an oocyte that was fertilised and transferred predictive of live birth?

Summary answer: Live birth is associated with a distinct transcriptomic signature in cumulus cells isolated from an oocyte that was fertilised and transferred.

What is known already: The selection of the best embryo for transfer is currently based on standard morphological assessment, which is intrinsically subjective, and time-lapse monitoring, which fails to show benefit in predicting pregnancy. Alternative non-invasive selection methods are in development including analysis of cumulus cells (CCs) isolated from oocytes that resulted in an eligible embryo for transfer. However the majority of the existing studies were conducted using outdated methodologies such as transcriptomic analysis with PCR and microarrays. Moreover, the studies associating CCs' transcriptome with clinical and embryological outcomes lack consensus due to non-homogenous cohorts while the outcome of live birth is inadequately explored.

Study design, size, duration: CC samples (n = 17), collected between 2018 and 2021 and biobanked, were retrospectively selected for RNA sequencing on the basis of their embryo transfer outcome. Thus, we analysed CCs associated with an oocyte that resulted in pregnancy (n = 6), no pregnancy (n = 7), live birth (n = 5) and pregnancy without live birth (n = 5). Differential gene expression analysis identified the differentially expressed genes (DEGs) between the groups in relation to pregnancy and live birth.

Participants/materials, setting, methods: The samples were retrieved from subfertile couples undergoing controlled ovarian stimulation and intracytoplasmic sperm injection with subsequent unbiopsied fresh or frozen embryo transfer at the IVF centre of University Hospital Zurich. Maternal age was below 43 years. Libraries from CCs were prepared using the Smart-seq2 pipeline and the RNA sequencing was performed on the NovaSeq 6000 system. The DESeq2 workflow was conducted and the threshold for the adjusted p-values (FDR) was set to 0.05.

Main results and the role of chance: No DEGs emerged when comparing the transcriptomic profiles of cumulus cells between the "no pregnancy" and "pregnancy" groups. However, 139 DEGs were identified in the comparison between the "pregnancy without live birth" and "live birth" groups. 28 DEGs were familiar and relevant to competent cumulus-oocyte-complexes: *CTGF* (fold change: 4.38, FDR: 0.005), *SERPINE2* (fc: 3.08, FDR: 0.0002), *PCK1* (fc:3.04, FDR: 0.0001), *HHIP* (fc:2.69, FDR: 0.006), *HS3ST* (fc:2.45, FDR: 0.009) and *BIRC5* (fc: -2.69, FDR 0.0001) among others. Functional enrichment analysis of DEGs revealed that the incidence of live birth is associated with promotion of pathways such as runt-related transcription factor 1 (RUNX1/2) signalling, glycosaminoglycan biosynthesis and sonic hedgehog signalling (SHH), and reduction in pathways such as glycosphingolipid biosynthesis and pyrimidine metabolism in cumulus cells.

Limitations, reasons for caution: We reveal the transcriptome associated with live birth in CCs analysed with RNAseq on a limited number of samples. Consolidation of these findings via utilisation of a validation prospective cohort is pending.

Wider implications of the findings: CCs' transcriptome could inform the development of non-invasive biomarkers to predict the chance of live birth across a group of embryos eligible for transfer. The transcriptomic findings inspire further explorations in the field of cumulus-oocyte bidirectional communication and establishment of oocyte competence.

Trial registration number: N/A

Abstract citation ID: dead093.616

P-258 In-vitro oocyte maturation (IVM) in the ART clinic – What can we learn from our failed cycles?

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Study question: Why do some IVM cycles remain unsuccessful? Which factors contribute to failure in producing a usable embryo in IVM cycles?

Summary answer: Failure to obtain usable embryos after IVM was associated with oocyte dysfunction, inappropriate patient selection and unanticipated low oocyte yield.

What is known already: In-vitro maturation of oocytes retrieved from antral follicles has been offered as a mild-ART-approach to subfertile women with increased functional ovarian reserve, ovarian resistance to gonadotropins and in the context of fertility preservation.

When using monophasic IVM culture systems, oocyte maturation, embryological development and pregnancy outcomes are generally lower compared to conventional ART (cART). Women who yield high numbers of cumulus oocyte complexes from a single egg retrieval procedure, such as those with polycystic ovary syndrome (PCOS), have been considered the best candidates for IVM, to compensate for the lower maturation rate and lower embryo yield from IVM cycles.

Study design, size, duration: This was a single-tertiary center, retrospective cohort study between May 2018 and February 2022 including 277 patients: 53 patients with failed IVM and 224 patients with a successful IVM cycle (inter-patient analysis). All ART cycles from patients with one failed IVM were investigated (intra-patient analysis). Failed IVM cycles were grouped: (i) ≤20% oocyte maturation; (ii) fertilization failure; (iii) embryo development failure. Patients with FSH resistance, fertility preservation or egg donation were excluded.

Participants/materials, setting, methods: Only patients with AMH >2.27 ng/ml were included. Oocytes retrieved in minimally stimulated non-hCG triggered IVM cycles were matured in a monophasic IVM system. Embryos were vitrified at cleavage stage and transferred in a hormonally substituted (HRT) cycle. Baseline patient characteristics, endocrinology and embryological data were analyzed. Intra-patient analysis was done using all ART cycles (failed IVM, IVM and cART) performed in one patient.

Main results and the role of chance: Inter-patient analysis revealed similar age, BMI and free testosterone, whereas AMH was lower in patients with a failed cycle (8.8 ± 3.5 vs 10.9 ± 6.4 ng/mL, p = 0.03). As expected, maturation, fertilization and useable embryos differed significantly in failed vs successful cycles: respectively 31% vs 48%; 40% vs 65%; 0% vs 50%; p < 0.0001.

Out of 53 patients with a failed IVM cycle, 6 had oocyte maturation problems, 11 had failed fertilization and 36 had impaired embryological development.

Intra-patient analysis revealed that in the failed oocyte maturation group, 2/6 patients had maturation problems that persisted in cART cycles, indicating oocyte dysfunction. Similarly, recurrent fertilization failure was noted in 3/11 patients, due to insufficient sperm quality (n = 2). In 8/36 patients, insufficient embryo quality was observed both after IVM and cART. Hence 13/53 (24.5%) failed IVM cycles were attributed to intrinsic patient characteristics. A subgroup of patients with a failed IVM cycle had normal maturation (3/6), fertilization (7/11) or good embryos (18/36) in other IVM or cART cycles. Factors associated with cycle failure included technical issues during egg retrieval resulting in unanticipated low oocyte yield (n = 14/40), and suboptimal inclusion criteria (no PCOS, n = 3/40).

Limitations, reasons for caution: This was a retrospective study and holds the possibility of unmeasured confounding factors.

Wider implications of the findings: Risk factors for IVM failure should be identified (e.g. non-PCOS, poor operator experience) and any underlying intrinsic patient conditions should be managed within a multi-disciplinary team. Optimal patient selection is key. Analysis of failed cycles helps clinicians to estimate the prognosis of future ART cycles in women with elevated AMH.

Trial registration number: not applicable

Abstract citation ID: dead093.617
P-259 Intracytoplasmic sperm injection conducted within a micro 3D printed device that requires no holding pipette or vacuum reduces porcine oocyte invagination during microinjection

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Study question: Can the use of a custom-designed and 3D printed microICSI™ device reduce the degree of oocyte invagination during intracytoplasmic sperm injection (ICSI)?

Summary answer: Invagination was significantly less in porcine oocytes when ICSI was performed in microICSI™ device compared to conventional ICSI.

What is known already: ICSI is a difficult procedure for embryologists to master, yet until recently, technically little had changed. In particular, the holding pipette (HP) with vacuum to hold the oocyte has not been surpassed. However, a source of shear-stress during ICSI of oocytes is the invagination of the zona pellucida and cytoplasm that occurs during microinjection, due to the pressure of the injection pipette against the small surface area of the HP. A recent study demonstrated microinjection within a two-piece 3D printed device. Here we evaluated a design refinement that displaced injection pressure more evenly across the oocyte to improve embryo quality.

Study design, size, duration: A control (conventional ICSI) and study group (microICSI™) were compared over three replicates investigating the level of invagination (3-point score, 1 is lowest and 3 is greatest) during ICSI. Incidence of cytoplasmic lysis was also recorded 24 h following ICSI.

Participants/materials, setting, methods: Porcine oocytes collected by follicle aspiration of abattoir-sourced ovaries were matured for 39-44 h in NCSU-17 medium supplemented with eCG, hCG, porcine follicular fluid, EGF and insulin. Mature oocytes, stripped of cumulus, were randomly allocated to either conventional ICSI or microICSI™. ICSI was performed, with treatment order randomised, and the injected oocytes were cultured in NCSU-17 culture medium at 39 C.

Main results and the role of chance: The microICSI™ device was modelled as a one-piece structure with contoured surfaces within a microwell array to support the oocyte during injection without the requirement for a holding pipette. Two-photon polymerisation 3D micro printing was used to print and assess multiple prototype geometries to optimise the support of the oocyte during ICSI. Over the three replicates (n = 10-20/treatment/replicate), the degree of invagination was consistently lower in the microICSI™ group compared with conventional ICSI (Mean ± SEM): Replicate 1: 1.40 ± 0.16 vs. 2.00 ± 0.19; Replicate 2: 1.20 ± 0.11 vs. 2.00 ± 0.13; Replicate 3: 1.42 ± 0.12 vs. 1.78 ± 0.15, with a paired t-test showing a significant difference between the two groups ($p = 0.035$). The incidence of lysis was not significantly different between the two treatments (ranging from 15-20% across both groups and varied between replicates).

Limitations, reasons for caution: The use of a porcine ICSI model may not sufficiently replicate what occurs with human ICSI as human oocytes experience greater levels of invagination (typically scored out of 4 rather than 3).

Wider implications of the findings: Reducing the invagination due to the injection process may reflect a less stressful procedure, with improvement to fertilization rates and embryo quality.

Trial registration number: not applicable

Abstract citation ID: dead093.618
P-260 A more physiological approach to support oocyte activation in cases with fertilization failure

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Study question: Can we use a physiological compound to trigger oocyte activation in couples undergoing ICSI with history of fertilization failure due to sperm activating factor deficiency?

Summary answer: In a pilot sibling oocyte study, oocyte activation using recombinant human phospholipase C-zeta (rhPLCζ) yielded a comparable fertilization compared to conventional method using ionomycin.

What is known already: ICSI overcomes most forms of male gamete defects, however partial or total fertilization failure still occurs regardless of adequate semen parameters and optimal oocyte maturity. This has been attributed to the lack of a cytosolic factor identified as PLCζ. To address this, various approaches of assisted oocyte activation have been proposed, including gamete exposure to chemical or electrical stimuli. The most popular approach remains to be ionomycin. Despite its apparent safety, this stimulus is unnatural and may be aggressive on the oocytes. Therefore, we propose a more physiological approach by utilizing a recombinant form of this specific enzyme.

Study design, size, duration: In the past 2 months, couples with poor fertilization at their initial ICSI cycles were identified and counseled for assisted oocyte activation (AOA) in the subsequent cycle after confirmation of a PLCζ deficiency. Oocytes were separated into 2 equal cohorts activated either by ionomycin or co-injection with recombinant human rhPLCζ during ICSI. Fertilization rates between ionomycin-activated and rhPLCζ-activated cohorts were compared, as well as with the couples' history cycles.

Participants/materials, setting, methods: Six couples with suboptimal fertilization outcomes consented for the study (IRB 0712009553) and were offered AOA with their subsequent ICSI cycles. Conventional AOA was carried out by exposing post-ICSI oocytes to 50 mM ionomycin. The rhPLCζ had a confirmed >95% purity. Our physiological rhPLCζ-activation method was performed by co-injecting spermatozoa with 0.4pL of a rhPLCζ (5mg/mL in glycerol) during ICSI.

Main results and the role of chance: Couples (n = 6, female age: 36.2 ± 6yrs, male age: 38.0 ± 7yrs) had negative infertility workups with normal semen parameters (3.3 ± 3 mL volume, 42.1 ± 35 x10^6/mL concentration, 30.8 ± 23% motility, 2.0 ± 1% normal morphology). They underwent 7 unsuccessful ICSI cycles with 20.0% (12/60) fertilization rate and yielded a 91.7% cleavage rate, of those, 3 were transferred at cleavage stage but did not yield clinical pregnancy. A total of 2 blastocysts were obtained but were all aneuploid.

In their subsequent treatment cycles, overall AGT achieved a significantly improved fertilization rate of 51.6% (32/62) ($P<0.001$). We then compared the efficiency between the two AGT methods in equally distributed sibling oocyte cohort. Although mathematically insignificant, we observed a trend that rhPLCζ-activation outperforms ionomycin-activation in total activation

(67.9% vs 50.0%) as well as fertilization (57.1% vs 47.0%). The new rhPLCζ-activation method yielded a comparable embryo cleavage rate at 87.5% compared to the conventional method at 81.3%. Although fresh embryo transfer at cleavage stage did not yield pregnancy rate yet, rhPLCζ-activated cohort had one euploid blastocyst while the ionomycin-activated cohort did not yield euploid blastocyst.

Limitations, reasons for caution: While we observed that a more physiological rhPLCζ-activation yields a comparable fertilization compared to the conventional ionomycin method, the study is preliminary, and a confirmatory clinical outcome is not yet available.

Wider implications of the findings: This is the first attempt to use recombinant sperm-specific protein to mimic physiological oocyte activation for patient treatment. If this data is confirmed in a larger series, this may represent a way to normalize fertilization in cases of sperm activating factor deficiency even in different sources of spermatozoa.

Trial registration number: N/A

Abstract citation ID: dead093.619

P-261 Embryo compaction based on time-lapse imaging is a valuable parameter for selecting blastocysts with the highest developmental potency and associated with a ploidy state

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Study question: Does embryo compaction positively correlate with blastocyst development and further embryonic ploidy status?

Summary answer: Fully compacted embryos develop into good-quality blastocysts, have shorter developmental times, and are related to ploidy status.

What is known already: To succeed in assisted reproduction technique (ART), it is necessary to select embryos that have the highest potential. So, numerous studies make an effort to establish parameters for selecting embryos. A Time-lapse system (TLS) allows embryologists to understand dynamic embryo change through continuous monitoring. In development, embryos undergo dynamic functional changes during compaction, which play a crucial role in blastocyst formation. It is also known that embryonic genome activation can be seen with compaction. Incomplete compaction leads to blastocyst developmental failure. Nevertheless, the details about the compaction of human embryos have not been paid sufficient attention, so still rarely known.

Study design, size, duration: This was a retrospective cohort study including couples that underwent an IVF cycle at the CHA Fertility Center, Gangnam, between January 2019 to October 2022. A total of 371 reached the blastocyst from 113 patients cultured in the TLS were analyzed. Among these, 94 blastocysts were analyzed by preimplantation genetic testing for aneuploidy (PGT-A). Statistical analysis was performed by prism9 using a t-test and chi-square test. P values <0.05 were regarded as statistically significant.

Participants/materials, setting, methods: Embryos were classified into two categories by compaction pattern: fully compacted (Group1, N = 194) and partially compacted (Group2, N = 177). Blastocyst quality was determined by morphology and divided into three groups (Good, Average, and Poor). The developmental time ranging from morula to blastocyst was annotated based on the embryo scope image. The surface of the blastocyst was measured every hour starting at blastocyst formation (tB) by using the ellipse tool of the Embryo Viewer software.

Main results and the role of chance: Good and average quality blastocysts are significantly higher in Group 1 than in Group 2 (21.6% vs. 3.4%, $p < 0.01$; 47.9% vs. 26.6%, $p < 0.01$, respectively). In contrast, poor-quality blastocysts are lower in Group 1 than in Group 2 (30.4% vs. 70.1%, $p < 0.01$). The beginning and completion of compaction, and blastocyst formation times of embryos from Group 1 were significantly shorter than those of embryos that Group 2(78.6h vs. 82.4, $p < 0.01$; 87.0h vs. 92.2h, $p < 0.01$; 100.2h vs. 103.7, $p < 0.01$, respectively). Also, there is a significant difference in longitudinal surface area at 3, 6, and 12h from the time of tB between the two groups. Consequently, the average expansion rate in Group 1 was significantly faster than in Group 2 (653.6 μm^2/hour vs. 499.2μm^2/hour, $p < 0.05$). According to the result of PGT-A, Group 1 had statistically significantly higher euploid and lower aneuploidy rates compared to Group 2 (47.2% vs. 36.2%, $p < 0.001$; 52.8% vs. 63.8%, $p < 0.001$, respectively). However, in the PGT-A group, there was no significant difference in developmental time between the two groups, regardless of fully or partial compaction. Meanwhile, the average expansion rate in euploidy blastocyst was significantly faster than in aneuploidy blastocyst (747.8 μm^2/hour vs. 564.3 μm^2/hour, $p < 0.05$).

Limitations, reasons for caution: The main limitation is the single-center retrospective approach. Therefore future prospective research is needed to verify and extend our findings. Another one, in the PGT-A there was no difference in developmental time regardless of fully or partially compaction. The reason is that we selected blastocysts of sufficient quality for biopsy.

Wider implications of the findings: Using the TLS, we have observed the dynamics of compaction in detail. We found a positive relationship between compaction and blastocyst quality and its association with embryo ploidy. The assessment of compaction is a significant parameter for predicting competent embryos and should be given priority when selecting blastocysts.

Trial registration number: Not applicable

Abstract citation ID: dead093.620

P-262 Comparative ability of iDAScore and embryo morphology grade to predict clinical pregnancy: Retrospective cohort study of 1510 fresh and frozen elective single embryo transfers

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Study question: Can iDAScore predict ongoing clinical pregnancy (CP) with a sensitivity and specificity equivalent to that associated with manual morphology assessment and grading?

Summary answer: The fully automated iDAScore was able to predict CP with equivalent performance to manual morphology assessment and grading in this retrospective cohort study.

What is known already: iDAScore is an artificial intelligence (AI) based algorithm developed by applying machine learning to morphokinetic time laps (TL) image data of embryos with a known treatment outcome. Embryos are automatically assessed on day 5 of culture and ranked according iDAScore, ranging from 1 to 9.9. Embryos may be prioritised for transfer based on highest sore. Along with other published AI algorithms, iDAScore has been proposed to optimise the chance of CP following ET by improving objectivity of embryo assessment compared with manual scoring systems. The fully autonomous assessment of embryos by AI algorithms has beneficial implications for laboratory workload.

Study design, size, duration: Retrospective audit of 787 fresh and 723 frozen single embryo transfer cycles which took place from April 2019 to September 2022. All recipient, surrogacy, warmed oocyte, embryo biopsy, cleavage stage and slow thaw frozen ET cycles were excluded.

Participants/materials, setting, methods: Selection for transfer was based on blastocyst morphology grade. iDAScores were obtained retrospectively. The area under the receiver operator characteristic (AUROC) curve and sensitivity and specificity for CP prediction of both iDAScore and blastocyst morphology grade was compared overall and in two stratified analyses. The first assessed fresh and frozen cycles separately, the second compared their performance in female age groups of ≤ 35 and >35 years. CP was defined by ultrasound detection of foetal heartbeat.

Main results and the role of chance: The mean grading of blastocysts by iDA was 8.34 $\pm$ 1.4, with a strong correlation to classic morphological scoring ($r = 0.69$, $P < 0.001$). The clinical pregnancy rate for fresh and frozen embryo transfer was 31.0% (95%CI 27.9-34.3) and 44.0% (95%CI 40.8-48.0) respectively. iDA score was positively associated with clinical pregnancy rates in both fresh (adjOR 1.69, 95%CI 1.44-1.99) and frozen embryo transfers (adjOR 1.45, 95%CI 1.26-1.67), independent of maternal age. There was no

difference in the AUROC for iDA (AUC 0.64, 95%CI 0.62-0.67) versus conventional morphology (AUROC 0.63, 95CI 0.61-0.66) when all eSETs were considered, or when fresh eSETS (AUROC for fresh iDA 0.66 95%CI 0.62-0.70 vs morphology 0.65 95%CI 0.62-0.69) or frozen iDA 0.63 95%CI 0.59-0.67 vs morphology 0.61 95%CI 0.57-0.64) eSETS were considered separately. The iDA score exhibited slightly better performance in the age stratified analyses, with a higher AUROC in women >35 years; iDA 0.68 95%CI 0.64-0.71 vs morphology 0.64 95%CI 0.60-0.67, p = 0.021) but no difference was observed in younger women. This age difference in iDA performance was primarily driven by frozen embryo transfers (p = 0.002). For women >35years an iDA score of 8.75 was associated with an AUROC of 0.63 and 67% sensitivity and 60% specificity for prediction of clinical pregnancy.

Limitations, reasons for caution: This was a retrospective single centre study and the performance of iDA needs to be confirmed prospectively in a multi-centre trial. That selection of embryos for transfer was based on morphology, may have contributed to overestimation of model performance.

Wider implications of the findings: The application of AI based embryo ranking algorithms to embryo selection for transfer, has the potential to maintain clinical pregnancy rates while reducing the time burden associated with conventional morphology / morphokinetic assessments.

Trial registration number: Nil

Abstract citation ID: dead093.621
P-263 Increased live birth rate, blastocyst formation and quality when embryos were cultured in EmbryoScope time-lapse incubators compared to MINC™ benchtop incubators

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Study question: Does embryo culture using the EmbryoScope or EmbryoScope+ time-lapse incubators affect blastocyst development and birth rate compared to MINC™ benchtop incubators?

Summary answer: Culture in the EmbryoScope or EmbryoScope+ resulted in higher blastocyst quality and utilisation rates, and birth rates from fresh blastocyst transfers was increased.

What is known already: Time-lapse incubators enable continuous monitoring of embryos so that morphological changes can be observed, and embryo selection software applied to select the best embryo for transfer. However, there has been considerable debate over the benefits of such systems and a review of 8 randomized controlled trials (Armstrong et al., 2019; Cochrane Database Syst Rev (5)) was inconclusive. Interpretation and comparison of results are complicated by differences among imaging systems and variations in culture protocols between clinics. EmbryoScope time-lapse incubators may also have better temperature and pH stability than benchtop incubators, improving embryo development.

Study design, size, duration: Retrospective data from 1189 cycles in the EmbryoScope or EmbryoScope+ and 1381 MINC cycles conducted concurrently in the same laboratory over 18 months. All embryos from each patient cycle were cultured in one incubator type. All patients from a treating clinician were allocated to one incubator type.

Participants/materials, setting, methods: All IVF/ICSI cycles from a single clinic. Cycles with vitrified eggs or embryos were excluded. Embryos were cultured in Vitrolife G-TL media under 5% O_2, media was refreshed on Day 5. Embryos were scored using the Gardner grading system. For embryos cultured in the Embryoscope, selection of embryos for transfer was based on a combination of Gardner grading and the KIDScore D5 v2 algorithm.

Main results and the role of chance: There was an increase in Day 5 top quality blastocysts (AA) from embryos in the Embryoscope compared to the MINC (15.3% vs.9.7%, p < 0.0001), and Day 5 good quality blastocysts (≥BB, 24.4% vs.16.9%, p < 0.0001). Overall, the utilisation rate was significantly higher from embryos in the Embryoscope (51.3% vs.46.0%, p < 0.0001). From fresh single blastocyst transfers, there was a significant increase in detection of fetal heart by ultrasound (34.7% vs 28.0% p < 0.01) and live birth rate (30.9% vs 24.6%, p<0.01) from embryos cultured in the Embryoscope (n = 972) compared to the MINC (n = 633).

Interestingly, pronuclei were not observed during fertilisation assessment for 84 embryos that subsequently formed blastocysts cultured in the MINC, compared to only 4 embryos in the Embryoscope, suggesting that continuous monitoring increases accuracy in determining fertilisation.

Limitations, reasons for caution: Assessment using the EmbryoViewer compared to an inverted microscope may result in discrepancy in blastocyst grades. The use of KIDscore combined with Gardner grading for embryo selection in the Embryoscope but not the MINC may influence transfer outcomes. As data was collected retrospectively, patients were not randomised to incubator types.

Wider implications of the findings: Culture in the Embryoscope increases the frequency of high-quality usable blastocyst development when compared to a MINC. Importantly, this translates to a higher pregnancy rate and live birth rate from fresh embryo transfers. This may be due to the microwell dishes, incubator performance, embryo selection or all of these.

Trial registration number: Not applicable

Abstract citation ID: dead093.622
P-264 Correlations between the artificial intelligence scoring system (iDAScore v1.0) and live birth outcomes in preimplantation genetic testing for aneuploidy cycles

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Study question: Is the iDAScore v1.0 score correlated with pregnancy outcomes in single embryo transfer (SET) cycles following preimplantation genetic testing for aneuploidy (PGT-A)?

Summary answer: We demonstrated that elevated iDAScore v1.0 scores are positively correlated with the probabilities of pregnancy and live birth (LB) in SETs following PGT-A.

What is known already: To select the blastocysts with high development potential in advance, several groups have proposed the implementation of artificial intelligence (AI)-based software is capable of predicting implantation in women undergoing *in vitro* fertilization (IVF). It has also been known that using high resolution next generation sequencing preimplantation genetic screening (hr-NGS) for PGT-A can enable the exclusion of aneuploid embryos before embryo transfers and thus improve pregnancy outcomes of SETs. However, the capacity of AI-based assessments has remained unclear in IVF cycles with PGT-A.

Study design, size, duration: This retrospective study, approved by the Institutional Review Board of Chung Sun Medical University, was performed to assess the dataset of 317 SET cycles after PGT-A from January 2017 to September 2019. A single euploid or mosaic blastocyst with an AI score calculated using the iDAScore software (v1.0) was selected for frozen embryo transfer according to the given priority of blastocyst morphology. The iDAScore groups were categorized by quartiles of AI scores.

Participants/materials, setting, methods: Embryos were cultured in a time-lapse incubator and qualified blastocysts underwent next generation sequencing (NGS)-based PGT-A. The confounding factors associated with LB were evaluated using logistic regression analysis in generalized estimating equations (GEEs). The differences between iDAScore groups were assessed using the Mann–Whitney U test or Fisher's exact test, as applicable. The receiver operating characteristic (ROC) curve analysis was used to estimate the predictive powers. A P value < 0.05 was considered statistically significant.

Main results and the role of chance: The results revealed the patient age, anti-Müllerian hormone, body mass index, oocyte sources (autologous or donor), PGT-A results (euploidy, low-level mosaicism, or high-level mosaicism), aberrant chromosome types (none, whole chromosome, segmental

chromosome, or whole with segmental chromosome), and aberrant chromosome sites (0, 1, 2, or > 2) were not correlated with LB. However, embryo biopsy days (day 5 vs. day 6, OR = 2.271, 95% CI = 1.534–14.824), blastocyst morphology scores (OR = 1.185, 95% CI = 1.034–1.359), and AI scores (OR = 1.640, 95% CI = 1.231–2.183) were significantly correlated with LB. The AI scores were then divided into quartiles (group 1: 5.0–7.9; group 2: 8.0–8.5; group 3: 8.6–8.9; and group 4: 9.0–9.5). The ongoing pregnancy (29.1% vs. 54.2%–57.5%) and live birth (27.8% vs. 52.8%–56.3%) rates of group 1 were significantly lower than other groups. The blastocyst morphology score (5.3 ± 1.2), and KIDScore D5 scores (v1 = 3.0 ± 1.7; v3 = 4.6 ± 1.5) were also lowest in group 1. The ROC curve analysis confirmed a significant but limited LB prediction capability for iDAScore v1.0 (AUC = 0.6), which was similar to the KIDScore D5 (AUC = 0.61–0.62).

Limitations, reasons for caution: Because of the retrospective nature, the major limitation was the lack of randomization, which may present a risk of selection bias. According to the criteria of blastocyst selection for SETs, the embryos with low (< 5.0) or high (> 9.5) AI scores were not present in this dataset.

Wider implications of the findings: Although prediction capability of iDAScore remains perfectible, the AI score is still significantly associated with LB probability. Euploid or mosaic blastocysts with low AI scores (<8.0) processed a decreased LB rate, indicating the potential of annotation-free iDAScore system as a decision support tool for deselecting embryos with poor post-implantation development.

Trial registration number: not applicable

Abstract citation ID: dead093.623

P-265 The inner cell mass (ICM) hatching levels of blastocyst before vitrification associated with clinical pregnancy rates after single embryo transfer in PGT-A cycles

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Study question: Is there an association between ICM hatching levels and clinical pregnancy rate after single blastocyst transfer (SET) in preimplantation genetic testing for aneuploidy (PGT-A) cycles?

Summary answer: A blastocyst with ICM hatching level ≤ 50% has a better clinical pregnancy rate than that in a blastocyst with ICM hatching level > 50%.

What is known already: The zona pellucida (ZP) breaching and trophectoderm (TE) herniating by laser assisted hatching before blastocyst stage is commonly used in blastocyst biopsy. The degree of hatching levels of ICM and TE is different between blastocysts before vitrification. However, completely hatched embryos may be more fragile due to the biopsy and vitrification/warming processes and thus more susceptible to potential cellular damage prior to transfer. For the moment, no published studies have explored the entire set of information on morphological evaluation of biopsied blastocyst before vitrification including TE, ICM hatching levels, and clinical outcomes.

Study design, size, duration: A retrospective study including 578 PGT-A cycles with SET was conducted at Lee Women's Hospital between January 2020 and May 2022 (CS1-21156). The women age <20 or > 45 years were excluded in this study. The vitrification of expanded blastocysts on day 5 or day 6 was performed after TE biopsy. The hatching levels before vitrification were classified according the degree of TE or ICM hatching out from ZP.

Participants/materials, setting, methods: The groups of TE hatching levels were divided into (1) without hatching, (2) ≤25% hatching, (3) >25% <100% hatching and (4) hatching out. The groups of ICM hatching levels were divided into (1) ≤50% and (2) >50% hatching. The primary outcome measure was the clinical pregnancy rate. Statistical analysis was performed using the generalized estimating equations (GEE), Spearman's correlation, Kruskal-Wallis test, Fisher's exact test and c2 test.

Main results and the role of chance: The average of women age was 36.8 ± 4.4 (24-45) years. The overall clinical pregnancy, miscarriage and ongoing rates after SET were 65.4% (378/578), 10.3% (39/378) and 58.5% (338/578), respectively. Spearman's correlation analysis indicated that the TE hatching levels, the ICM hatching levels and the ICM grade were associated with the clinical pregnancy rate ($p < 0.01$). According to multivariate regression analysis, the ICM hatching level ≤ 50% (OR: 1.863, 95% CI: 1.034-3.358, $p = 0.038$) and ICM grade A (OR: 1.662, 95% CI: 1.037-2.667, $p = 0.035$) were positively associated with clinical pregnancy probability. No significantly associations were found between women age, embryo day, TE hatching levels and clinical pregnancy rate ($P > 0.05$) after multivariate regression analysis. The clinical pregnancy rate in the blastocyst with ≤ 50% hatching (68.7% (328/477)) was significantly higher than that in the blastocyst with > 50% hatching (49.5%, 50/101; $p < 0.001$). Furthermore, the blastocyst with grade A and ≤ 50% hatching had a highest clinical pregnancy rate (76.9% (93/121)) and the blastocyst with grade B and > 50% hatching had a lowest clinical pregnancy rate (46.3%, 38/82; $p < 0.001$). The abortion rates between all groups were no significantly difference.

Limitations, reasons for caution: A smaller sample size in the group of ICM hatching > 50% and further study with a larger sample size was needed to investigate putative impacts on clinical outcomes after SET.

Wider implications of the findings: These results demonstrate that a blastocyst with high degree of ICM herniating after biopsy and vitrification protocols decreased the clinical pregnancy probability. Selecting a biopsied blastocyst with either low ICM hatching level and grade A morphology for SET has a best pregnancy outcomes.

Trial registration number: not applicable

Abstract citation ID: dead093.624

P-266 Study on the mechanisms of ovol1/2 during the morula-to-blastocyst transition

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Study question: Screen the key gene groups that determine the development of blastocyst. Explore the effect of *ovol1/2* gene on early embryonic development.

Summary answer: We founded that there were insufficient activation of the Major ZGA genes in the AE group,and focused on candidate gene *ovol1/2*.

What is known already: At present, the clinical success rate of assisted reproductive technology (ART) is less than 50%. A large number of embryos cultured in vitro can not develop to blastocyst, and there are cleavage or morula stage arrest. The developmental arrest of preimplantation embryos is an important reason for treatment failure of infertile patients. However, the cause of early embryonic development arrest is not clear, and there is also a lack of effective methods to improve embryo quality.

Study design, size, duration: In this study, fertilized embryos were obtained through ICSI and a single cell from eight-cell embryo was biopsied through micromanipulation for single-cell RNA sequencing (scRNA-seq). Subsequently, we tracked the developmental potential of remaining cells and divided them into high-quality blastocyst(Bla) group and high-quality eight-cell failed blastocyst formation(arrest embryo, AE) group. In addition,we employed a RNAi approach to study the function of candidate gene *ovol1/2*, by injecting *ovol1/2*-targeting small interfering RNAs (si*ovol1/2*) into wild-type (WT) zygotes.

Participants/materials, setting, methods: The study collected 21 human blastocysts and 5 arrest embryos. Human immature oocytes from ICSI treatments were clinically discarded and donated by women after signing informed consent by donors. C57B6 background mouse strains were used in this study. Mice were maintained under specific pathogen free conditions in a controlled

environment of 20–22 °C,with a 12/12 h light/dark cycle, 50–70% humidity, and food and water provided.

Main results and the role of chance: The results showed that the gene expression of embryos in AE group was significantly different from that of Bla group: 79.5% of differential expression genes were down-regulated, including 48.3% of the Major ZGA gene. Then we focused on the candidate gene *ovol1/2*. When *ovol1/2* were knocked down alone, there was no significant difference in the development rate of embryos from 2-cell to blastocyst between knockdown group and normal control group. When *ovol1/2* were knocked down at the same time, there was no significant difference in the development rate of embryos among three groups before the morula stage. But in the morula developed to the blastocyst stage, there were significant differences among three groups of embryos: about 73% of embryos in the normal control group formed blastocyst; about 39% of the embryos in the low-dose knockdown group; the high-dose knockdown group embryos were all arrested in the morula stage. Further investigation revealed that the number of inner cell mass(ICM) cells in low-dose knockdown group did not change significantly, while the number of trophoblastic ectoderm(TE) cells significantly decreased. In addition, the TE functions of low-dose knockdown group such as blastocyst cavity size, paved area, hatching and adhesion rate were damaged.

Limitations, reasons for caution: Further research of the mechanisms is needed.

Wider implications of the findings: This is significant to understand molecular mechanisms of early embryonic development, and will provide important theoretical basis for clinical diagnosis and intervention of embryonic development arrest. Our study provides clues for the causes of abnormal blastocyst formation and new ideas for possible clinical improvements in the success rate of ART.

Trial registration number: not applicable

Abstract citation ID: dead093.625

P-267 Artificial intelligence (AI) can successfully predict pregnancy despite of the visual differences of fresh and frozen-thawed embryos

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Study question: Can AI accurately predict pregnancy from fresh and frozen-thawed embryo images that may exhibit visual differences?

Summary answer: AI was able to distinguish between fresh and frozen images based on visual differences, but these differences did not affect the accuracy of pregnancy prediction.

What is known already: Frozen-thawed embryos may experience changes to their structure and composition, though these changes are difficult to detect with the human eye. AI has been shown to predict pregnancy by analyzing embryo images in IVF cycles. However, there has been no evidence to suggest that the morphologic changes caused by cryopreservation affect AI's pregnancy predictions. In this study, we developed an AI model to distinguish between images of fresh and frozen-thawed embryos and evaluated if the visual differences affected pregnancy prediction.

Study design, size, duration: We performed a retrospective study of single static images of 2,237 Day 5 blastocysts from two in vitro fertilization (IVF) clinics between February 2001 and December 2021. The images were collected from standard optical light microscopes and matched with metadata such as pregnancy outcomes, cryopreservation and assisted hatching information. We defined a positive pregnancy indication as the presence of a gestational sac (G-SAC).

Participants/materials, setting, methods: We constructed two CNN models to verify two hypotheses. The first model was a classification model that utilized day 5 images of fresh and frozen-thawed embryos from two IVF clinics. The proportion of fresh to frozen-thawed at each clinic was 603 to 667 and 402 to 565, respectively. The second model was a CNN designed to predict pregnancy and its performance was compared after incorporating the cryopreservation label through internal validation.

Main results and the role of chance: The first AI model classified frozen-thawed and fresh embryos with high AUROCs (0.848 and 0.912) and accuracy (0.846 and 0.912) at each clinic. The Grad-CAM images revealed that the AI learned to differentiate the two types of images based on features mainly in the embryo's zona pellucida region. The AUROCs of the second AI model for pregnancy prediction were 0.730 and 0.663 at each clinic, respectively. Adding the cryopreservation label did not significantly change the AUROCs, which remained at 0.734 and 0.650. The study found that the visual differences between fresh and frozen-thawed embryos had no effect on the performance of the pregnancy prediction model. It's important to note that assisted hatching was used in 88% of the frozen-thawed cycles in the study, which may have compensated for the changes to the zona pellucida caused by cryopreservation.

Limitations, reasons for caution: This study was validated using data from two IVF clinics, and a larger dataset from multiple centers is needed for external validation. Further research on non-assisted hatching (AH) cycles is necessary to confirm the role of AH in frozen-thawed cycles.

Wider implications of the findings: The study found that AI can accurately differentiate between fresh and frozen-thawed embryos by analyzing the zona pellucida region. The visual differences between the two types of embryos did not impact the accuracy of pregnancy prediction. Assisted hatching may also mitigate any negative effects on frozen-thawed embryos.

Trial registration number: not applicable

Abstract citation ID: dead093.626

P-268 Artificial intelligence (AI) image analysis outperforms patient age as a surrogate marker for oocyte quality, demonstrating an increased accuracy in predicting blastocyst development

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Study question: Can an AI image analysis tool (VIOLET) provide better predictions of oocyte potential than the current standard, patient age, as it relates to blastocyst development?

Summary answer: While increasing patient age slightly correlates with decreases in blastocyst development, VIOLET provides personalized assessments of individual oocytes correlated to blastocyst development with improved accuracy.

What is known already: Increasing patient age correlates to decreases in oocyte competency, leading to greater challenges in successful fertility treatment. Clinically, patient age is used to estimate probabilities of success based on national databases. Such as the case in oocyte cryopreservation cycles, considering only age and number of mature oocytes vitrified. However, oocyte quality may vary widely between patients of the same age, and even within each cohort of oocytes. VIOLET is an AI tool that assesses images of mature denuded oocytes to provide an analysis shown to significantly correlate with subsequent blastocyst development and quality, consistently outperforming embryologists in this task.

Study design, size, duration: This large-scale retrospective study assessed 9,120 mature denuded oocytes retrieved during 2014-2022, representing 1,384 patients between ages 19-49, attending 7 fertility clinics across 5 countries. The VIOLET prediction model is based on image analysis of mature denuded oocytes, without incorporating clinical variables, such as age. Patient

age was used to build a separate predictive model of blastocyst development (10,947 training and 3,750 validation samples) to assess the predictive value of age in comparison to VIOLET.

Participants/materials, setting, methods: Blastocyst development was determined by embryos achieving a Gardner grade by Day5/6 post-ICSI. Blastocyst rates per oocyte cohort were calculated by number of blastocysts divided by total number of mature oocytes retrieved. Various machine learning techniques were trialed to build the predictive model strictly using age; with Random Forest model providing the best-balanced performance. VIOLET and the age model assessed the images and age of 9,120 mature denuded oocytes, respectively, providing predictions of blastocyst development.

Main results and the role of chance: Blastocyst development was significantly different between patients <35 years old compared to those ≥35 years old [47% vs 42%; $p < 0.05$ by Two Proportion Z-test]; however, not when the older age group was stratified further. On a patient level, the cohort of oocytes was evaluated by the blastocyst development ratio. Among four age groups (<35, 35-37, 37-40, >40), the blastocyst development ratios per cohort were very similar, with overlapping distributions of 84-94% using Kernel Density estimates. Thus, patient age group does not provide enough information to explain blastocyst development success for an individual oocyte or within an oocyte cohort.

In comparison, VIOLET probability is significantly correlated to blastocyst development ($p < 0.05$; Welch's Two sample t-test). And blastocyst development rates display a stepwise positive correlation that is significantly different between VIOLET probability quartiles [24% vs 39% vs 47% vs 53%; $p < 0.05$ by Two Proportion Z-test], providing meaningful information on individual oocytes.

Additionally, the predictive model built with age as the only feature had poor ability to predict blastocyst success of an individual oocyte with a limited area-under-the-curve (AUC) of 0.5—unable to separate positive and negative classes. This was outperformed by VIOLET, which displayed an AUC of 0.62 on the same unseen dataset.

Limitations, reasons for caution: Increasing maternal age causes increased chromosomal abnormalities in oocytes, which translates to lower efficacy with treatment outcomes; therefore, further research to assess VIOLET correlation with PGT-A and implantation outcomes is needed and underway.

Wider implications of the findings: Patient age correlates with blastocyst development on a general level; however, it does not provide meaningful insights to distinguish rates of blastocyst success on an individual oocyte or oocyte cohort level. VIOLET augments oocyte understanding over the current standard of care, which can be utilized to support personalized clinical decision-making.

Trial registration number: Not applicable.

Abstract citation ID: dead093.627

P-269 trophectoderm grade is associated with the risk of placenta previa in frozen-thawed single blastocyst transfer cycles

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Study question: Dose obstetric and perinatal complications differ among different blastocyst developmental parameters after frozen-thawed single blastocyst transfer (SBT) cycles?

Summary answer: Blastocysts with grade C trophectoderm (TE) were associated with an increased risk of placenta previa compared to those with grade A TE.

What is known already: Existing studies investigating the effect of blastocyst morphology grades on birth outcomes have mostly focused on fetal growth and have produced conflicting results, while the risk of obstetric complications has rarely been reported. Additionally, growing evidence have suggested that the appearance of TE cells could serve as the most important parameter for predicting implantation and live birth. Given that the TE ultimately develops into the placenta, it is plausible that this independent predictor may also impact placentation.

Study design, size, duration: This was a retrospective cohort study conducted at a single tertiary-care academic reproductive center. A total of 6168 patients who underwent frozen-thawed SBT and resulted in singleton delivery beyond 20 weeks of gestation between January 2017 and December 2021 were analyzed.

Participants/materials, setting, methods: Main outcomes included placenta previa, placental abruption, placenta accreta, pregnancy-induced hypertension (PIH), preeclampsia, preterm birth (PTB), low birth weight (LBW) and small for gestational age (SGA). Multivariate logistic regressions were performed to evaluate the effect of blastocyst developmental stage (day 5 and 6), embryo expansion stage (stages 3, 4, 5 and 6), inner cell mass (ICM) grade (A, B and C), and TE grade (A, B and C) on measured outcomes adjusting for potential confounders.

Main results and the role of chance: The overall rates of placenta previa, placental abruption, placenta accreta, PIH, preeclampsia, PTB, LBW and SGA was 2.8% (n = 173), 0.4% (n = 26), 1.3% (n = 83), 5.3% (n = 328), 4.3% (n = 267), 6.8% (n = 417), 3.7% (n = 230), and 2.8% (n = 172), respectively. Specifically, the incidence of placenta previa derived from blastocyst with TE of grade C was higher compared with those derived from blastocyst with TE of grade A (1.8%, 2.5% and 3.9% for A, B and C, respectively, $P = 0.003$ for all comparisons). No such differences were observed for any other outcomes. After adjusting for potential covariates (maternal age, maternal BMI, maternal education, duration of infertility, gravidity, parity, previous caesarean section, infertility diagnosis, IVF or ICSI, previous embryo transfer, and type of endometrial preparation), TE grade C blastocyst had 2.58 times the likelihood of resulting in the risk of placenta previa compared to TE grade A blastocysts (adjusted odds ratio [AOR] 2.58, 95% confidence interval [CI] 1.05-6.31). No statistically significant associations were detected between any other measured outcomes and blastocyst developmental parameters. Furthermore, when restricted to pregnancies above 28 gestational weeks, the risk of placenta previa remained significantly higher with TE of grade C (AOR 2.56, 95% CI 1.05-6.27) than with TE of grade A.

Limitations, reasons for caution: The retrospective design, lack of controlling for residual confounding factors, and inter-observer variability limited this study.

Wider implications of the findings: The study extends our knowledge of the potential downstream effect of TE grade on placental abnormalities.

Trial registration number: not applicable

Abstract citation ID: dead093.628

P-270 The timing of intracytoplasmic sperm injection does not impact live birth rate and neonatal outcomes

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Study question: Does the timing of intracytoplasmic sperm injection (ICSI) relative to ovulation trigger impact live birth rates and neonatal outcomes?

Summary answer: ICSI timing relative to ovulation trigger impacts neither live birth rates nor neonatal outcomes.

What is known already: When conducting ICSI, the timing of the insemination relative to ovulation trigger is difficult to standardise in clinical practice, especially in busy ART laboratories. Flexibility when scheduling ICSI procedures would therefore be beneficial from a laboratory management perspective. However, the impact of this timing on live birth rates and neonatal outcomes remains inconclusive in the literature.

Study design, size, duration: This retrospective study included a total of 1547 consecutive single autologous embryo transfer cycles (maternal age 35.2 ± 4.8 years) performed at Fertility North between January 2017 and August 2021. ICSI was performed immediately after denudation without timing restrictions relative to ovulation trigger. A total of 422 (27.3%) transfers

resulted in a live birth, including 415 singletons, 5 twin deliveries, and 2 lost to follow-up. Only singleton babies were included for neonatal outcome analysis.

Participants/materials, setting, methods: ICSI timings relative to ovulation induction trigger (mean ± SD 42.5 ± 1.4 hours, range 37.0-46.5 hours) were extracted from the electronic witnessing database. Cycles were grouped according to ICSI timing quartiles (groups Q1-4: <41.7, 41.7-42.7, 42.8-43.4 and 43.5+ hours). Logistic/linear regressions were used to assess impacts of ICSI timing on the subsequent live birth and birthweight outcomes in the singleton babies. Mixed effect model was used to account for clustering effect from repeat cycles by same patients.

Main results and the role of chance: Q1 group had significantly lower live birth rate (22.7%) but higher maternal age (37.3 ± 5.2 years) in comparison to Q2 (28.5% and 34.5 ± 4.6, $P < 0.05$ respectively), Q3 (30.2% and 34.8 ± 4.5, $P < 0.05$ respectively) and Q4 groups (27.7% and 34.4 ± 4.4, $P < 0.05$ respectively). However, following multivariate logistic regression adjusting for potential confounders including maternal age, body mass index, aetiology, sperm origin (ejaculated or surgical), sperm type (donor/partner), embryo stage at transfer (cleavage or blastocyst), and fresh/frozen transfer; observed differences in live birth rates became no longer significant (Q2 vs Q1, aOR = 0.982, 0.692-1.394, $P = 0.919$; Q3 vs Q1, aOR = 1.058, 0.884-1.266, $P = 0.538$; Q4 vs Q1, aOR = 0.969, 0.859-1.094, $P = 0.615$; Q3 vs Q2, aOR = 1.153, 0.830-1.600, $P = 0.396$; Q4 vs Q2, aOR = 0.963, 0.818-1.134, $P = 0.649$; Q4 vs Q3, aOR = 0.836, 0.601-1.164, $P = 0.289$). Gestational age and birthweight of singletons arising from 4 groups were also comparable (Q1 38.1 ± 2.2 weeks and 3209.1 ± 581.4 grams, Q2 38.2 ± 1.6 weeks and 3159.9 ± 508.2 grams, Q3 38.7 ± 1.8 weeks and 3304.7 ± 546.9 grams, and Q4 38.4 ± 2.0 weeks and 3297.5 ± 526.9 grams; $P > 0.05$ respectively). Multivariate linear regression further confirmed the absence of significant impacts (standardised coefficient = 0.001, $P = 0.980$) by ICSI timing on the birthweight of resulting newborns, taking into account gestational age and baby sex plus all above mentioned 7 potential confounders.

Limitations, reasons for caution: Our study is limited by its retrospective nature, where it is impossible to control all known and unknown confounding factors. The varied denudation duration as a result of different egg numbers was unable to be analysed, although ICSI was aimed to be performed immediately after completion of denudation.

Wider implications of the findings: Our findings indicate a wide time window in human oocytes to initiate fertilization via ICSI. This data offers reassurance in applying flexible ICSI timing without impacting live birth rates and neonatal outcomes. This is clinically valuable by providing evidence-based guidance to daily practice and patient counselling.

Trial registration number: not applicable

Abstract citation ID: dead093.629
P-271 How much should you tolerate? The tolerance level of gas mixtures percentage in premixed gases

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Study question: Does the percentage of tolerance level of gas mixtures for premixed gas affect blastocyst formation rate?

Summary answer: A lower percentage of gas tolerance level can improve the blastocyst formation rate by providing a more optimal pH in the culture microenviroment.

What is known already: Benchtop incubators that use premixed gases are regarded as one of the most common incubators used in many IVF setups. The level of CO_2 in the mixture has a direct impact on the media's pH whereby an increase in the CO_2 level will result in a lower pH and vice versa. Most media companies require a percentage of 6.0-6.5% to achieve an acceptable pH range of 7.2-7.4 for optimal culture microenvironment. However, it is often taken for granted that the CO_2 and pH of the medium is within the acceptable range due to the "certificate" provided by these gas companies.

Study design, size, duration: This is a retrospective study looking at the average blastocyst formation rate from January 2018-December 2022 on 1665 cycles.

Participants/materials, setting, methods: The total MII collected and inseminated was 21,631. The mean women's age was 37 ± 4.2 years old. All the embryos were cultured in the same laboratory conditions using BT 37 Planer (Origio) and K-Minc (Cook) incubators. From January 2018 to July 2020, all the patient's embryos were cultured with premixed gas of 5% tolerance level (n = 940) and from Aug 2020-December 2022, all of the embryos were cultured with premixed gas of 2% tolerance level (n = 725).

Main results and the role of chance: It was found that the average blastocyst rate was significantly increased when the gas tolerance level was 2% (57.09%) when compared to when the gas tolerance level was 5% (52.11%) ($p < 0.05$).

Limitations, reasons for caution: Different gas companies may use different methods of measuring the gas percentage levels. Therefore, it is advisable to be cautious and impose internal QC on the gas mixture percentage on individual cylinders.

Wider implications of the findings: A lower gas tolerance level may provide a more optimal accuracy of gas percentage to achieve the desired pH for embryo culture and to improve IVF outcomes.

Trial registration number: not applicable

Abstract citation ID: dead093.630
P-272 The impact of CHLOE-EQ and embryologist seniority on the ability and confidence to predict ploidy

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Study question: Can embryologists and CHLOE-EQ predict ploidy? Does their confidence and ability to predict vary with embryologist seniority?

Summary answer: High inter-observer variability between embryologists on prediction of ploidy. CHLOE-EQ provided a consistent prediction of ploidy.

What is known already: Previous studies have demonstrated the value of using Artificial Intelligence (AI)-based tools, such as CHLOE-EQ (Fairtility), to support, quantify and standardize embryo assessment. CHLOE-EQ uses AI-based algorithms to predict implantation. Recent studies have demonstrated that the algorithms also have ploidy predictive capabilities. Little is known about the ability of human embryologists to predict ploidy of blastocysts deemed suitable for biopsy, and whether this ability to predict varies with seniority or confidence level.

Study design, size, duration: Cohort study including 141 patients treated in a Memorial Sisli Hospital ART and Reproductive Genetics Center between January 2020-August 2022, with at least 4 blastocysts with different PGT-A results per cycle, leading to a total of 734 embryos. The same blastocysts were blindly assessed by CHLOE-EQ and by a senior and a junior embryologists working in the same clinic. Intraobserver variance of senior embryologist was also evaluated.

Participants/materials, setting, methods: Embryologists were asked to predict whether a blastocyst was euploid or aneuploid, their confidence of this determination (confident, neutral, not confident), the rank of the embryos based on chance of being euploid. The same embryos were assessed using CHLOE-EQ, scoring embryos from 0 to 10. The extent of mosaicism (from 35-70%) was quantified. Trophectoderm biopsy was performed to embryos at least 3BB, four low quality blastocysts (Aneuploid:5AC,4AC,6CB; Euploid:2AA) were also included into the study.

Main results and the role of chance: The average patient age was 33 ± 4 years (ranging from 22 to 39 years). Embryologists agreed on euploid prediction in a minority of blastocysts(48%,324/670). Agreement of the

senior embryologist on same embryos in a different time was higher (Kappa = 0.42, moderate) than with the junior embryologist [Kappa = 0.19(slight)/0.26(fair)], suggesting seniority affected prediction consistency. CHLOE-EQ ranking had fair agreement with the embryologists (Kappa = 0.22,0.22,0.23), bringing consistency to prediction irrespective of seniority. Confidence was not affected by seniority (senior vs junior: 'Confident'/'Neutral'/'Not confident':60/26/14% vs 57/27/16% vs 66/24/10%,NS). Efficacy of prediction of ploidy reduced with junior embryologist (senior: AUC = 0.58,0.57 vs junior:AUC = 0.55). The senior embryologist was able to predict ploidy with a greater accuracy when 'confident' (AUC = 0.60, n = 441, p<0.001) compared with 'not confident' (AUC = 0.50, n = 107, NS). This was not the case with the junior embryologist ('Confident' AUC = 0.56,NS vs 'Not Confident' AUC = 0.54,NS). Euploidy rate was greater in high scoring embryos(CHLOE-EQ 5.1-10) than low(0-5) scoring (60%, 272/457 vs 48%,135/280;p<0.005,AUC = 0.58). This was maintained in blastocysts where the senior embryologist was 'confident'(64% vs 54%) and 'not confident' (57% vs 36%), showing consistency in assessment irrespective of confidence. CHLOE-EQ score reduced with increasing degree of mosaicism (Euploid 6.3 ± 2; Mosaic<35% 5.98 ± 2.87; Mosaic>50% 4.31 ± 2.80; Mosaic>70% 2.85 ± 4;p = 0.04). Euploid embryos had a higher CHLOE-EQ score than aneuploid/mosaic embryos (6.3 ± 2, n = 396 vs 5.78 ± 2,n = 319;p = 0.008).

Limitations, reasons for caution: Mostly optimal quality blastocysts were included in this study. There is a need to better understand the role of Artificial Intelligence in improving consistency of selection of blastocysts for biopsy, and to extend this study for viable lower quality blastocysts to be included rather than discarded.

Wider implications of the findings: Effective prediction of ploidy can (i) improve biopsy criteria so that viable embryos are not discarded, (ii) reduce the cost of PGT-A by prioritizing embryos with increased chances of being euploid. CHLOE-EQ's ploidy prediction improved consistency can support both PGT-A programs, and cycles where PGT-A is not an option.

Trial registration number: Not applicable

Abstract citation ID: dead093.631

P-273 Heavy oil and conventional mineral oil produce similar Day 5 embryological outcomes. A prospective, single centre case study on 546 sibling 2PN zygotes

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Study question: Does heavy oil improve embryological outcomes compared to conventional mineral oil?

Summary answer: Heavy oil produces no improvement in embryological outcomes.

What is known already: Oil overlays are integral part of the culture system as they buffer gas and temperature exchanges in the culture media and reduce the risk of contamination and evaporation. However, slight variations in their composition, oxidative status, storage and utilisation can have a direct effect on culture system performance, eventually resulting in reduced embryo development and subsequent decrease in clinical outcomes. In an attempt to improve the performance of oil overlays, new formulations have recently reached the market. Nonetheless, their positive impact on embryological outcomes, as well as best practice in handling them, are yet to be broadly recognised.

Study design, size, duration: This is a prospective case-study conducted as part of routine consumable validation procedures undertaken when evaluating the use of new products in the IVF laboratory of a public hospital. A total of 546 sibling 2PN zygotes derived from 52 treatments were cultured to Day5 of development. All cases attending the clinic between March 2022 and January 2023 and leading to the collection of more than 10 oocytes were enrolled in this validation exercise.

Participants/materials, setting, methods: Sibling oocytes from IVF and ICSI treatments were cultured under the same conditions until fertilisation check (IVF) or after ICSI. Oocytes from each treatment were then equally split between conventional mineral oil (CONTROLS) and heavy oil (CASES) study groups. All embryos were individually cultured in 25µL drops of single step media at 37°C, 5% CO2 and 5% O2. Embryological outcomes on Day3 and Day5 were compared across the study groups using Chi-square test.

Main results and the role of chance: CONTROLS and CASES showed similar embryological outcomes, including fertilisation (for ICSI only; 77.2% vs. 79.77%, respectively p = NS), good quality Day3 (69.5% vs. 75.6%, p = NS), good quality Day5 (30.2% vs. 24.0%, p = NS), blastulation (58.9% vs. 53.9%, p = NS) and utilisation rates (34.9% vs. 29.5%, p = NS). However, a sub-analysis was performed on the basis of oil handling/storage method. When heavy oil was pre-aliquoted and stored in 15mL tubes prior to usage, negative effects were measured on embryological parameters. In this subset of cases (n = 28 treatments; n = 304 2PN zygotes), although fertilisation and good quality Day3 rates were comparable (80.0% vs 81.6% and 69.1% vs. 73.0% in CONTROLS and CASES, respectively), CASES revealed a significantly lower rate of good quality Day5 blastocysts (Gardner grade ≥B for ICM and TE and expansion ≥grade3) compared to CONTROLS (32.2% vs. 21.7%, p = 0.04). Moreover, CASES showed significant lower embryo utilisation (36.8% vs. 25.7%, CONTROLS and CASES, respectively, p = 0.03) and a trend towards lower blastulation rate (61.2% vs. 50.7%, respectively, p = 0.06). Although the equal split of sibling oocytes between the two groups has minimised population bias, multiple variables may have influenced the performance of the tested product. We note that adequate storage/handling of products is required to avoid suboptimal results.

Limitations, reasons for caution: Although designed with sibling oocytes/embryos, this study is limited in population size. Moreover, only one product brand has been tested, limiting the universality of the conclusions about heavy oil performance in IVF culture systems.

Wider implications of the findings: Introducing a new product in the IVF laboratory should undergo a reliable validation exercise. Despite the commercial claims, heavy oil failed to improve our results. Untested variations may have detrimental effects on clinical performance and, if implemented without control, their effects may become impossible to identify at later stages.

Trial registration number: not applicable

Abstract citation ID: dead093.632

P-274 Clinical limitations of manual oocyte quality scoring by embryologists are overcome by an artificial intelligence (AI) oocyte image analysis tool

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Study question: Do manual embryologist annotations (individual/combined) of dysmorphisms within mature denuded oocytes display better correlation with blastocyst development compared to an AI oocyte assessment tool?

Summary answer: Manual scoring cannot reliably determine oocyte quality in comparison to oocyte assessments by an AI image analysis tool (MAGENTA), which significantly correlates with blastocyst formation.

What is known already: Despite the importance of oocyte quality on blastocyst development, there is no validated visual oocyte assessment employed in clinical practice. While individual oocyte dysmorphisms can be identified, none have been shown to consistently correlate with laboratory and reproductive outcomes. Even when manual oocyte scoring systems have been employed to aggregate multiple dysmorphisms, correlations to blastocyst development fall short. An AI image analysis tool (MAGENTA) has been trained to correlate parameters of the mature denuded oocyte, including those that may be imperceptible to the human eye, to reproductive outcomes, producing more objective, accurate and time-saving assessments.

Study design, size, duration: A retrospective dataset consisting of 1009 mature denuded oocytes from 166 IVF-ICSI patients attending Bahçeci Umut ART Centre (Istanbul, Turkey) during 2016-2019 was assessed. 879 oocytes

were included in the final data analysis, after removing oocytes with missing manual morphological assessments, lab outcomes (fertilization and blastocyst development), or whose static images could not be linked to outcomes. The resulting 879 oocytes correspond to 149 patients from ages 22-45 (mean age of 33.9 years old).

Participants/materials, setting, methods: Two embryologists, blinded to outcomes, retrospectively annotated oocyte images for 7 dysmorphism categories: shape/size, cytoplasmic granularity, polar body fragmentation, zona pellucida thickness, perivitelline space thickness, smooth endoplasmic reticulum (SER) presence, and vacuole presence. Annotations were assigned points and combined into an overall manual score ranging from 0-10 based on a recent review (Bartolacci 2022). Each, previously unseen, oocyte image was analyzed by MAGENTA, assigning a score (0-10), where increasing scores correlate to greater blastocyst development.

Main results and the role of chance: The correlation between each of the 7 observed morphological categories and blastocyst outcome were assessed individually using the Chi-squared test. Only the presence of SER was found to be significantly correlated with whether an oocyte became a blastocyst ($p < 0.05$), however the sample size was small (27 out of 879; 3% of the dataset).

Manual and MAGENTA scores were each assessed for correlation to blastocyst outcome by Welch's two-sample t-test. The mean manual score for the whole dataset is 9.2. No differences in mean manual score were observed between oocytes that developed into blastocysts (9.1) and those that did not (9.2). The manual scoring system assigns a score of 10 unless dysmorphisms are noted, defaulting to the assumption that oocytes are of high quality unless noted otherwise. This is reflected in the skewed distribution of most oocytes receiving high scores due to a lack of visible dysmorphisms. Conversely, the mean MAGENTA score for the whole dataset is 4.2. Oocytes that developed into blastocysts had a significantly higher mean MAGENTA score (4.7) compared to oocytes that did not (4.2) ($p < 0.05$). The distribution of the MAGENTA scores were more evenly distributed across the entire spectrum of scores (0-10).

Limitations, reasons for caution: To avoid reducing sample size, male factor influence on blastocyst development was not removed. Manual oocyte annotations were performed retrospectively, consequently allowing embryologists more time to assess than typically available. The number of oocytes with polar body fragmentation was too few to appropriately assess with the Chi-squared test.

Wider implications of the findings: Because oocyte dysmorphisms are rare, subjective, and don't consistently correlate with blastocyst development, annotating them is clinically nonbeneficial and an inefficient use of an embryologist's time. MAGENTA oocyte assessments are immediate, objective, correlate with blastocyst development, and are easily incorporated into a laboratory workflow for improved clinical insights and decision-making.

Trial registration number: Not applicable.

Abstract citation ID: dead093.633

P-275 Oxidation reduction potential (ORP) levels in blood plasma could represent the ORP status in follicular fluid from oocyte donors and patients

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Study question: Does the evaluation of oxidation-reduction potential (ORP) levels in blood plasma from subfertile patients and oocyte donors represents the ORP status in follicular fluid?

Summary answer: ORP levels in blood plasma from oocyte donors and sub fertile patients could be an indicator of oxidative stress in follicular fluid

What is known already: Oxidation-reduction potential (ORP) measurement of seminal plasma is one method to diagnose oxidative stress in male patients, which can help physician to recommend antioxidants administration when it is elevated. Seminal plasma is easily accessible by masturbation. An empirical analogue for semen could be follicular fluid (FF). Unfortunately, it is very difficult to access to FF, unless a surgical procedure is performed. Hence, it is necessary to find indirect parameters to evaluate the ORP status in FF. Since blood plasma (BP) circulation provides antioxidants to the FF, we studied the BP ORP measurement to indirectly determine ORP levels in FF.

Study design, size, duration: Prospective study conducted at CITMER, Mexico from December 2022 to January 2023. We included under informed consent 15 oocyte donors (age 26.8 ± 4.06 years old) and 55 patients (age 35.2 ± 4.8 years old) undergoing IVF/ICSI.

Participants/materials, setting, methods: ORP levels in BP and FF from dominant follicles were measured at the same time of oocyte collection with MiOXSYS system. Oocytes were inseminated and zygotes were cultured until blastocysts stage. We calculated correlation of a) ORP levels in FF vs BP, b) ORP of FF and BP vs number of retrieved oocytes, c) ORP of FF and BP vs number of fertilized oocytes, d) ORP of FF and BP vs number of blastocysts

Main results and the role of chance: A total of 172 oocytes from oocyte donors were collected. After fertilization check, 135 zygotes (78%) were observed. A total number of 55 blastocysts (31%) were observed at day 5 + day 6.

For patients, a total of 550 oocytes were collected, 360 zygotes were observed (65%) and 164 blastocysts at day 5 + day 6 were obtained (45%).

The correlation between ORP levels from FF and BP for oocyte donors was 0.90 and 0.51 for patients. The overall ORP levels in FF and BP in patients were 136.2 ± 14.9 mV and 148.3 ± 15.97 mV, respectively. For oocyte donors, ORP levels in FF and BP were 160.3 ± 13.8 mV and 170.28 ± 17.67mV, respectively. There was a mild correlation of ORP levels in BP with numbers of oocytes retrieved ($r = 0.25$). The correlation of BP ORP with oocyte fertilization was 0.24 in patients with autologous oocyte utilization.

The mean ORP levels of FF were 136.2 ± 14.9 mV, versus a mean ORP of 148.3 ± 15.97 mV in BP. The FF ORP levels between 100-150 mV [LRD1] were moderately correlated and predictive of blastocyst development [LRD2].

For donor oocytes there was a weak negative correlation [LRD3] of FF ORP with oocyte fertilization (-0.06) and a weak negative correlation for blastocyst formation was found (-0.18).

Limitations, reasons for caution: The MiOXSYS System (Careous Biotechnology, Lituania) is a device calibrated to measure ORP levels in seminal plasma but not to measure ORP levels in FF nor BP. The number of analyzed data should be increased.

Wider implications of the findings: According to this study, the use of blood plasma to surrogate the measurements of ORP levels in follicular fluid could represent a further strategy to include female patients to be treated by antioxidants before an IVF treatment.

Trial registration number: None

Abstract citation ID: dead093.634

P-276 Telomeres cooperate in zygotic genome activation by affecting DUX4/Dux transcription

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Study question: Whether the activation of DUX4, a key inducer in the process of zygotic genome activation (ZGA), is associated with telomere length.

Summary answer: Telomeres regulate the expression of DUX4/Dux through chromatin remodeling and are thereby involved in ZGA.

What is known already: In human early embryos, the expression of DUX4 is activated as a key inducer in the initial stage of ZGA, and it, in turn, activates hundreds of genes in the cleavage-stage embryo. Human DUX4 is localized to the subtelomeric region 4q35.2 with a D4Z4 repeat of approximately 10 to 100 units that encodes a homeodomain transcription factor. DUX4 expression is inversely proportional to the telomere length in myoblasts/myotubes derived from FSHD patients

Study design, size, duration: We characterize the dynamics of telomeres during preimplantation development, and assessed the relationship between the expression of DUX4/Dux and telomere length in preimplantation embryos and human embryonic stem cells.

Participants/materials, setting, methods: All sperm and immature oocytes were collected after obtaining written informed consent from the donor couples. Telomere length in gametes and early embryos by telomere-specific quantitative fluorescence in situ hybridization (Q-FISH).

Main results and the role of chance: Zygotic genome activation (ZGA) is initiated once the genome chromatin state is organized in the newly formed zygote. While telomeres are specialized chromatin structures at the ends of chromosomes and are reset during early embryogenesis, the details and significance of telomere changes in preimplantation embryos remain unclear. We demonstrated that the telomere length was shortened in the minor ZGA stage and significantly elongated in the major ZGA stage of human and mouse embryos. Expression of the ZGA pioneer factor *DUX4/Dux* was negatively correlated with the telomere length. ATAC-sequencing suggested that the chromatin accessibility peaks on the *DUX4* promoter region (i.e., the subtelomere of chromosome 4q) were transiently augmented in human minor ZGA. Reduction of telomeric heterochromatin H3K9me3 in the telomeric region also synergistically activated *DUX4* expression with p53 in hESCs. We propose herein that telomeres regulate the expression of *DUX4/Dux* through chromatin remodeling and are thereby involved in ZGA.

Limitations, reasons for caution: Since the mouse Dux gene is not located at the end of the chromosome, the classical telomere position effect (TPE) pathway may not involve in its activation in early embryos. 3D analysis as presented by Hi-C may be able to make a greater breakthrough in confirming the relationship.

Wider implications of the findings: We herein provided detailed data on changes in the telomere length during ZGA in human and mouse preimplantation embryos, explored the possibility that the TPE affects regulation of DUX4/Dux gene expression in embryos, and suggest that telomere chromatin remodeling is involved in the ZGA process.

Trial registration number: not applicable

Abstract citation ID: dead093.635

P-277 Can a validated, livebirth predictive, in-house morphokinetic algorithm, score embryos according to maternal age; providing more information, for patients, regarding chance of success?

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Study question: How does a livebirth (LB) prediction algorithm, built in-house on data from 6228 known outcomes from all ages, perform when categorised by female age?

Summary answer: The algorithm scored and ranked embryos accurately for clinical outcome (FH) regardless of female age; with proportions of poor prognosis embryos highest in older patients.

What is known already: Time-lapse incubation enables development of predictive algorithms using key morphokinetic variables to score embryos according to potential for clinical outcomes. Maternal age is associated with embryo quality and implantation. Understanding whether kinetic events are influenced by increasing age may benefit embryo selection and patient information provision. There is little evidence of a correlation between advancing maternal age and morphokinetics, although incidence of abnormal cleavage is linked to aneuploidy and reduced blastulation. Predictive algorithms are generally applied to embryos irrespective of oocyte age but are they effective in selecting the optimum embryo for transfer comparatively across differing maternal age groups?

Study design, size, duration: A retrospective, comparative analysis of 10034 transferred embryos from 2011 to 2022 in 11 sister fertility clinics. Embryos were cultured for 4 - 6 days in time-lapse incubation before transfer (Embryoscope, Vitrolife). A score was derived using a prospectively validated in-house statistical algorithm, assessing 6 manually annotated morphokinetic variables (t3,t4,t5,t8,tSB,and tB) and trophectoderm grade. Embryos were scored 1 to10 for increasing live birth (LB) potential.

Participants/materials, setting, methods: All transferred embryos were assessed for age of the patient or donor at treatment, traceable to an algorithm score (1-10) with known implantation, defined by presence of fetal heart (KID-FH). Embryos were assigned to two age groups, < 38 years (n6948) or >=38 years (n3086) and compared according to the distribution of algorithm scores. Comparative significance of the two groups, against each other and the whole cohort, was assessed for significance using chi-squared.

Main results and the role of chance: Comparison of the KID-FH per score, for age groups revealed increasing implantation rates and effective ranking per score in each age group, <38 KID-FH scores 1 to10; 9.6%,17.8%, 25.7%, 24.3%, 28.3%, 38.2%, 41.4%, 48.4%, 54.6% and 57.8% and for >=38 score 1-10; 2.0%, 3.8%, 5.9%, 9.9%, 10.8%, 17.9%, 22.2%, 22.0% 31.6% and 36.8%.

KID-FH increased incrementally with increasing score in both maternal age groups, demonstrating that the algorithm is predictive irrespective of maternal age.

The percentage incidence of scores were compared, <38 years, score 1 to 10; 3.8%, 4.6%, 4.5%, 5.0%, 6.2%, 7.5%, 9.3%, 12.4%, 16.8%, 29.8% versus >=38 years 1 to 10; 8.0%, 9.4%, 7.1%, 9.2%, 8.7%, 9.4%,10.5%,11.0%, 11.2%,15.4%. There were significantly higher numbers of embryos with lower scores (p=0.01) in the >=38 age group (scores 1-6) than the <38 age group. The younger ages conversely, had significantly greater high scoring embryos 9-10, (p=0.01), score 7 to 8 were comparable across the age groups.

The algorithm score is effective in determining implantation potential irrespective of maternal age. Embryos with poorer implantation potential are present in significantly greater numbers in the older age group and this age group attains significantly fewer high potential embryos when compare to younger patient embryos.

Limitations, reasons for caution: The data for this analysis was determined from multiple clinics within a fertility group operating under uniform practices. The derived algorithm variables and outcomes may not be transferable to another clinic setting. Follow up is required for known live birth outcomes to confirm comparative prediction to KID-FH per age group.

Wider implications of the findings: The algorithm score infers embryo competence irrespective of age with differences existing only in the prevalence of low and high scores. This algorithm differentiation promises to inform clinicians and patients regarding their chance of success, when utilised to assess embryos. Morphology alone does not have this breadth or predictive capability

Trial registration number: N/A

Abstract citation ID: dead093.636

P-278 What are the effects of ICSI procedure time intervals on in vitro fertilization outcomes?

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Study question: Do time intervals between oocyte pick-up (OPU), oocyte denudation (OD), and Intra-cytoplasmic Sperm Injection (ICSI) impact ICSI outcomes?

Summary answer: ICSI time intervals impact oocyte maturation, blastulation and pregnancy rates. There is no effect on fertilization and cleavage rates.

What is known already: Cytoplasmic and nuclear oocyte maturation are crucial for the success of in vitro fertilization and embryo development. Cumulus cells enhance oocyte maturation. Therefore, prolonged oocyte culture prior to denudation may induce oocyte apoptosis. There is no consensus for optimal time intervals between different ICSI procedures and their effects on fertilization, embryo and blastocyst, development and pregnancy rates.

Study design, size, duration: A retrospective analysis of 794 ICSI fresh cycles was performed at the Alyssa Fertility Group Center, between Juin 2021 and December 2022.

Participants/materials, setting, methods: ICSI cycles were performed on women aged ≤40 years, with at least 3 mature oocytes. Semen partner concentrations greater than 1million/mL were included. Time intervals between OPU, OD, and ICSI were recorded. We evaluate the effects of these timing intervals on oocyte maturation, fertilization, embryo development and pregnancy rates.

Statistical analysis was performed using SPSS 22.0. Kolmogorov–Smirnov, Student *t, Pearson* tests, and ROC curve analysis were used. Level of signification p-*value* <0.05.

Main results and the role of chance: The mean women's age was 33,2 years (SD 4,4). Mean times in hours for OPU/OD, OD/ICSI, and OPU/ICSI were respectively: 01:28(SD 00:37); 02:35(SD 01:01); 04:03(SD 01:08). Positive correlations were seen between maturation rate and OPU/OD; OPU/ICSI intervals respectively (r = 0,217, p = 0,002; r = 0,193, p = 0,005).There were no significant correlation between ICSI time intervals, fertilization and cleavage rates. Top quality embryos rates were positively associated with OPU/OD time interval (r = 0,136: p = 0,049). The blastulation rate was positively associated with OPU/OD, OD/ICSI, and OPU/ICSI time intervals respectively(r = 0,183: p = 0,008; r = 0,211: p = 0,002; r = 0,279: p < 0,001).Pregnancy rates were positively correlated to OD/ICSI and OPU/ICSI respectively: (r = 0,158: p = 0,022; r = 0,188: p = 0,006).

The ROC curves analysis revealed that the cut off value of time intervals between: OPU/OD was 01:10 (AUC = 0,554; IC 95% 0,476-0,632), p = 0,185; OD/ICSI was 02:20 (AUC = 0,600; IC 95% 0,516-0,669), p = 0,023 and for OPU/ICSI: 03:50, (AUC = 0,600; IC 95% 0,524-0,676), p = 0,014.

Limitations, reasons for caution: Trigger time is an important factor for oocyte maturation that should be noted. This study is limited by its retrospective nature. Potential unmeasured confounding factor cannot be excluded

Wider implications of the findings: Time intervals between all the ICSI procedures affect reproductive outcomes. According to our findings, excessive oocyte incubation time before denudation should be avoided. ICSI should be performed 2 hours after OD. Time intervals should be adjusted according to laboratory practice to improve ICSI outcomes.

Trial registration number: Not applicable

Abstract citation ID: dead093.637

P-279 Comparison of AI model performance in predicting blastulation in old and young mice groups

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Study question: Is there a difference in AI performance in predicting blastulation between young and old mice?

Summary answer: AI performance in predicting blastulation is higher in young mice than in old mice.

What is known already: Previous studies have demonstrated AI's ability to predict oocyte development using oocyte or embryo images, but there has been no study to determine if AI performance varies with the age of the female. Maternal age influences oocyte quality and development, but other factors such as diet and environment also play a role. Using mouse experiments can minimize these confounding factors. The study aims to determine if there is a difference in AI performance for predicting blastulation using oocyte images from old and young mice.

Study design, size, duration: We collected oocyte images from 673 B6D2F1 (BDF1) mice at a single research center between July 2022 and January 2023. The samples were separated into two groups based on maternal age, 386 from young mice and 287 from old mice. The development stages including fertilization and blastulation were recorded through daily observation, and the data was divided into a training set of 3 batches and a test set of 2 batches.

Participants/materials, setting, methods: The mice were divided into two groups: young mice (7-9 weeks old) and old mice (62-68 weeks old). The study used images of MII oocytes collected after superovulation, taken with a 200x inverted microscope, and cultured in a single drop. A CNN was trained to predict blastulation and compared for accuracy between the young and old mouse groups. Blastulation was determined by observing the presence of expanded blastocysts or above on day 4.

Main results and the role of chance: The study used 203 and 182 oocyte images of young and old mice respectively for training, and 183 and 105 images for testing. Three convolutional neural network models (VGG16, ResNet50, and DenseNet121) were used to predict blastulation, and ResNet50 was selected as the final model. Without considering age, the accuracy, sensitivity, and specificity of the AI for predicting blastulation were 0.75, 0.74, and 0.76, respectively. The AI model used in the study had accuracy of 0.77, sensitivity of 0.75, and specificity of 0.79 in the young mice group. In the old mice group, the model had accuracy of 0.72, sensitivity of 0.70, and specificity of 0.74. When a t-test was performed, there was a statistically significant difference in accuracy (p = 0.014) with better accuracy in young mice. On the contrary, the sensitivity in the old mice group was the lowest, making it difficult to predict blastulation. As oocyte morphology does not always represent aneuploidy, the AI model using oocyte images may not perform well in the old mice group due to increased aneuploidy.

Limitations, reasons for caution: The limitations of this study include its retrospective design using oocyte images from only one research center. A larger, multicenter study would be beneficial.

Wider implications of the findings: The study found that AI accurately predicted blastulation using mice oocyte images, with higher accuracy for both positive and negative outcomes in young mice. However, accuracy was lower in old mice, particularly for positive outcome, suggesting the need for additional tests like genetic testing for predicting blastulation in old mice.

Trial registration number: not applicable

Abstract citation ID: dead093.638

P-280 Continuous in-situ monitoring of female hormone levels as a tool to improve the efficacy of assisted reproductive technology (ART) and in-vitro fertilization (IVF) treatment

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Study question: Is it possible to remotely monitor a patient's hormone levels during an ART cycle using an unobtrusive and subcutaneously injectable biosensor for real-time monitoring?

Summary answer: It is possible for in-situ remote continuous monitoring implantable devices to successfully measure patient blood hormone levels over a clinically relevant concentration range.

What is known already: Worldwide 48.5 million couples suffer from infertility. In the UK, assisted reproductive treatments (ART) have increased by almost ten-fold in the last 20 years. Hormonal imbalances are the leading cause of female infertility and the success rate of ART tremendously depends on the accurate measurement of the levels of hormones, namely Luteinizing Hormone, Progesterone and Beta Estradiol. These hormone levels change and pulsate during each patient cycle, requiring women undergoing ART treatments to have a blood draw and endocrinology analysis every 2-3 days, making ART treatment highly invasive to a patient's lifestyle and at a high cost.

Study design, size, duration: In order to determine the feasibility and potential of the study question, a minimally invasive biosensor to monitor ART hormone levels in real time was developed. The biosensor was tested in vitro for the range of 1 pg/ml to 1 μg/ml in three analytical replicates. The resulting sensitivity of testing was measured in order to evaluate real-work applications.

Participants/materials, setting, methods: A gold working electrode was utilized and activated using cyclic voltammetry. After activation, a self-assembled monolayer (Thiol-EDC-NHS), was formed on the gold surface, followed by the separate addition of anti-Progesterone4 and Beta-estradiol antibodies. After the addition of each layer, the Differential Pulse Voltammetry (DPV) of the gold electrode was measured. Based on the peak current, calibration curves were plotted and the sensitivity of the sensors was calculated. All electrochemical measurements were performed using CHI potentiostat.

Main results and the role of chance: This electrochemical hormone biosensor technology) is based on the detection of Progesterone4 and Beta-estradiol biosensor electrodes which can continuously detect hormone levels with a limit of detection (LOD) and limit of quantification (LOQ) of 2.01 and 6.72 pg/ml, respectively. Further to this, the biosensor covers the detection of a wide range of hormone concentrations in the blood from 1 pg/ml to 1 μg/ml. The sensor was also characterised by microscopy techniques such as scanning electron microscope (SEM) and Raman microscopy. Next, the biosensors will be validated in-vivo by conducting animal trials allowing for the main parameters of the biosensors to be optimized according to the in-vivo study.

Limitations, reasons for caution: This novel implantable biosensor technology requires more upcoming validation in animal models before then being tested clinically to order to assess its ability for widespread use.

Wider implications of the findings: The development and implementation of an unobtrusive remote and continuous monitoring implantable system for hormonal concentrations monitoring that are vital for success in an ART procedure could transform the entirety of the process as well as positively influence patient's access to care.

Trial registration number: NA

Abstract citation ID: dead093.639

P-281 Single-cell RNA sequencing analysis of granulosa cells in preovulatory follicles in normal ovarian reserve patients undergoing progestin-primed ovarian stimulation and GnRH-antagonist

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Study question: Could the difference in controlled ovarian stimulation protocol between progestin-primed ovarian stimulation and GnRH-antagonist induce change in the expression genes of human granulosa cells?

Summary answer: The mitochondrial DNA (mtDNA) gene expression of granulosa cells in patients who underwent progestin-primed ovarian stimulation was significantly higher than the GnRH-antagonist.

What is known already: The progestin-primed ovarian stimulation (PPOS) protocol has attracted attention and many studies have reported similar pregnancy outcomes for both PPOS and the GnRH-antagonist (GnRH-ant). However, our previous study demonstrated that pregnancy rate was significantly lower in PPOS compared to GnRH-ant (38th Hybrid Annual Meeting of the ESHRE, O-130). In this study, we evaluated the gene expression of granulosa cells (GCs) which affects reproductive outcome. Many studies have shown a close association between the mitochondrial status of GCs and embryo's quality. To date, the change in gene expression of GCs with different controlled ovarian stimulation protocols have not been investigated.

Study design, size, duration: From November 2021 to January 2022, all samples were obtained from the patients who provided written informed consent before the inclusion and underwent controlled ovarian stimulation cycles at a single-institution. Single cell RNA-seq (scRNA-seq) was performed on 2,224 cells corresponding to GCs of the MII-oocyte follicles from a total of 16 patients with normal ovarian reserve (aged $<$ 40 years, AMH $\geqq$1.1 ng/mL) undergoing PPOS with chlormadinone acetate (n = 8), or GnRH-ant with cetrorelix (n = 8).

Participants/materials, setting, methods: The GCs were obtained by follicular aspiration from 16 patients undergoing oocyte retrieval. The follicular fluid (FF) from each follicle was collected and all FF were mixed together in MII-oocyte for sequencing. The GCs were isolated from the blood cells and cellular debris using Percoll gradient centrifugation. Samples for sequencing were prepared using Chromium Next GEM Single Cell V(D)J Reagent Kits. Libraries were sequenced in DNBSEQ-G400, and reads processed with CellRanger and visualized with CellxGene.

Main results and the role of chance: Two population of GCs were used in this investigation: PPOS and GnRH-ant of MII-oocyte. After the quality control, 1,197 cells for PPOS and 1,027 cells for GnRH-ant, respectively, were analyzed. The GCs of MII-oocyte were clustered into 6 groups based on Leiden algorithm and visualized by UMAP analysis. There was no significant difference in the gene expression and cell distribution in each cluster. In the two population of GCs, we identified 12 differentially expressed genes (DEGs). Interestingly, we observed significant increase in expression of mtDNA genes in PPOS of MII-oocyte as compared to GnRH-ant of MII-oocyte. The data were confirmed by qRT-PCR analysis.

Limitations, reasons for caution: Our conclusions are limited due to the inclusion of Asian patients at a single-institution. The results need to be validated across different centers and other ethnicities. Due to the small sample size, these results should prompt further study to confirm our findings.

Wider implications of the findings: These findings describe, for the first time, the DEGs of GCs investigated in different COS protocols at single-cell level. This study suggests that progestin for PPOS induce elevation of the mt DNA gene expression of GCs which may be associated with reproductive outcomes.

Trial registration number: Not applicable

Abstract citation ID: dead093.640

P-282 The role of activin A in the development of embryo: a preliminary insight using a mouse in vitro model

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Study question: Is activin A involved in the preimplantation development of mouse embryo? Do maternal-derived and zygotic protein have a different impact on embryogenesis?

Summary answer: Deletion of zygotic activin A does not impair the preimplantation development of mouse embryos. However, reduced level of maternal activin A affects female fertility.

What is known already: Activin A is a protein secreted by the reproductive system of females, both in mouse and human. Disruption of its signalling pathway is correlated with the occurrence of ectopic pregnancies and miscarriages. Activin A is also a known regulator of the hormone FSH, affecting the growth of ovarian follicles and, consequently, the formation of functional gametes. Furthermore, expression of this protein is detected in embryos themselves and is imperative during postimplantation development. Although activin has been reported to be present from the preimplantation stages, due to the multiple sources of secretion, its role during this period of embryogenesis remains elusive.

Study design, size, duration: In the first part of our project, we compared the phenotype of zygotic knockout mouse embryos (*Inhba*$^{-/-}$, n = 43) with stage-matched heterozygotes (*Inhba*$^{+/-}$, n = 113) and wild-type (*Inhba*$^{+/+}$, n = 42) embryos. In addition, the blastocyst outgrowth assay has been used as an *in vitro* implantation model. The second part of our studies focuses on the protein provided by the mother. Using hypomorphic mutants, we investigated the impact of reduced level of activin A on mouse female fertility.

Participants/materials, setting, methods: We analysed the morphokinetic parameters of *Inhba*$^{-/-}$, *Inhba*$^{+/-}$ and *Inhba*$^{+/+}$ embryos by recording their development using time-lapse microscopy. Then blastocysts were immunostained and the percentage of each cell lineage was quantified using Imaris software. To assess the fertility of hypomorphic mice, we mated them with wild-type individuals. We evaluated the time needed to receive the offspring and the size of the litter. Furthermore, we examined the functionality of oocytes of hypomorphs by *in vitro* fertilisation.

Main results and the role of chance: We revealed that the deletion of zygotic activin A does not affect the rate and course of development of embryos from the zygote to the blastocyst stage. *Inhba*$^{-/-}$ embryos have similar timing of cleavage divisions, compaction, cavitation and synchrony of the cleavage rounds to the stage-matched *Inhba*$^{+/-}$ and *Inhba*$^{+/+}$ embryos. Moreover, *Inhba*$^{-/-}$ embryos do not significantly differ in the number and percentage of cells contributing to the cell lineages of a blastocyst. Despite the reports on the possible involvement of activin A in the embryo implantation process, the obtained *Inhba*$^{-/-}$ outgrowths have normal morphology and a similar surface area to the corresponding control embryos. Therefore, our results indicate that zygotic activin A is indispensable for preimplantation development and embryo implantation. To study the effect of activin A secreted by the maternal reproductive system on embryogenesis, we used mice with a hypomorphic mutation of the activin A gene. We revealed that homozygotic mice have a characteristic phenotype and significantly reduced fertility. Crossing hypomorphic homozygous females with wild-type males resulted in the pregnancy of only one of the seven females. These results, combined with successful oocyte *in vitro* fertilisation experiments, point to the role of maternal-derived activin A in mouse reproduction.

Limitations, reasons for caution: Literature data indicate that oocytes have a pool of protein that is a product of the maternal genome. This maternal activin A can partially compensate for the lack of zygotic protein. Therefore, our further research will address the consequences of maternal and maternal-zygotic activin A knockout on embryo development.

Wider implications of the findings: Separate examination of zygotic and maternal-derived activin A allowed a comprehensive investigation of the contribution of this protein to mouse reproduction and preimplantation embryo development. Despite using a mouse as a model organism, the obtained results may become an argument for discussion of the impact of activin on human pregnancy.

Trial registration number: not applicable

Abstract citation ID: dead093.641
P-283 Is it time to abandon the practice of double embryo transfer?

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Study question: Can single embryo transfer (SET) with euploid embryos be an effective strategy for improving livebirth rate (LBR) and reducing multiple pregnancy rate (MPR)?

Summary answer: SET of euploid embryos leads to higher LBR and lower MPR per embryo transfer cycle compared to DET of untested embryos in all age groups.

What is known already: Multiple pregnancy, IVF, and advanced maternal age are independently associated with adverse obstetric outcomes and their coexistence is likely to lead to aggravation of obstetric risks. DET is often recommended in women aged over 37 with the aim of improving LBR but at the cost of significantly higher MPR.

Preimplantation genetic testing for aneuploidy (PGT-A) was pioneered to select euploid embryos for transfer. We evaluate whether utilization of PGTa with a subsequent transfer of a single euploid embryo can be an effective strategy for reducing MPR and achieving higher LBR compared to DET of untested embryos.

Study design, size, duration: This is retrospective national cohort study using data from the UK Human Fertilisation and Embryology Authority (HFEA). We analysed 137, 186 IVF treatment cycles performed between 2017-2018 in the UK. Other than grouping by maternal age, no further confounders were controlled for. Both fresh and frozen transfers were included.

Participants/materials, setting, methods: We included all IVF treatment cycles. Cycles undertaken for donation or embryo storage were excluded. Cycle parameters including age, ethnicity, the number of previous cycles, previous infertility history, previous pregnancy outcomes, gestational age at delivery, birthweight and early pregnancy outcomes were evaluated.

We compared the LBR and MPR per embryo transfer cycle following a SET of a euploid embryo (PGTa SET) vs. a DET of untested embryos.

Main results and the role of chance: PGTa was utilised in 1990 IVF treatment cycles, of which 1521 had a SET of a euploid embryo (76%, 95% CI 75-78%). Of 135, 195 IVF cycles in which PGTa was not utilised, there 37, 281 had DET of untested embryos (28%, 95% CI 27-28%). There were 319 IVF treatment cycles in which there were no euploid embryos to transfer following PGTa (16%, 95% CI 14-18%). In the untested group, there were 16, 680 IVF treatment cycles in which there were no embryos to transfer (12%, 95% CI 12-13%).

Per embryo transfer cycle, the LBR was significantly higher and the MPR was significantly lower in all age groups in PGTa SET group vs. DET of untested embryos group (including those aged under 35) (43.7% vs. 31.4%, $p < 0.001$ and 0.3% vs. 8.4%, $p < 0.0001$, respectively).

Limitations, reasons for caution: It is unclear whether the HFEA dataset includes all intended collections when assessing outcomes, thus we were unable to report on outcomes per cycle started.

Wider implications of the findings: Advanced maternal age is associated with increased perinatal risk and maternal morbidity. This population are particularly vulnerable to multiple pregnancy. PGTa followed by SET of a euploid embryo is an effective strategy for reducing MPR and improving LBR in all age groups.

Trial registration number: not applicable

Abstract citation ID: dead093.642
P-284 Time-lapse embryo deselection: development of an interpretable numerical prediction algorithm based on 10320 embryos

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Study question: Is it possible to build a numerical Day 3 prediction model based on calculated weightings of time-lapse deselection measures?

Summary answer: A numerical Day 3 prediction model was developed incorporating computed weightings for a range of contributing factors, showing satisfactory performance.

What is known already: The transferability issue of time-lapse embryo selection algorithms is gaining wider attention, with growing evidence revealing altered embryo morphokinetics in response to different culture conditions and patients' profiles. In fact embryo deselection using abnormal cleavage patterns, such as direct cleavage or reverse cleavage, have been shown to produce superior inter-laboratory reproducibility. However data is limited in the literature on the weightings between individual measures for prognosis prediction, where disagreement often exist amongst embryologists. In this study, we aimed to build a mathematical blastocyst prediction model for improved transferability and robustness, by incorporating computed weightings for a range of contributing factors.

Study design, size, duration: Time-lapse annotation data were retrospectively extracted from 10320 Day 3 embryos created at Fertility North between January 2017 and June 2022. A subset of 3432 embryos were excluded due to poor quality according to standard Day 3 criteria. Data training and model development were based on 6019 embryos with subsequent blastulation outcomes up to Day 6. Further validation was conducted using another 969 embryos with known implantation outcomes following single fresh Day 3 transfers.

Participants/materials, setting, methods: Embryos were assessed using both static morphology criteria and time-lapse annotation. Time-lapse deselection parameters included direct cleavage (DC), reverse cleavage (RC) and <4 intercellular contact points at 4-cell stage (<4ICCP). For DC and RC, the number of affected blastomeres was also recorded for the 1st (1-cell), 2nd (2-3-cell), and 3rd cleavage cycles (4-8-cell), respectively. Fivefold cross validation (n = 6019) was used to develop the numerical model, followed by additional validation (n = 969) via receiver operating characteristics (ROC).

Main results and the role of chance: Multivariate logistic regression identified 11 variables that were independently associated with blastulation, including insemination methods (IVF or ICSI, odds ratio or OR = 0.757, 95% confidence interval or CI 0.676-0.847, P < 0.001), maternal age (OR = 0.937, 95% CI 0.926-0.949, P < 0.001), <4ICCP (OR = 0.733, 95% CI 0.599-0.895, P = 0.002), number of DC blastomeres at the 1st (OR = 0.036, 95% CI 0.022-0.058, P < 0.001), 2nd (OR = 0.139, 95% CI 0.109-0.176, P < 0.001) or 3rd cleavage cycle (OR = 0.375, 95% CI 0.270-0.521, P < 0.001), number of RC blastomeres at the 1st (OR = 0.082, 95% CI 0.028-0.238, P < 0.001), 2nd (OR = 0.304, 95% CI 0.231-0.400, P < 0.001), or 3rd cleavage cycle (OR = 0.482, 95% CI 0.411-0.567, P < 0.001), cell number (OR = 0.890, 95% CI 0.849-0.934, P < 0.001) and degree of fragmentation (OR = 0.675, 95% CI 0.598-0.763, P < 0.001) on Day 3. A mathematical model was constructed using coefficients of 11 variables via fivefold cross validation (AUCs ranged from 0.765 to 0.777 in 5 development subsets and 0.757 to 0.782 in 5 testing subsets), giving rise to a blastulation prediction score (range 0-10). Blastulation rates rose (5%, 11%, 28%, 47%, 70% to 84%) along score increments (<4, 4-4.9, 5-5.9, 6-6.9, 7-7.9, 8+, respectively). Further validation of the proposed model using a separate dataset with known implantation outcomes also showed satisfactory performance (AUC = 0.626, 95% CI 0.590-0.662, P < 0.001).

Limitations, reasons for caution: Our model was developed using a clinic-specific dataset so might not generalize to other clinics with different laboratory setups and patient profiles. Prospective validation, ideally using randomized controlled design, is required to validate its effectiveness in clinic practice.

Wider implications of the findings: In comparison to most machine learning or black box-based embryo selection algorithms, the development of our model only engaged mathematical methodologies with complete interpretability. We acknowledge the transferability issue of embryo selection algorithms and would invite external validation of our algorithm by sharing the full details of our mathematical formula.

Trial registration number: N/A

Abstract citation ID: dead093.643

P-285 Analysis, including morphokinetic data, of IVF/ICSI cycles with oocytes containing aggregates of smooth endoplasmic reticulum (SER)

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Study question: Is there a difference in ART outcomes and morphokinetic parameters for SER+ and SER- cycles and oocytes?

Summary answer: Fertilisation and blastulation rates are lower in the SER+ group but no difference was seen in pregnancy or live birth rates.

What is known already: The SER is an organelle found in eukaryotic cells, including oocytes. SERs synthesize lipids and steroids, and store and metabolise calcium ions, needed for fertilization and early embryonic development. SERs can aggregate in the oocyte, forming an intracytoplasmic dimorphism, thought to interfere with reproductive outcomes. It is suggested that SER aggregates in an oocyte may negatively effect embryo development and implantation. Oocytes without SERs, but which arise from the same oocyte cohort as SER+ oocytes may also affect outcomes. Evidence around the safety and use of these SER+ and SER- oocytes is conflicting, with widespread variations in clinical practice.

Study design, size, duration: A retrospective review was conducted of all IVF/ICSI[MW1] cycles between January 2019 and December 2020 at Merrion Fertility Clinic, Dublin. Cycles containing SER+ oocytes were identified, and their outcomes were compared with those of a matched control group of SER- cycles. Within the SER+ group, results were compared for SER+ and SER- oocytes within the same cohort.

Participants/materials, setting, methods: Once participants had been identified, a chart review was undertaken noting demographics, IVF protocol used, duration of stimulation, ART and neonatal outcomes and morphokinetic parameters in the embryoscope. Statistical analysis was performed using PRISM.

Main results and the role of chance: There were 77 SER+ cycles with a total of 135 SER+ oocytes and 645 SER- oocytes, accounting for 8% of total cycles for the study period. These were matched to 64 control cycles with 528 SER- oocytes. 12 embryo transfers occured using blastocysts derived from SER+ oocytes. SER+ cycles had a higher mean oocyte number than controls (9.3 vs 7.3) and 92% of oocytes were mature compared to 88% of the control group. Fertilisation rates were significantly lower in the study group (60% SER+cycle; 68% in SER-cycle), related to the SER+ oocytes in that cohort. Good/top quality blastocyst rates differed (SER+ cycle 28%, SER- cycle 36%) but pregnancy(SER+ cycle 53%, SER- cycle 51%) and live-birth (SER+31%, SER- 32%) rates were similar, even when blastocysts derived from SER+ oocytes were transferred.

5 morphokinetic variables were studied – time to 2-cell (t2), 3 cell (t3) 4 cell (t4), 5 cell (t5) and time to blastulation (tB). T5 appears to be shorter in embryos derived from SER+ oocytes than in controls, with the other parameters being comparable. In terms of neonatal outcomes there were no congenital malformations reported in the SER+ groups and one case of upper limb amelia in the control group.

Limitations, reasons for caution: This study is limited by its retrospective nature. We only have embryoscope data on 46 out of 77 patients with SERs, limiting numbers. Embryos transferred which derived from SER+ve oocytes were limited by the clinic policy to only transfer these embryos if no other embryos available. PGT was not performed.

Wider implications of the findings: This preliminary study shows that, while SER+ cycles have lower fertilisation rates than SER- cycles, pregnancy rates and live birth rates are comparable. Neonatal outcomes are reassuring. Initial analysis of morphokinetic data shows little effect. Larger numbers and further analysis are needed to fully understand the importance of these aggregates.

Trial registration number: n/a

Abstract citation ID: dead093.644

P-286 Clinical pregnancy after blastocyst culture at a stable temperature of 36.6 °C or 37.1 °C : a prospective randomized controlled trial

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Study question: Is there a difference in clinical pregnancy rate after single blastocyst transfer (SET) on day 5, in case of stable culture at 36.6 °C or 37.1 °C?

Summary answer: Clinical pregnancy rates (CPR, heartbeat at 7 weeks) do not differ between blastocyst culture at 36.6 °C and 37.1 °C for five/six consecutive days.

What is known already: Since the beginning of IVF, embryo culture has been performed at 37.0 °C; however, the optimal temperature remains unknown. Changes in incubator types have led to significant improvements in temperature control. Stable temperature control, with temperature gradients of 0.1 °C among chambers, is possible in G210 incubators (K-Systems, Coopersurgical). A previous prospective trial at our centre showed that embryo development on day 5/6 was not affected when embryos were cultured at a stable temperature of 36.6 °C or 37.1 °C. Though not powered for clinical pregnancy, culture at 37.1 °C resulted in an increased CPR when compared to culture at 36.6 °C (46.4% vs. 74.2%).

Study design, size, duration: A prospective randomized controlled trial was performed in a tertiary fertility centre between February 2017 and December 2022. Sample size of 89/89 patients with fresh SET was required achieving 80% power to detect a difference of 0.22 between group proportions (0.43-0.65) at a significance level of 0.05 using a two-sided z-test with continuity correction.

Participants/materials, setting, methods: Patients were recruited the day before oocyte retrieval based on inclusion criteria (SET on day 5, fresh or frozen ejaculated sperm, female age<40 years, BMI<35m^2/kg, <3 cycles for the current child) with final randomization after denudation once six mature oocytes were present. Primary endpoint was clinical pregnancy rate (CPR defined as heartbeat at 7 weeks); secondary endpoints were fertilization, blastocyst development, pregnancy (positive hCG), live birth rate (LBR) and cumulative live birth rate (CLBR).

Main results and the role of chance: A total of 304 patients were eligible for the study of whom 268 signed the consent, 234 were randomized and 181 received SET on day 5: 90 cultured at 36.6 °C and 91 at 37.1 °C. Patients were on average 32.4 ± 3.5 vs. 32.5 ± 4.2 years old, respectively. No differences were observed in embryological outcomes per cycle between both temperatures: 12.0 ± 3.8 vs. 12.1 ± 3.8 COCs retrieved ($p = 0.88$), 10.0 ± 3.1 vs. 9.7 ± 2.9 mature oocytes inseminated ($p = 0.68$) with a maturation rate of 83.2% (901/1083) vs. 81.3% (898/1104) ($p = 0.87$); 8.0 ± 3.1 vs. 7.9 ± 2.7 normally fertilized oocytes with a fertilization rate of 79.9% (720/901) vs. 80.0% (718/898) ($p = 0.96$), respectively. On average 1.5 ± 1.7 vs. 1.4 ± 1.9 ($p = 0.25$) and 1.1 ± 1.1 vs. 0.9 ± 1.0 ($p = 0.45$) blastocysts were vitrified on day 5 and day 6, respectively. Utilization rate per fertilized oocyte was 44.3% (319/720) vs. 42.1% (302/718) ($p = 0.14$). A single blastocyst transfer was performed for 181 patients, leading to a pregnancy rate of 72.2% (65/90) vs. 62.7% (57/91) ($p = 0.17$), respectively. CPR of the fresh cycle was 51.1% (46/90) vs. 48.4% (44/91) [OR (95%CI) 1.05 (0.55-1.96), $p = 0.71$]. To date, a CLBR of 71.1% (64/90) vs. 65.9% (60/91) ($p = 0.64$), was achieved respectively; with seven patients in each group with remaining blastocysts and no live birth yet.

Limitations, reasons for caution: Only a selected patient population with expected good prognosis was eligible for the study.

Wider implications of the findings: Embryos are capable to tolerate small changes in temperature deviations, demonstrated by their similar implantation potential.

Trial registration number: NCT03548532

Abstract citation ID: dead093.645

P-287 Morphokinetic analysis of early embryos subjected to double stranded DNA damage provides evidence for cell cycle checkpoint activity

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Study question: Is there evidence of cell cycle checkpoint activation in preimplantation embryos with double stranded breaks (DSBs) in their DNA, induced using CRISPR?

Summary answer: Cells with persistent double strand DNA damage exhibit longer timeframes to reach key morphological milestones than those with successful repair, consistent with checkpoint activation.

What is known already: In most cell types, DNA damage leads to activation of cell cycle checkpoints that halt the cycle while DNA repair is attempted. It has been hypothesised that early embryos lack (or have relaxed) checkpoints, and that this may be necessary to allow the embryo to divide rapidly and synchronously during the first few mitoses. It is thought that maternal (oocyte derived) inhibitors may be responsible for suppressing checkpoints until activation of the embryonic genome. Inadequate checkpoint control may be responsible for the genetic instability and sensitivity to DNA damage seen during early preimplantation stages, which is of clinical importance.

Study design, size, duration: 84 embryos were generated for research in an IRB approved study. For this purpose, donor oocytes were fertilised with donor sperm using ICSI. 51 of the resulting embryos served as controls, while in the other 33 double strand DNA breaks were created in a highly controlled fashion, directed at specific genomic sites using CRISPR-Cas9 technology. Successfully fertilised oocytes underwent culture in a time-lapse incubator and the duration of key developmental events were carefully timed.

Participants/materials, setting, methods: Precise induction of DNA damage involved injection of CRISPR-Cas9 ribonuclear complex (RNP), along with the sperm at the time of fertilization (ICSI). All embryos were disaggregated on day-3 and their cells subjected to whole genome amplification. Amplified products underwent low-pass next generation sequencing to detect segmental aneuploidy related to failure of DNA repair, while the site targeted using CRISPR-Cas9 was PCR amplified and sequenced to confirm whether it had successfully undergone repair.

Main results and the role of chance: Embryos that successfully repaired the induced DNA damage showed timings of pronuclei appearance/disappearance and cell divisions that were indistinguishable from control embryos. Rates of embryo arrest prior to completion of the second mitotic division were also similar between the two groups (25% versus 24%). In contrast, embryos with unresolved DNA damage, as evidenced by the detection of chromosomal fragments involving a breakpoint at the site targeted using CRISPR, were delayed in reaching the same developmental milestones ($p < 0.0001$ for time to first cleavage division) and displayed a much higher incidence of arrest (63% before the second mitotic division; $p = 0.0002$). The delay in mitotic progression in blastomeres with DNA damage represents strong evidence that a checkpoint is active in the cells of cleavage stage embryos, sensing double strand DNA breaks and slowing the cell cycle. However, the fact that 36% of embryos with unresolved DNA damage continued to progress, albeit at a slower rate, is consistent with the notion that checkpoint control is less stringent in early human embryos. This relaxation of control is likely to contribute to the genetic instability seen at the cleavage stage, including a risk of unresolved DNA damage and a high frequency of chromosomal malsegregation (causing mosaicism).

Limitations, reasons for caution: While altered cell cycle timings are consistent with checkpoint activation, other factors could conceivably influence the duration of key morphokinetic events. Complimentary experiments to

test the functionality of specific checkpoints and/or the activity of their individual components will be required for definitive proof that embryonic checkpoints are active but weakened.

Wider implications of the findings: Segmental aneuploidy, micronucleation and mosaicism are frequently seen in human preimplantation embryos and are associated with reduced likelihood of ongoing pregnancy. Such abnormalities are symptomatic of genetic instability associated with excessively permissive checkpoints. These results highlight the need for culture systems that protect embryos from cellular stressors, especially DNA damage.

Trial registration number: N.A.

Abstract citation ID: dead093.646
P-288 Chat Generative Pre-trained Transformer (ChatGPT) Proves to be an Effective Assistant for Clinical Embryologists in Laboratory Tasks: A Pilot Cross-sectional Study

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Study question: What are the capabilities of ChatGPT in troubleshooting, fact-checking and generating report templates in the *in vitro* fertilization (IVF) laboratory?

Summary answer: Clinical embryologists perceived ChatGPT as accurate and comprehensive in troubleshooting, generating standard operating procedures (SOP), writing reports and fact-finding in the IVF laboratory.

What is known already: ChatGPT is an artificial intelligence (AI)-driven chat robot (chatbot) with 175 billion parameters in its natural language processing model. It is remarkable for its concise, human-like answers to user inquiries. With advanced AI technology, ChatGPT provides in-depth responses, handles complex problems, and addresses intricate questions. This chatbot has received significant recognition and is anticipated to encourage users to employ it for practical applications, including in the IVF laboratory. However, the abilities of ChatGPT in executing various tasks (such as troubleshooting, generating SOPs) in the in vitro fertilization (IVF) laboratory have not been investigated yet.

Study design, size, duration: The aim of this cross-sectional study is to assess the proficiency of ChatGPT in four tasks commonly performed by embryologists: troubleshooting, designing SOPs, composing reports, and fact-checking. An anonymous online survey of clinical embryologists (n = 40) was conducted to achieve this aim. It was performed between December 2022 and January 2023.

Participants/materials, setting, methods: Clinical embryologists participated in a five-point Likert scale (1-Very disagree to 5-Very agree) questionnaire. Participants were presented with eight vignettes generated by ChatGPT related to the investigated four tasks. Embryologists provide ratings about both their perceived accuracy and perceived completeness of the provided answers. We then asked about their intention to incorporate ChatGPT into their daily tasks.

Main results and the role of chance: The median years of experience of survey participants was 11.5 years (IQR 8-18). Among the participants, 37.5% held an undergraduate bachelor's degree, while the largest proportion, 62.5%, held graduate degrees (Masters and doctorates). Embryologists rated ChatGPT as having an accurate (mean Likert score of 3.45) and comprehensive (mean Likert score of 3.36) value for troubleshooting. They also considered it to be accurate (mean Likert score of 3.34) and comprehensive (mean Likert score of 3.26) for writing SOP templates. Furthermore, they found ChatGPT to have an accurate (mean Likert score of 3.71) and comprehensive (mean Likert score of 3.66) value for report writing. Additionally, they deemed ChatGPT to have both an accurate and comprehensive value (mean Likert score of 3.67) for verifying facts. Overall, experienced embryologists perceived an added value of ChatGPT (Likert scores $\geq$3). On average, they rated ChatGPT as accurate (average score of 3.54) and comprehensive (average score of 3.49) in all tasks tested. They also expressed an intention to use ChatGPT in their laboratory work (average Likert, 3.58).

Limitations, reasons for caution: The embryologists who participated in the survey were highly experienced.

Wider implications of the findings: The model has the potential to assist clinical embryologists in resolving issues and performing administrative duties, making it a valuable resource. Embryologists may benefit from integrating ChatGPT into their educational and certification processes, as well as their daily tasks.

Trial registration number: Not applicable

Abstract citation ID: dead093.647
P-289 Evaluation of AI-based, non-invasive and annotation free EMBRYOAID software with embryologists: time and prediction

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Study question: Is an AI model as good as an experienced embryologist?

Summary answer: Properly trained AI models can perform as good as embryologists with respect to accuracy, improving in the same time decisiveness.

What is known already: There are many attempts to solve the embryo selection problem. One way is to determine the specific features of the embryo and on this basis calculate the final score. The non-algorithmic approach uses the professional knowledge of the embryologist to perform the visual analysis and score the embryos. The most promising attempts are using deep learning, specifically CNNs to directly predict pregnancy probabilities from a given image or set of images. Although these tools deliver high quality answers they have rather low intra-embryologist agreement.

Study design, size, duration: Comparing scoring of AI algorithms with embryologists is a challenge, as they miss a common scale, e.g., total ranking of embryos. In order to overcome this problem we have designed a test containing150 pairs of day-5 embryo time-lapses. For each pair of embryos only one gave the pregnancy (implantation based on beta-hCG). We compared our algorithm with the decisions of 10 embryologists with 10 years of experience on average.

Participants/materials, setting, methods: We have created a web questionnaire for the test. It displayed time-lapses for a pair of embryos and allowed the embryologists to choose the more promising one. We have invited doctors from several clinics to take part in the study.

The AI model was tested on the same data, i.e., its goal was to choose between two transferred embryos.

After collection of data, the effectiveness of the embryologists and the model were compared.

Main results and the role of chance: The results of the comparison are as follows. The accuracy of predicting the embryo that gave the pregnancy was:

- 66.9 (CI 63.1 - 70.7) for our model,
- 63.8 (CI 62.6 - 65.0) on average for the embryologists.

The decisions taken by the algorithm are slightly better, however, this holds with rather low statistical significance. Some decisions taken by the doctors have high variance, e.g., there were cases where 5out of 10 decisions indicated one embryo.

In order to understand these variances better, we have divided the test-set into two parts:

a) 57 cases where all doctors agreed on the decision,

b) 93 cases where there were some differences.

On the a) set the decisions of the algorithm agreed with the experts in 95% of cases. While for the set b) the correlation between expert decisions or the algorithms with the ground truth was rather weak,i.e., p-score of approximately 0.1.

The last aspect of this study was the time of making the decision. The average time for all experts was 54 seconds for each decision, while our algorithm took decisions in 2 seconds on average.

Limitations, reasons for caution: The experiment shows high agreement between algorithms and experts in the case when experts agree. However, the difference between the average accuracy scores shows low statistical significance.

Wider implications of the findings: The model returns the result of the analysis almost immediately, thus it can speed up the process of selecting the most significant embryos. The model agrees with the experts in the case when experts agree.

Trial registration number: not applicable

Abstract citation ID: dead093.648

P-290 Choosing the appropriate fertilization method would increase fertilization rate and improve embryo quality

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Study question: The impact of Conventional IVF versus ICSI on embryo morphokinetic is still scarce. Would proper selection of fertilization method affect embryonic development in different subgroups?

Summary answer: In unexplained or tubal factor subgruops, convential IVF was preferable resulting in higher fertilization rate and better embryo quality expressed by less discarded embryos.

What is known already: IVF requires a sperm cell to penetrate the cumulus cells and the zona pellucida, but ICSI can induce insemination without certain processes taking place. Moreover, ICSI is more invasive than conventional IVF. For example, ICSI oocytes are exposed to hyaluronidase and intense light during the denuding process and are damaged by mechanical pipetting. It was found that ICSI-fertilized 4-cell embryos spent approximately 2.5 hours less time in the 2-cell stage than IVF-fertilized 4-cell embryos, and that the 3-cell stage was longer in ICSI-fertilized oocytes. In addition, the first cleavage has been reported to be slow in conventional IVF.

Study design, size, duration: A retrospective analysis of prospective collective data of all fresh ART (assisted reproductive technique) cycles, conducted and underwent in a single IVF clinic center.

Participants/materials, setting, methods: The study collected a total of 11472 fertilized oocytes that were studied using a time-lapse system (Embryoscope). A total of 2085 oocytes were fertilized by insemination (conventional IVF group) and 9387 were fertilized by ICSI (ICSI group). All oocytes in the study were sibling oocytes. All embryos were investigated for the developmental events.

Main results and the role of chance: Significantly Younger maternal age (34.6 vs. 34.1; $P < 0.001$) and more male factor infertility (43% vs. 14%; $P < 0.001$) were in the ICSI group. Normal fertilization (2PN) was significantly more in the conventional IVF (98% vs. 89.9%). Significantly more embryos were transferred and discarded (37% vs 33%; $P < 0.001$(in the ICSI group and more frozen in the conventional IVF group 59% vs. 46.5%; $P < 0.001$).

In the ICSI group all parameters of fertilization and morphokinetics starting in the time to pronuclear appearance till time to morula stage were significantly shorter. KID score was comparable between the groups for all transferred and frozen embryos (3.23 vs 3.3 $P = 0.08$), however, significantly more embryos were discarded in the ICSI group (16.5% vs 8% $p < 0.001$).

Limitations, reasons for caution: This is a retrospective study. We can't report our cumulative pregnancy rate since not all frozen embryos were transferred yet.

Wider implications of the findings: Regardless of male factor, given the debate between IVF vs ICSI techniques in daily IVF cycles, our results spotlight the better results of conventional IVF for anovulation, unexplained infertility and tubal factor and enable us to offer a personalized treatment.

Trial registration number: Trial registration number number is 026-20-HWMC

Abstract citation ID: dead093.649

P-291 Seeking Arrangements: A New Cleavage-Stage Biomarker from 3D Morphokinetics

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Study question: What can we learn from 3D reconstructions of cleavage-stage embryos derived from Hoffman modulation contrast (HMC) time-lapses?

Summary answer: A simple spatial biomarker extracted from 3D embryo reconstructions at the t4 and t8 stages is associated with blastulation, blastocyst quality, pregnancy and live birth.

What is known already: Several works have demonstrated significant associations between t4 cell arrangement and blastulation potential. However, no studies have investigated the impacts of cell arrangement beyond the t4 stage in a clinical setting owing to difficulties visualising the 3D structure of embryos in a safe, cost-effective manner. In the previous ESHRE meeting, He et al. presented a deep learning system for the 3D reconstruction of cleavage-stage embryos from HMC focal stacks recorded in standard timelapse incubators. In this work, we use the aforementioned system to understand the spatial networks present in cleavage-stage embryos and investigate their associations with clinical outcomes.

Study design, size, duration: The study was a retrospective analysis of two imaging datasets from two different clinics. The first dataset (DS1) consisted of 162 t4 embryos with information on blastulation and Gardner grade. The second dataset (DS2) consisted of 202 embryos at t4 and t8 with

information on blastocyst grade, pregnancy, live birth and PGT-A. All data was captured at 11 focal planes on Embryoscope incubators between 2018 and 2020.

Participants/materials, setting, methods: The system proposed by He et al. was used to reconstruct the 3D structure of embryos from their focal stacks. Networks of cell contacts were extracted from the resulting embryo 3D models and each embryo's mean contacts per cell was computed. Statistical analysis of average cell contacts with respect to outcomes was carried out using unpaired t-tests. Moreover, cell contact networks from different embryos were compared to identify embryos with similar cell arrangements.

Main results and the role of chance: At t4, a higher average number of contacts per cell was associated with greater rates of blastulation in DS1 (2.59 vs 2.36, blastulated vs non-blastulated, $p = 0.029$) and blastocyst quality in both DS1 (2.59 vs 2.37, good vs poor, $p = 0.010$) and DS2 (2.51 vs 2.35, good vs poor, $p = 0.017$) where a 'good' embryo is defined as having an embryologist-provided Gardner grade with EXP>2, ICM>C and TE>C. At t8, a higher average number of contacts was associated with increased blastocyst quality (3.36 vs 3.09, good vs poor, $p = 0.017$), pregnancy (3.32 vs 2.87, pregnant vs not pregnant, $p = 0.003$) and live birth (3.40 vs 2.90, live birth vs no live birth, $p = 0.0003$). No associations were found with miscarriage or aneuploidy. Moreover, average contacts at t4 were not correlated with those at t8 ($r = 0.15$, 95% CI [-0.043, 0.34]).

While 4-cell embryos fell neatly into 9 distinct cell arrangements with the 5 most common (tetrahedral, pseudotetrahedral, planar, closed-Y and linear) accounting for 95% of embryos, 8-cell embryos displayed a great degree of variation with 59 distinct cell arrangements, the largest such group representing only 8 embryos.

Limitations, reasons for caution: The datasets used in this study were small. Moreover, DS2 was subject to survivorship bias as it only contained embryos with PGT-A results which necessarily entailed successful blastulation. Furthermore, the 3D reconstruction system was not applicable to all cleavage-stage embryos, especially those obscured by the well or having undergone compaction.

Wider implications of the findings: This work provides evidence for the clinical relevance of cleavage-stage cell arrangement in the human preimplantation embryo beyond the 4-cell stage, which may improve selection techniques for D3 transfers. Moreover, our work provides a strong case for further investigation into spatial biomarkers derived from 3D embryo reconstruction and 3D morphokinetics.

Trial registration number: N/A

Abstract citation ID: dead093.650

P-292 It is feasible to carry out a large-scale multi-national Assisted Reproductive Technology Real World Data (RWD) collection prospectively, encompassing collected patient and treatment cycle information

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Study question: Is it feasible to carry out a large-scale multi-national Assisted Reproductive Technology Real World Data (RWD) collection prospectively, encompassing collected patient and treatment cycle information?

Summary answer: The creation of an international database collecting RWD is feasible. The quality and quantity of the data allow them to be used for research purposes.

What is known already: Real World Evidence (RWE) may help to answer clinical research questions that are relevant to a broad population of patients.

For 40 years, Assisted Reproductive Technology (ART) treatment cycle data have been collected in national registries. The aggregated national data are further collated on an international level. These data are useful for describing secular trends in ART utilization and associated outcomes. However, registries often fall short in providing patient-level and treatment-cycle-level information for data collation and analysis. Furthermore, national registries may restrict access of raw data for open research, for political, infrastructural, or legal reasons.

Study design, size, duration: In a first phase, the OPERA database (Observational retrospective ProjEct for a Research database for ART procedures) included data from cycles recorded between January 2016 and December 2020 in eighteen clinics, providing a robust sample size for Real World Data analyses.

Fondazione per la Ricerca Ospedale di Bergamo (FROM), together with expert clinicians in the ART field, promoted the OPERA project with the financial support from two pharmaceutical companies (Merck KGaA, IBSA SA).

Participants/materials, setting, methods: The Eighteen clinics located in Germany and Italy joining the OPERA data collection, have proven expertise in ART procedures and use MedITEX® DB. The study protocol was reviewed by the competent Ethic Committees and Data Protection Officers. Among the outcomes collected were the mean number of cycles, the pregnancy and birth rate per Embryo Transfer, and the pregnancy and birth rates as a function of female age. The first extraction was scheduled for December 2022.

Main results and the role of chance: Around 75,000 ART cycles were collected from the first ten participating centers. Among the around 45,000 cycles that have available fertilization information , 11,553 (25.7%) were the In-Vitro Fertilization treatments (IVF), 31,202 (69.3%) Intracytoplasmic Sperm Injection treatments (ICSI), and 2,282 (5.1%) a combination of the two techniques. Of the adopted stimulation protocols, 38.43% (10,538) of the total were with variable antagonists, 31.27% (8,574) were with a single antagonist, 24.31% (6,665) were with a long agonist, 5.51% (1,511) were with a short agonist, and 0.35% (96) were with fixed antagonists. The average number of oocytes retrieved is 11.28, with a median of 10.0. The average number of 2 Pronuclear Oocytes (2PN) obtained is 3.7, with a median of 3.0. Moreover, the average number of 8 cell embryos at day 3 is 2.0, with a median of 2.0. It was also recorded the rate of unsuccessful cycles with no oocytes retrieved (4.8%). Among the cycles collected, 2.9% of the total were from frozen oocytes. Most of the ET (40.0%) were performed 5 days after the oocyte retrieval.

Limitations, reasons for caution: Amassing ART cycle information does not compensate for the well-known insufficiencies of primary data quality, of information on confounders and of access to patient level data. Moreover, the technical risk of this project is the potential loss of participant confidentiality, mitigated through a full anonymization.

Wider implications of the findings: The OPERA project may facilitate the transition of ART surveillance from a national aggregate level to a large-scale individual patient and treatment cycle level. Furthermore, as an evolutionary path, the OPERA project may help building large and longitudinal databases, suitable for appropriate epidemiological research in ART.

Trial registration number: not applicable

Abstract citation ID: dead093.651

P-293 Is time-lapse monitoring a safe alternative? Obstetric and neonatal outcomes of a multicenter randomized controlled trial

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Study question: Are there obstetric or neonatal risks associated with time-lapse monitoring and uninterrupted embryo culture compared to interrupted standard culture and conventional embryo selection?

Summary answer: The application of time-lapse monitoring using the Geri+ incubator is a safe alternative to standard culture and selection in terms of obstetric and neonatal results.

What is known already: There is very limited evidence regarding the safety of time-lapse monitoring (TLM) from prospective randomized controlled trials (RCT). Recent RCTs have demonstrated that the application of time-lapse monitoring does not increase (cumulative) live birth rates or the time to pregnancy within one year. However, while most studies only report pregnancy rates, it is essential to also study the safety of this commonly used method.

Study design, size, duration: The obstetric and neonatal outcomes of patients scheduled for day three single embryo transfer who participated in our multicenter RCT on TLM were studied. Three groups were compared: 1) TLE (Time-Lapse Eeva): embryo selection based on the Eeva® Test (a day three TLM algorithm, used adjunctively with morphology) and uninterrupted culture. 2) TLR (Time-Lapse Routine): routine morphological embryo selection and uninterrupted culture. 3) CON (Control): routine morphological embryo selection and interrupted culture.

Participants/materials, setting, methods: In total, 1731 IVF/ICSI patients undergoing their first, second or third oocyte pickup were randomized. Obstetric and neonatal data were registered for all pregnancies occurring after fresh and cryo embryo transfers associated with the initial oocyte pickup cycle as well as natural conceptions within one year. Adjusted relative risks with 95% CI were calculated; in view of the three groups and many comparisons only p-values are presented.

Main results and the role of chance: A total of 838 women had a live birth during the follow-up period (TLE = 281, TLR = 280, CON = 277; p = 0.98). The rate of serious pregnancy complications was not significantly different between the three groups (TLE = 10.7%, TLR = 10.9%, CON = 11.0%; p = 0.99). Mean gestational age at birth was 39.3 (2.0) weeks, 39.4 (1.6) weeks and 39.3 (2.0) weeks, respectively (p = 0.59). The rate of preterm and very preterm birth did not differ significantly between the three groups (<37 weeks: p = 0.50; <32 weeks: p = 0.20). Average weight at birth was 3396 (598) grams, 3394 (565) grams and 3363 (592) grams, respectively (p = 0.76). Low and very low birthweight also did not differ significantly between the three groups (<2500g: p = 0.77; <1500 g: p = 0.88). Health problems immediately after delivery were reported for eight babies in the TLE group, twelve in the TLR group and eleven in the CON group (p = 0.66). Major congenital malformations occurred in four children in the TLE group, four in the TLR group and seven in the CON group (p = 0.54). Minor congenital malformations were found in five children in the TLE group, three in the TLR group and five in the CON group (p = 0.73). No significant differences were observed for the mode of delivery and the APGAR scores.

Limitations, reasons for caution: This study reports the safety of time-lapse monitoring using the Geri+ time-lapse incubator, while more systems are currently available.

Wider implications of the findings: Uninterrupted embryo culture with or without the use of the Eeva® Test selection algorithm does not lead to increased obstetric or neonatal risks when compared to conventional interrupted embryo culture. Our results suggest that TLM is a safe and effective alternative to standard culture and selection.

Trial registration number: NTR5423

Abstract citation ID: dead093.652

P-294 The impact of audit in embryology laboratory and its outcome - A multicentric study

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Study question: Would establishing an audit division in the embryology department of a well-organized multicentric IVF chain improve compliance thereby improving laboratory Key Performing Indicators (KPIs)?

Summary answer: The internal audits not only helped in increasing compliance but also KPIs like blastocyst rate, good blastocyst rate and β-HCG rate across various centers.

What is known already: Audit plays an important role in multiple industries worldwide. It has been proven to improve compliance with system, process and productivity of the organization. It helps an organization accomplish its objectives by using a systematic, disciplined approach to evaluate and improve the effectiveness of risk management, control and governance processes. As every organization has its own set of SOPs and KPIs there's no universally accepted audit process; hence we introduced and implemented an indigenously designed audit system to ensure compliance and optimize laboratory performance as per the organizational Standard Operating Protocol (SOPs) to verify the Quality Management System (QMS) implementation.

Study design, size, duration: A retrospective cohort study was conducted in 30 centers of Indira IVF across India. Audits were planned in 2021/22 and KPIs were compared with pre and post-audit periods. Study duration was April-2019 to March-2022. Various parameters were used to analyze compliance based on checklist of 120 checkpoints divided into 7 categories like facility & lab management, laboratory process, SOP implementation, etc. Audit covered every critical area with 360-degree approach including people, equipment, facility & processes.

Participants/materials, setting, methods: The compliance rate was considered as the primary outcome and blastocyst, good blastocyst and β-HCG

rate were taken as secondary outcomes. Pre-audit (Group-1) and post-audit (GrouP-2) outcomes were compared by paired T-test. The change in compliance rate was calculated as (Average Compliance after 2nd Audit – Average compliance in 1st Audit)/ Average Compliance after 2nd Audit*100. All $p < 0.05$ were considered statistically significant. Statistical analyses were performed using Statistical Package for social science (IBM SPSS), V28.0.

Main results and the role of chance: The average percent increase in compliance rate (74.03 ± 7.49 to 80.0 ± 7.94; $p < 0.001$) was statistically significant. Considering a team of 100 plus embryologists and 175 plus doctors panned across India, this improvement was substantial. The increase in average total blastocyst rate (48.48 ± 15.01 vs 54.52 ± 12.19; $p < 0.001$), average good blastocyst rate (29.15 ± 9.85 vs 31.05 ± 8.37; $p < 0.02$) and average β-HCG success rate (71.73 ± 9.97 vs 75.16 ± 8.28; $p < 0.001$) in G1 and G2 respectively was statistically significant. With respect to 30 individual centers, a statistically significant increase was found in 15 centers for blastocyst rate, in 16 centers for good blastocyst rate and in 7 centers for β-HCG success rate. Leaving 11 centers with blastocyst rate, 7 centers with good blastocyst rate and 17 centers with β-HCG success rates showed improvement though it was non-significant. All audits were carried out by independent competent and qualified auditors. Considering the above-mentioned data, we observed that audit is playing a very vital role in quality management systems. It helps to establish compliance with systems, processes and SOPs thereby improving immediate laboratory KPIs and overall outcomes for even established organizations.

Limitations, reasons for caution: Due to the Covid-19 pandemic, data from April 2020 to March 2021 could not be collected for analysis. The study has been conducted till β-HCG outcomes. Further, implantation rate and live pregnancy has not been analyzed.

Wider implications of the findings: "Audit system" plays a very vital role in the IVF laboratory for even SOP-driven organizations to ensure stringent adherence with regards to implementing and ensuring compliance. It is highly recommended that an audit system should be a part of every organization to improve QMS and overall outcomes.

Trial registration number: not applicable

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P-295 Application of a Time-Lapse Optical Coherence Tomography (OCT) approach in a pilot study to visualise oocytes and embryos in depth

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Study question: Can we visualise model oocytes and embryos during embryo development using OCT non-invasively?

Summary answer: This approach, new to embryology, OCT, allows visualising cells in a 3D perspective, providing more detailed information than standard microscopy from the oocyte or embryo.

What is known already: Time-lapse is a well-established technique in human IVF, but it is limited in its depth of view due to the limitations of classical microscopy. To overcome this limitation, the approach of Optical Coherence Tomography (OCT), allows non-invasive visualisation through optical cross-sections of the embryo or oocyte to produce 3D images. The obtained information can be extended to 4 or 5 dimensions to track movement and elasticity, using a low-power light source and no staining to ensure the minimum effect on the cell. Here, a pilot study was performed in model (porcine) samples using a newly developed "in incubator" system.

Study design, size, duration: A pilot basic study was performed where 16 oocytes and 16 cleavage embryos were obtained and prepared to be visualised using OCT.

Participants/materials, setting, methods: Pig ovaries were obtained from a slaughterhouse, from which follicles were aspirated in order to retrieve oocytes. The best-quality oocytes were cultured for maturation for 44 hours and 16 oocytes were selected for OCT imaging. before fertilisation for 2 hours. Sperm was previously prepared by Percoll gradient. Zygotes were cultured for 6 days until visualisation through OCT. For imaging, samples were prepared in a 16-well Primo Vision dish.

Main results and the role of chance: Oocytes and embryos were successfully imaged, allowing the identification of distinct cellular features. In oocytes, it was possible to identify the germinal vesicle (nucleus) and polar bodies, individual blastomeres in cleavage stage embryos and trophectoderm, ICM and blastocoel in blastocysts. As images were taken throughout different optical sections, it was possible to correlate the position of the areas within the oocytes/embryos and create a 3D image from a blastocyst, understanding the size of the inner cell mass compared with the embryo's overall size. Interestingly, images were obtained non-invasively and under optimal conditions (in an incubator), demonstrating the future utility of OCT for embryo imaging.

Limitations, reasons for caution: An experimental OCT system was developed and placed in the incubator, and all images obtained were in a format of a pilot study. Future imaging sessions are planned to obtain more data points and assess the viability of the embryo.

Wider implications of the findings: Although in the present study, OCT was applied in pig oocytes and embryos, this new approach can be employed in future for human IVF to overcome the current imaging limitations, proving more information on the oocytes and embryos' quality and assisting Artificial Intelligence analysis.

Trial registration number: not applicable

Abstract citation ID: dead093.654
P-296 Supernumerary blastocysts resulting from abnormal cleavage patterns: freeze or not to freeze? A practical question!

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Study question: After eSET, the supernumerary blastocysts that showed cleavage anomalies can be vitrified as second choice embryos. Do they display enough implantation chance to be frozen?

Summary answer: No pregnancy was obtained after frozen transfer of blastocysts with direct uneven cleavage and chaotic cleavage. It is not recommended to freeze those spare embryos.

What is known already: The use of time-lapse incubation offers stable culture conditions and provides comprehensive information on dynamic embryo development. Studies found that abnormal cleavage affects the genome of human embryos and can reduce its developmental potential with embryonic arrest. Aneuploidy contributes also to failed implantation. It was also reported that cleavage anomalies such as direct uneven cleavage and chaotic cleavage affect negatively implantation and live birth. Even if the good survival rate of blastocysts after vitrification has dramatically improved clinical outcome after frozen embryo transfer, implantation and live birth are correlated to embryonic ploidy and healthy cellular machinery.

Study design, size, duration: Retrospective qualitative assessment of time-lapse images for 186 vitrified blastocysts resulting from extended culture of 871 embryos showing morphokinetic abnormalities such as direct uneven cleavage first mitosis (DC1), direct cleavage second mitosis (DC2), fast cleavage (FC), complete chaotic cleavage (CC) and multiple anomalies (MA), during 2021-2022 in a single private hospital, and analysis of the clinical outcome after warming and SET.

Participants/materials, setting, methods: From 871 embryos in Embryoscope + (Vitrolife), 445 total blastocysts and 251 top quality were obtained according to Gardner's grading. 186 of those were vitrified for further transfer. In 5 subgroups of anomalies (DC1, DC2, FC, CC, MA) pregnancy (PR) and ongoing pregnancy rate (OPR) per warming cycle and per transfer were calculated.

The results were compared by SPSS software to overall 2021 center's results.

Main results and the role of chance: The mean female age was 35.4 ± 0.5 years. From the 186 top quality blastocysts that were vitrified, 96 were warmed in 94 frozen cycles with a survival rate of 98.3%, resulting in 93 SET transfers.

A total of 32 pregnancies were obtained from which 21 were ongoing pregnancies. There was no statistical difference in PR per warming cycle or per transfer, and in OPR per warming cycle or per transfer between the overall study group compared to the center's annual results 2021: 34.0% *vs* 40.2%, 34.4% *vs* 40.1%, 22.3% *vs* 30.1%, and 22.6% *vs* 30.7%.

Reasonable PR and OPR per transfer were obtained with blastocysts from DC2, FC and MA patterns, with caution to small sample size: 32.0% and 20.0%, 44.1% and 32.3%, 40.0% and 25.0%. The results were statistically comparable to the center's annual result.

All the transfers from DC1 and CC pattern failed to achieve a pregnancy.

Limitations, reasons for caution: One limitation of the study was its retrospective nature and the other was the small sample size in each subgroup of anomaly. The results should be confirmed prospectively and should compel the live birth rate after delivery of the ongoing pregnancies obtained during the second semester of 2022.

Wider implications of the findings: Abnormal cleavage patterns such as DC and CC are known to affect negatively implantation and live birth. Deselection of embryos showing these specific patterns could significantly increase successful implantation for the accordingly selected embryos. It is therefore non optimal to freeze the spare blastocysts resulting from these peculiar abnormal patterns.

Trial registration number: NA

Abstract citation ID: dead093.655

P-297 Comprehensive artificial intelligence-powered investigation of blastocyst expansion dynamics: associations with competence

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Study question: Are blastocyst expansion dynamics from time of starting blastulation (tSB) to time of biopsy (t-biopsy) indicative of embryo competence?

Summary answer: Early expansion dynamics across 5hours after tB, embryo-proper area (emb-A), zona-pellucida-area (zp-A), ZP-thickness (zp-T) at t-biopsy and their t-biopsy/tB ratios are associated with competence.

What is known already: Blastocyst expansion is the very first morphogenetic event common to several species. Time-lapse-technology (TLT) implementation in IVF allowed deeper understanding of blastocyst expansion process. Some studies leveraged TLT and Artificial-Intelligence to investigate blastocyst expansion timings and dynamics for their association with embryo competence. Huang's group, in particular, designed a quantitative standard expansion assay (qSEA) showing promising results. However, data about qSEA reproducibility are missing. Here we comprehensively investigated the expansion processes between tSB and t-biopsy through Artificial-Intelligence, and adapted the qSEA to our setting that encompasses PGT-A without day3-hatching and single-euploid-blastocyst-transfer.

Study design, size, duration: Retrospective study including 2184 blastocysts cultured in EmbryoScope during 786 PGT-A cycles conducted across 2013-2020. Videos were analyzed through an Artificial-Intelligence-powered tool (CHLOE^{TM}, Fairtility). The software automatically extracted timings in hours-post-insemination and measures as proportions of video frames occupied by each feature under investigation (single pixel = 300μm; wells' area = 90,000μm^2) recorded every 30min from tSB. These data were tested for their association with euploidy and live-birth after 548 euploid transfers via multivariate regressions.

Participants/materials, setting, methods: ICSI, trophectoderm biopsy on fully-expanded blastocysts without day3 zp drilling, and qPCR/NGS to assess full-chromosome non-mosaic aneuploidies were performed. The timings assessed were tSB, tB, tEB and t-biopsy (= end of video). At these timings and every 30min across 5hours after tSB the software recorded the following measures emb-A, zp-A, zp-T, inner-cell-mass area (ICM-A), and ICM-to-trophectoderm ratio. Also increase/decrease ratios for all measures between timings were assessed. Putative confounders (e.g., maternal age, blastocyst quality) were considered.

Main results and the role of chance: Larger emb-A and zp-A at t-biopsy and zp-T at both tEB and t-biopsy were associated with euploidy. Similarly, the ratios zp-A at t-biopsy/tB and t-biopsy/tEB highlighted a larger expansion among euploid versus aneuploid blastocysts. All these differences were confirmed when adjusting for maternal age, morphological quality and tB ($p < 0.01$). zp-A t-biopsy/tB ratio (aneuploid:+68.8% versus euploid:+79.9%) showed a more relevant association than final zp-A at t-biopsy per se (24082 ± 5763 versus 25438 ± 5969μm^2). The ratios zp-T at t-biopsy/tB and t-biopsy/tEB were also significantly associated with euploidy, even when adjusting for confounders ($p < 0.01$). In this case, zp-T at t-biopsy (8.1 ± 3.2 versus 7.1 ± 2.7μm) per se showed a stronger association than zp-T t-biopsy/tB ratio (-50% versus -55%). ICM-A and ICM-to-trophectoderm ratio showed no association with euploidy. All features showed no association with LBs (N = 233/548 euploid transfers).

The qSEA every 30min across 5hours after tB outlined different early expansion dynamics between euploid and aneuploid blastocysts, with the former expanding more (larger areas and thinner zp) and sooner. The differences became significant already after 2.5-3 hours, due to rather constant expansion rates in both groups, but faster among euploid. The same significant trend was reported for euploid blastocysts resulting in a LB versus not.

Limitations, reasons for caution: Retrospective single-center study. Previous studies on qSEA were based on 10 measurements every hour from tB, instead of 10 measurements every 30min. To properly assess the association between expansion dynamics and timings with LB, more transfers are required. To outline a predictive power, instead, a prospective randomized design is warranted.

Wider implications of the findings: Blastocyst expansion dynamics, timings and ratios measured through Artificial-Intelligence, already during the 5hours following tB, provide objective quantitative data associated with embryo competence. qSEA is a promising clinical strategy, user-friendly and easily applicable, that deserves further appraisal. Basic research on the mechanisms that govern blastocyst expansion processes is warranted.

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P-298 Women undergoing IVF-ICSI cycles after mild Covid-19 infection do not show a poor ovarian response: A prospective randomized controlled study

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Study question: Does mild COVID-19 infection in women undergoing IVF-ICSI cycles affect their ovarian reserve ?

Summary answer: A recent past mild COVID-19 infection does not seem to alter the ovarian reserve testing and ovarian response to stimulation in women undergoing IVF-ICSI cycles.

What is known already: Since December 2019, the world has been facing a COVID-19 pandemic. Besides its effect on mortality, COVID-19 infection raises questions about short and longterm effects on general health. Clinical manifestations are highly heterogeneous and involve many different organs. The SARS-CoV-2 virus penetrates human cells by directly binding with angiotensin-converting enzyme 2 (ACE2) receptors present on the cell surface.The ACE2 receptors are present in testes and in ovarian tissue. In the ovary, ACE2 plays a role in the response to gonadotrophins, steroidogenesis regulation, and in follicle development, angiogenesis and degeneration and therefore, SARS-CoV-2 could be responsible for adversely affecting ovarian reserve.

Study design, size, duration: A prospective randomized controlled study was conducted between June 2020 and December 2021. Women with primary or secondary infertility undergoing IVF-ICSI between the age of 25-40 years were included. All the women underwent a COVID testing by RT-PCR and were subsequently tested for ovrian reserve by assessing their AMH and AFC (USG).

Participants/materials, setting, methods: The study population consisted of 128 women, 22% of whom were COVID RT-PCR positive. None of the tested women presented with a history of severe COVID-19 infection. They were randomized into two groups depending on whether they were positive for past COVID-19 infection or negative. These women were then subjected to AMH testing and estimation of AFC by transvaginal ultrasonography.

Main results and the role of chance: The difference between the initial AMH concentration and AMH concentration tested during ART treatment was not significantly different between the COVID RDT positive group and COVID RDT negative group (–1.24ng/ml [–0.35 to –1.61) versus –0.56ng/ml [–0.15 to –1.11], P = 0.22). Similarly the AFC (Antral Follicle Count) was also not significantly different between the two groups of patients.

The results of this prospective study showed that, based on AMH concentrations, mild COVID-19 infection did not affect ovarian reserve in our population of asymptomatic women who underwent an ART protocol susequently.

Limitations, reasons for caution: First, it has been shown that the AMH concentration is modified during ART treatment as this hormone is secreted by granulosa cells of small growing follicles thus reflecting the granulosa cell activity. Second, a relatively small number of women were included in the analysis and larger study groups are required.

Wider implications of the findings: The wider implication of our study is that AMH concentrations were tested in the same women at different time points and could, therefore, analyse any potential modification of the ovarian reserve after COVID-19 infection. This information can be used to reassure the population who have been afflicted by COVID-19 infection.

Trial registration number: not applicable

Abstract citation ID: dead093.657

P-299 Influence of one carbon metabolism supplements over developmental dynamics and gene expression of genes related to DNA methylation machinery in mice

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Study question: Does betaine and the active metabolite 5-MTHF influence cleavage dynamics and expression of genes related to methylation during preimplantation embryos?

Summary answer: Supplementation of betaine and 5-MTHF had no influence over morphokinetics of mice preimplantation embryos but induced transcriptional variations over methylation activity related-genes.

What is known already: After fertilization, the emerging genome is reprogrammed to enable the control of cellular differentiation all over downstream events and lead further developmental progression. One of the most determining processes defining these new genomic states in cell daughter is mediated by methylation. S-adenosylmethionine (SAM) is the main donor of methyl groups to DNA and its availability during highly active conditions can be sensitive to external conditions and dependent on one carbon metabolism (OCM). DNA methyltransferases (DNMT1, DNMT3a and DNTM3b) and methionine adenosyltransferase (MAT2a and MAT2b) are influenced by OCM and key components of DNA methylation machinery.

Study design, size, duration: This is a prospective experimental study conducted from November 2022 to January 2023. F1 hybrid (B6/CBA) fresh mice embryos were exposed to different culture conditions. A primary analysis consisted of the morphokinetics assessment of embryo development through time-lapse system up to expanded blastocyst (105 hours), including a score assessment based on blastocyst appearance. Subsequently functional analysis based on gene expression of selected genes was carried out over individual embryos.

Participants/materials, setting, methods: The zygotes were collected from the oviduct, washed and culture in KSOM supplemented with 5-MTHF and betaine at respectively concentrations of 50µM and 50µg/mL (THF/bet(50)), 10µM and 10µg/mL (THF/bet(10)) and no supplements for control. Geri incubator was used for culture at 37 °C, 6%CO_2 and 5%O_2. In a new assay, 4cells and 8cells embryos exposed to THF/bet(50) and control conditions were individually collected for pre-amplification and subsequent RT-qPCR quantification using GADPH as housekeeping gene.

Main results and the role of chance: A total of 54 mice embryos were used for the present study. The timings required for embryos to reach different developmental stages (t2, t4, t8 and tB) were manually selected and their comparison did not show any overall differences. A slightly faster trend could be observed for the THF/bet(50) group during the 3 first cleavage events (t2, t4 and t8), this group showed proportionally better morphology of blastocysts.

Expression analysis showed no variation for DNMT1; by contrast, greatest changes were observed for DNMT3a and DNMT3b whose relative expressions were downregulated at 4 cells ~0.5 fold-changes ($p < 0.01$) and highly increased for more than 2 fold-change in 8 cells in THF/bet(50) group. In addition, early downregulation at 4 cells was observed for either MAT2a and MAT2b with ~0.5 fold-changes ($p < 0.01$); however, at 8 cells levels were reestablished in both similar levels than control.

One carbon metabolism induced changes in gene expression of enzymes involved in *de novo* methylation of DNA DNMT3a and DNMT3a. In addition, Methyl adenyl transferases MAT2a and MAT2b were also influenced, particularly MAT2a which has a pivotal role relevant for genome activation in the embryo.

Limitations, reasons for caution: The translational approach is still limited due to known differences between kinetics of mice and human embryo. Intracellular concentration of folates and betaine in embryos are still unknown and the range of dosage used has been decided accordingly to previous studies showing a response in mice embryos under similar conditions.

Wider implications of the findings: Folate supplements are widely accepted for pregnant and women attempting to become pregnant, these are also recently incorporated in some IVF mediums. The influence of OCM in surrounding environment during highly active methylation activities of early embryo is still unknown, benefits or risks of these supplies require further assessment.

Trial registration number: not applicable

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P-300 Not all DUCs are the same: Impact of DUC type on the blastulation, utilization and ploidy

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Study question: We have identified six categories of DUCs as identified automatically by CHLOE-EQ. Do these DUC categories differ in embryo competency?

Summary answer: DUC-1, Major chaotic DUC and Fragmented DUC have compromised blastulation, utilization and ploidy, compared to DUC2, minor chaotic DUC and not-direct DUC.

What is known already: CHLOE-EQ (Fairtility, an AI-based support tool) automatically annotates embryo morphokinetics and identifies embryo division anomalies, such as Direct Unequal Cleavage(DUCs). DUCs are defined as less than 5 hours from two cells to three cells. DUCs have been associated with being severely compromised, with lower chance of blastulating, being utilized, euploid, implanting or leading to live birth. In some clinics, DUCs are automatically discarded. Other clinics reported euploids and live births from DUC embryos, raising questions as to whether there are different types of DUCs with different competencies. In this study, we identified 6 types of DUCs and assessed their viability.

Study design, size, duration: Retrospective cohort study that took place between March to July 2022 at a private fertility clinic in Spain. This study included 1032 time-lapse videos of embryos with Direct unequal cleavage (DUCs) as identified by CHLOE-EQ. CHLOE-EQ defines DUCs as (t3-t2)<5 hours. DUCS annotated by CHLOE-EQ were classified into 6 types by embryologists.

Participants/materials, setting, methods: DUC1(direct division from the 1-cell to 3 or more, without a visible 2-cell stage); DUC2 (Direct Division from 2-cells where either cell divides directly from one to three cells); Minor Chaotic DUC (asynchronous irregular divisions: cells still countable); Major chaotic DUC (asynchronous irregular divisions: cells/fragments are too chaotic to count); Fragmented DUC1 (resembles a DUC1, but the third 'cell' is a fragment); Not direct DUC (quick division from 1 cell to 2cells to 3cells).

Main results and the role of chance: CHLOE-EQ correctly identified 97.4%(875/898) of 2PN DUCs, based on the definition of DUC (t3-t2<5).

Most DUCs annotated by CHLOE-EQ, were classified by embryologists as minor chaotic [25%(231/921)], followed by DUC 1 [20.3%(187/921)], Not direct DUCs [18.1%(167/921)], major chaotic DUCs [14.6%(135/921)], DUC 2 [14%(129/921)], and lastly, fragmented DUC1 [7.8%(72/921)]. The average t3-t2 time did not differ between the six groups (1.6,1.5,1.5,1.6,1.5,1.6,respectively,p>0.05).

Among DUC embryos, Minor chaotic DUCs [85%(110/129)] and Not direct DUCs [66.9%(109/163)] and DUC2 [57%(73/128)] had similar blastulation rates ($p > 0.05$) and utilization rates [DUC2:44.2% (57/129), Minor Chaotics: 37.7%(87/231), Not Direct DUCs: 56.3%(94/167), $p > 0.05$].

These three groups had a higher blastulation rate than DUC1 [20.1% (37/184)], Major chaotic [21.6% (29/134)], Fragmented DUC1 [19.7% (14/71), $p < 0.05$)] and a higher utilization rate than DUC1 [11.8% (22/187)], Major chaotic [14% (19/135)], Fragmented DUC1 [11.1%(8/72), $p < 0.05$)].

Type of DUC was not affected by Age($p > 0.05$). Four live births from single embryo transfer of DUC embryos were recorded in this dataset.

Limitations, reasons for caution: DUCs were assessed by a single embryologist, further studies will assess intra and inter-operator variation in DUC classification across various clinics. It was particularly challenging to differentiate between fragments and cells. This study is ongoing to further understand implication of DUC types on clinical outcome.

Wider implications of the findings: Given that different DUC types have varied competency levels, the results of this study encourage embryologists not to discard DUC embryos simply because they are DUCs. It is important to assess the type of DUC when determining the fate of the embryo and when managing the expectation of affected patients.

Trial registration number: NOT APPLICABLE

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P-301 Assessment of ongoing clinical outcomes prediction of an AI system on retrospective SET data

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Study question: Would patients with fresh and frozen embryo transfer had achieved pregnancy before if the embryo was chosen by AI?

Summary answer: CHLOE (AI) can predict pregnancy, ongoing clinical pregnancy, and miscarriage following a single embryo transfer (SET).

What is known already: The use of time-lapse incubators has provided embryologists with more information to evaluate embryo development, resulting in varying clinical practices among clinics in prioritizing this information. However, manual annotation of time-lapse videos is time-consuming and prone to interoperator inconsistencies. To overcome these challenges, AI tools like CHLOE (Fairtility) can be utilized. CHLOE uses AI-based predictors to predict implantation and provides clarity on the biological factors driving these predictions. However, before incorporating AI tools into clinical practice, it is important to validate their effectiveness.

Study design, size, duration: Single study center that took place between July of 2021 and December 2022 at a private clinic in Spain. This was a retrospective cohort analysis that reviewed 118 time-lapse videos from single fresh embryo transfers and 92 time-lapse videos from single frozen embryo transfers with known ongoing clinical pregnancy outcome. CHLOE EQ score and CHLOE Rank efficacy of prediction of clinical outcomes and miscarriage was quantified using the metric AUC.

Participants/materials, setting, methods: Time-lapse videos were evaluated using CHLOE (Fairtility), an AI tool, to determine CHLOE EQ score and rank related to clinical outcomes (biochemical pregnancy, clinical pregnancy, and miscarriage) following fresh and frozen SET. CHLOE rank and embryology were compared with chi-square and AUC was calculated with logistic regression to measure prediction accuracy. T-test was used to check differences in CHLOE EQ score in different outcomes.

Main results and the role of chance: Embryologist vs CHLOE Ranking weren't significant ($p > 0.05$). In fresh SET the mean EQ score was 7.76, and in frozen SET was 7.07. Following fresh SET, CHLOE EQ score was not-significantly predictive of biochemical pregnancy (AUC = 0.53, n = 104, p = 0.462), clinical pregnancy (AUC = 0.51, n = 79, p = 0.949), and miscarriage rate (AUC = 0.50, n = 68, p = 0.949); CHLOE Ranking was more predictive than embryologist rank for biochemical pregnancy (embryologist vs CHLOE rank: AUC = 0.51, $p > 0.05$ vs AUC = 0.61, $p > 0.05$), clinical pregnancy (embryologist vs CHLOE rank: AUC = 0.51, $p > 0.05$ vs AUC = 0.70, $p > 0.05$) and miscarriage rate (embryologist vs CHLOE rank: AUC = 0.51, $p > 0.05$ vs AUC = 0.75, $p > 0.025$). Following frozen SET, only top 1 embryos ranked by embryologists were transferred, and CHLOE EQ score was predictive of biochemical pregnancy (AUC = 0.60, n = 85, p = 0.213), clinical pregnancy (AUC = 0.64, n = 60, p = 0.919), and miscarriage rate (AUC = 0.87, n = 52, p = 0.437); CHLOE Ranking was predictive of biochemical pregnancy (AUC = 0.59, $p > 0.05$), clinical pregnancy (AUC = 0.64, $p > 0.05$) and miscarriage rate (AUC = 0.90, $p > 0.05$).

Limitations, reasons for caution: This study is a single-center retrospective analysis where embryos were chosen for transfer by human embryologists and is part of a broader effort to validate the responsible integration of AI into clinical practice.

Wider implications of the findings: The use of AI-based tools has the possibility to enhance the consistency, efficiency, and effectiveness of embryo selection. The information from quantitative and qualitative morphokinetics

provided by AI tools like CHLOE brings greater clarity to predictions, enabling more personalized care for each individual embryo.

Trial registration number: Not Applicable

Abstract citation ID: dead093.660
P-302 Correlation of blastocyst shrinkage-pattern during vitrification and post-warming embryo performance

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Study question: Can blastocyst shrinkage-pattern during vitrification be predictive of post-warming survival and reproductive potential?

Summary answer: Blastocysts assuming a specific shrinkage-pattern during vitrification are liable to lower post-warming morphology and, potentially, degeneration.

What is known already: Blastocyst vitrification is a highly standardized technique. However, there are still possible factors than can compromise the outcome of the technique which should be minimized. For example, the degree of blastocoel expansion before vitrification may negatively influence embryo survival post-warming. There are studies which demonstrate that expanded blastocysts exhibit lower survival rate because of their higher blastocoel volume and amount of water, which is more prone to the detrimental ice crystal formation during vitrification. Moreover, shrinkage of expanded blastocysts during vitrification differs as some of them shrink in a uniform, spherical way while others shrink in a non-canonical way

Study design, size, duration: A retrospective study was conducted at IAKENTRO fertility clinic, Thessaloniki. A total of 2167 blastocysts vitrified between November 2018-December 2022, out of which 532 blastocysts warmed during the same period, were included in the present study. No biopsied or PGT-a tested embryos were included. Embryo vitrification was performed following the same protocol during the whole period of the study. For the clinical outcomes, 178 single-embryo transfers and 123 double-embryo transfers were analyzed

Participants/materials, setting, methods: During blastocyst exposure to the vitrification solution two shrinkage patterns were detected, allocating embryos into Group-1 for blastocysts presenting shrinkage without any detachment of the trophectoderm cells from the zona pellucida, leading to a ZP reforming which eventually resembled a "deflated" ball and into Group-2 for blastocysts that collapsed in a uniform way, having their TE cells fully detached from the ZP, which remained spherical throughout the exposure to the vitrification solution

Main results and the role of chance: In the total cohort of 2167 assessed blastocysts, 437 were allocated in Group-1 and 1730 in Group-2, which can be translated as 20,2% event occurrence. The Group-1 blastocysts were mostly of expansion degree 4 (76,7%) and degree 3 (23,3%), according to the Gardner system, while no smaller blastocysts were assigned in this group. As primary outcome was examined post-warming embryo survival with blastocysts in Group-1 presenting significantly higher degeneration rate (22/187, 11,7%) respect to blastocysts in Group-2 (7/345, 2%) (χ^2 test, $p < 0.0001$, OR = 5,798, 95%CI = 2,511 to 14,55). Moreover, in order to assess any potential decline in embryo morphology post-warming, we corresponded Gardner's system evaluation to a numerical score and we calculated the integrity in morphology before and after vitrification. Before vitrification, embryos' morphology score was similar in both groups (74,8% and 73,1% for Group-1 and Group-2, respectively) while, after warming, Group-1 blastocysts exhibited significantly lower integrity of their morphology (86,52% integrity in score) respect to Group-2 blastocysts (93,82% integrity in score) (t-test, $p < 0,0001$). However, when analyzing PR and CPR no significant differences were observed between the two groups

Limitations, reasons for caution: The retrospective aspect of the study is per se a general limitation. Moreover, given that the analysis of the data was not limited to freeze-all cases, the embryos chosen for the fresh embryo-transfers, which means those with the highest implantation potential, were excluded from the study's cohort

Wider implications of the findings: Our results show that blastocysts resembling a "deflated" ball during vitrification have compromised post-warming survival. Given that this phenomenon is exclusively observed in expanded blastocysts, whose ZP is thin and TE cells are stretched, identifying the expansion grade over which is happening could be beneficial in clinical practice

Trial registration number: Not applicable

Abstract citation ID: dead093.661
P-303 Outcomes of day 5 versus day 6 blastocysts: correlation of euploidy, implantation and embryo quality

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Study question: Are outcomes of day 5 (D5) blastocysts better than day 6 (D6) blastocysts in a PGT-A program?

Summary answer: Outcomes of day 5 embryos show more blastocysts, better quality, more euploidy due to better quality and higher implantation on same quality than day 6.

What is known already: Human embryos optimally reach the blastocyst stage after five days of culture, but some have a slower development. The superiority of day 5 blastocysts compared to day 6 blastocysts in fresh cycle transfers was previously demonstrated and attributed mainly to endometrial asynchrony.

Data from frozen blastocysts transfers showed conflicting results, some studies have announced higher pregnancy rates after day 5 transfer compared with day 6 transfer, while others have shown equivalent outcomes.

However, none of these studies have compared outcomes between same quality and euploid day 5 (D5) frozen blastocysts versus those frozen on day 6 (D6).

Study design, size, duration: Retrospective observational study performed in a private centre between May 2017 and December 2022.

The study includes the data analysis of 5599 D5 and D6 blastocysts undergoing PGT-A obtained from 819 patients following 1295 PGS cycles. 948 euploid blastocysts with known implantation outcome were transferred in 789 frozen embryo transfers.

Blastocyst morphology was scored in 3 groups: A: excellent (AA, AB, BA), B: good (BB), C: average and poor-quality embryos (BC, CB, CC). (Gardner-Schoolcraft classification)

Participants/materials, setting, methods: PGT-A with NGS technology was offered to patients of advanced maternal age and/or with repeated IVF failures. Trophectoderm biopsies were performed on D5 and/or D6 embryos, with laser assistance. (Navilase, OCTAX)

We compared both populations (D5 and D6) in terms of number of blastocysts achieved and distribution of embryo quality of each population. As primary outcome, same-quality group results of euploid rate and implantation were compared according to the day of blastocyst development (D5 and D6).

Main results and the role of chance: Vast majority of the blastocysts were biopsied on D5 (71.8%, n = 4017) versus (28.2%, n = 1582) biopsied on D6.

The proportion of three quality-categories of blastocysts according to D5/D6 is uneven. Quality A = 18.6% vs 3,5%. Quality B = 72,4% vs 59,6%. Quality C = 9,0% vs 36,9%, respectively, showing significative differences between D5/D6 (Chi2 p-values of 3 groups< 0.001), concluding that overall D5 quality is better than D6.

Overall euploidy rate on D5 population versus D6 was 31,3% vs 23,3% (p value <0.001), showing significant difference in favour of D5. But layering on 3 quality categories on D5/D6, the euploidy rate was 44,7% vs 51,8% for quality A, 29,6% vs 26,6% for quality B and 17,6% vs 14,9% for quality C, respectively, showing no significative differences D5/D6 (Chi2 p-values:

A = 0,331; B = 0,067; C = 0,280). Euploidy is not D5/D6 dependant but quality dependant.

Overall implantation on D5 (65,9%) is higher than D6 (40,4%) (p value <0.001). Layering again, on D5 the implantation was 75,0% vs 66,7% for quality A, 63,8% vs 46,4% for quality B and 35,3% vs 26,3% for quality C, respectively. Despite implantation being higher on D5 on all three groups only group B shows D5/D6 significative differences. (Chî2 p-values: A = 0,651; B < 0.001; C = 0,360). Implantation is embryo quality and D5/D6 dependant.

Limitations, reasons for caution: The study is limited by its retrospective nature and the low number of grade A D6-euploid blastocysts available to transfer. Additionally, it is common to transfer more than one grade C quality embryo to increase the chances of pregnancy, losing implantation track of these type.

Wider implications of the findings: It was uncontested that D5 blastocysts had better reproductive potential, but we managed to quantify this potential according to euploidy and implantation rates based on embryo quality, which remains the most important predictive biomarker for selecting the best embryo to transfer and reducing the time to achieve a pregnancy.

Trial registration number: not applicable

Abstract citation ID: dead093.662

P-304 Does imaging affect embryo health? Comparative analysis of discrete wavelengths of light on the developing embryo

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Study question: What is the effect of exposing the embryo to discrete wavelengths of light on preimplantation development and resultant offspring health?

Summary answer: Exposure of embryos to red or yellow wavelengths negatively impacted embryo health, pregnancy rate and resulted in offspring that were heavier at weaning.

What is known already: Previous studies have indicated a potential negative impact of shorter wavelengths of light on embryo health. Red and yellow wavelengths are widely considered benign and utilised clinically in time-lapse equipped incubators within IVF clinics. However previous studies had not uniformly and correctly irradiated embryos to enable a fair comparison between different wavelengths.

Study design, size, duration: A current aim of the field is to use optical imaging to predict embryo developmental potential. Such approaches use varying wavelengths of light. The impact of irradiating the embryo with discrete wavelengths of light is not fully understood. Here, we assess the impact of various wavelengths on the developing embryo and for the first time, ensured that the energy dose applied was consistent between wavelengths, thus mimicking fluorescence and time-lapse imaging (470 – 620 nm).

Participants/materials, setting, methods: Preimplantation mouse embryos were exposed daily to blue (470 nm), green (520 nm), yellow (590 nm) or red (620 nm) wavelengths and compared to embryos that were not exposed. We assessed embryo development, DNA damage, and postnatal outcomes following transfer to pseudopregnant recipients.

Main results and the role of chance: We found exposure to the yellow wavelength significantly impaired embryo development to the blastocyst stage ($P < 0.05$). While exposure to blue, green and red wavelengths resulted in significantly higher levels of DNA damage when compared to unexposed embryos ($P < 0.05$). The pregnancy rate was significantly lower when embryos were exposed to the red wavelength ($P < 0.05$). Interestingly, resultant offspring were significantly heavier when derived from red or yellow light exposed embryos compared to those derived from unexposed embryos ($P < 0.01$). Towards understanding the effect on offspring weight we assessed intracellular lipid abundance in the embryo. We found lipid abundance to be significantly elevated following exposure to yellow wavelength (1.8-fold, $P < 0.0001$) but not red. We believe that the role of chance is low as results were collected from multiple independent experimental replicates that were tested using appropriate statistical analyses.

Limitations, reasons for caution: While we demonstrate the distinct impacts of discrete wavelengths of light on the developing mouse embryos including post-natal effects, confirmation of these results in human embryos is required.

Wider implications of the findings: Red and yellow wavelengths are utilised clinically in time-lapse equipped incubators within IVF clinics. Our results demonstrate the potential need to re-evaluate these assumptions. Mapping the stress tolerance embryos show for each wavelength may be advantageous in identifying how damage can be mitigated in clinical manipulation and imaging techniques.

Trial registration number: not applicable

Abstract citation ID: dead093.663

P-305 Detection of GSTM1 polymorphism in women undergoing IVF protocols

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Study question: Is GSTM1 polymorphism related to infertility and IVF parameters?

Summary answer: Possible correlation between the absence of GSTM1 gene and estradiol levels as well as the fertilized oocytes number, the number of COCs and their quality.

What is known already: Glutathione S-transferase (GST) M1 belongs to a family of detoxification enzymes. GSTM1 detoxifies reactive chemical species, by catalyzing their conjugation to glutathione. Deficiency in enzyme activity is caused by homozygous deletion of the GSTM1 gene. It is known that oxidative stress is a condition that leads to pathophysiological mechanisms related to female infertility Additionally several studies have addressed the possible correlation between female infertility and GSTM1 polymorphisms

Study design, size, duration: One hundred and seventy four samples were collected and divided into 2 groups. The study group was consisted of blood samples from 125 women classified as infertile according to WHO. Blood samples from 49 women classified as fertile with at least one successful pregnancy and nomiscarriages were used for the control group

Participants/materials, setting, methods: For all samples, genetic material(DNA) was extracted. Polymerase chain reaction with specific GSTM1 gene primers followed by agarose electrophoresis was applied to detect the presence of polymorphism.

Main results and the role of chance: Our study showed a significant difference between the presence of GSTM1 gene in the control group when compared with the study group. The results showed for a subgroup of the infertile women,(n = 54), that the estradiol levels were significantly higher, in women who had the GSTM1 gene compared to those who lacked the gene. The fertilized oocytes as well as the number of COCs were significantly higher in women in which the GSTM1 was detected, compared to the women in which the GSTM1 gene was not detected.

Limitations, reasons for caution: Detailed data from all infertile women could further confirm our results.

Wider implications of the findings: The results of the current study may be used for further research related to female infertility and oxidative stress and may clarify the possible interactions between the microenvironment of oocytes and the free radicals. Additionally GSTM1 may be a useful biomarker for predicting IVF results.

Trial registration number: not applicable

Abstract citation ID: dead093.664
P-306 Generalizable AI model for microscopic and timelapse multifocal embryo images

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Study question: Can an AI model be applied to datasets with different characteristics?

Summary answer: The AI model predicting pregnancy developed on microscope images can be generally applied to multifocal images from time-lapse system.

What is known already: AI models require large datasets to be able to generalize and handle various scenarios, but it can be challenging to gather enough data for each case. Embryologists evaluate embryos using multiple focal planes and add color filters as needed, and timelapse images have varying features. AI models in past studies were trained using both microscopic and timelapse images, and embryologists question if images must be captured at a specific focal plane for AI to work effectively. In this study, the AI model trained on over 2,000 microscopic embryonic images was validated using timelapse images taken at multiple focal planes.

Study design, size, duration: We collected 2,555 microscopic images from 7 IVF clinics and 1299 timelapse images from 433 embryos from a single IVF clinic between July 2016 and December 2020. The timelapse images were divided into 3 groups, with Group 1 being the best visualized ICM images, Group 2 being 20 μm higher or lower than Group 1, and Group 3 being 20 μm higher or lower than Group 2.

Participants/materials, setting, methods: We built 2 CNN models. The "Microscopic model" and "Timelapse model" were trained and validated using 3-fold cross validation with 2,555 microscopic images, 433 timelapse Group 1 images, respectively. To examine whether the Timelapse model was able to infer well for images taken at different focal points, Group 1, 2, and 3 images were used to test the Microscopic model.

Main results and the role of chance: The AUROCs and accuracies in mean (SD) for the Microscopic model were 0.738 (0.003) and 0.705 (0.011) after 3-fold cross-validation. The AUROC and accuracy for the Timelapse model were 0.627 and 0.583. The AUROCs for Group 1,2, and 3 were 0.699 (0.014), 0.705 (0.001) and 0.701 (0.064), respectively. The accuracies for Group 1,2, and 3 were 0.655. (0.013), 0.647 (0.007), and 0.651 (0.007). The predictive power of the Microscopic model applied to the time-lapse images was better than that of the Timelapse model, although it was not as accurate as the Microscopic model applied to the microscope images.

Limitations, reasons for caution: The limitations of the study include its retrospective nature and a small dataset. Transfer learning of timelapse images to a microscopic model may be beneficial for analyzing timelapse images.

Wider implications of the findings: The study found that timelapse images with multifocal planes can be applied to an AI model built based on a large dataset of microscopic images collected from multiple centers. It may be prudent for a small IVF clinic to apply a generalizable model rather than building its own.

Trial registration number: not applicable

Abstract citation ID: dead093.665
P-307 Is multinucleation as bad as we ever thought?

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Study question: Could the presence of multicleation at early stages influence further embryo development?

Summary answer: The presence of multinucleation at 2 cell stages is independent on the success, but not at 4 cell stage.

What is known already: The presence of multinucleated blastomeres (MNB) in the cleaving embryo has been linked to poor embryo development and adverse in vitro fertilization (IVF) outcomes, when detecte on days 2 and 3 of embryo culture under inverted microscope inspections once a day. Multinucleation has been associated with a lower rate of blastocyst formation, increased rates of aneuploidy and chromosomal abnormalities, leading to chromosomal chaos and a decreased rate of implantation and arrested embryo development. With the introduction of Time-Lapse (TL) technology, it is been demostrated that multinucleation at the 2 cell stage is a very frequent issue.

Study design, size, duration: It is a retrospective observational study. 195 embryos from 28 patients that underwent and IVF treatment at the Unit of Medicine Reproductive of the Hospital General Universitario de Valencia were included in this study from 2017 to 2021 to study embryo development. Those patients whose embryos could be visualized with quality in a Time-lapse incubator were selected.

Participants/materials, setting, methods: The study of the 195 embryos from the 28 patients was carried out in the MIRI® TL6 Time-Lapse incubator. These embryos were checked at the 2-cell, 4-cell and blastocyst stages. The Shapiro-Wilk test was used to determine whether the sample had a normal distribution. and the Pearson test was used to determine if there were significant differences between the samples. Besides, the Chi-square test was used to determine the correlation between 2 qualitative variables.

Main results and the role of chance: 108 fertilized embryos were studied, 78.7% of them did develop blastocyst formation; 50% did not show multinucleation and 28.7% did, the analysis indicated that there was no correlation between blastocyst formation and the presence of multinucleation at 2 cell-stage ($p = 0.364$). The analysis indicated that multinucleated cells showed a prolongation of the division time, the analysis indicated that it had a normal distribution ($p < 0.001$) and it exist significant differences ($p < 0.001$). Pregnancy was obtained in 5% and 9% of the embryos with multinucleation at 2 cell-stages. Finally, analysis of autocorrections on day 2 showed that these occurred in 60% and 40% of 2 cell-stage.

Limitations, reasons for caution: As these data correspond to patient profiles from our unicenter study, the data could be different in other assisted reproduction centers. In addition, a larger study sample size is necessary in order to confirm our results.

Wider implications of the findings: These results can help us to decide the fate of the embryos and not to penalize those embryos that present multinucleations at the 2-cell stage.

Trial registration number: not applicable

Abstract citation ID: dead093.666
P-308 Abnormal Oocytes are more likely to lead to abnormal embryo divisions (Direct Unequal Cleavage,DUC), but do not compromise embryo quality as assessed using CHLOE-EQ score

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Study question: Do oocyte dysmorphisms lead to abnormal embryo divisions and compromised embryo quality?

Summary answer: Oocyte cytoplasmic abnormalities (granularity, Smooth Endoplasmic Reticulum,SER) did not affect CHLOE-EQ score; whilst zona abnormalities (thickness and unevenness) and SER tend to lead to DUCs.

What is known already: Oocyte dysmorphisms include extracytoplasmic [zona pellucida (ZP) evenness and thickness] and cytoplasmic abnormalities [SERs, inclusions, darkness, granularity]. The impact of these abnormalities on embryo development and viability as reported in the literature is contradictory. CHLOE-EQ score is an Artificial Intelligence (AI) based algorithm designed to support embryologists in assessing embryo viability, and has previously been demonstrated to automatically detect embryo development anomalies (such as DUCs), to be predictive of blastulation, utilisation, selection for transfer, ploidy, implantation and live birth. Therefore, CHLOE-EQ is a metric of embryo viability. The impact of oocyte dysmorphisms on CHLOE-EQ and DUCs is poorly understood.

Study design, size, duration: Retrospective cohort analysis of 742 embryo time-lapse videos, cultured at a private fertility clinic between June and July 2022.

Participants/materials, setting, methods: The clinic provided annotations on extracytoplasmic abnormalities (ZP thickness and uniformity) and cytoplasmic abnormalities [SERs, inclusions, darkness, granularity]. CHLOE-EQ (Fairtility) automatically annotated morphokinetics and DUCs and further quantified embryo viability scores (CHLOE-EQ and Blast Score).

Main results and the role of chance: CHLOE-EQ score was not affected by the oocyte having cytoplasmic abnormalities (no vs yes: 4.2 ± 4, n = 122vs4.1 ± 4, n = 359, NS): dark (4.1 ± 4, n = 476vs3.1 ± 4, n = 5, NS), granular (4.1 ± 4, n = 179vs4.1 ± 4, n = 302, NS), SER (4.1 ± 4, n = 455 vs 3.8 ± 4, n = 26, NS), inclusion (4.1 ± 4, n = 430vs4.1 ± 4, n = 51, NS); or ZP abnormalities [overall (4.2 ± 4, n = 379vs3.7 ± 4, n = 102, NS), non-uniformity (4.1 ± 4, n = 409vs4.2 ± 4, n = 72, NS), thick ZP (4.2 ± 4, n = 457vs2.8 ± 4, n = 24, NS), thin ZP(4.1 ± 4, n = 475vs2.1 ± 4, n = 6, NS].

DUC embryos were two times more likely to be derived from oocytes with thick ZP (9/98, 9%oocytes) than oocytes without thick ZP (15/383, 3.9%, p = 0.03). DUCs were more likely to have a non-uniform ZP compared to non-DUCs [DUCs: 7%(7/98) vs Non-DUCs: 17%(65/383), p = 0.015]. DUCs were not associated with the following oocyte cytoplasmic dysmorphias: SER (DUC vs Non-DUCs: 6/98,6% vs 20/388,5%,NS), dark (0/98 vs 5/383, NS), granular (61/98 vs 241vs383, NS), inclusions (11/98 vs 40/383, NS).

DUCs had lower blastulation rate than non-DUCs [DUC:1.8%(2/113) vs Non-DUCs:77%(298/389), p < 0.001]. DUCs were 4-fold less likely to be multinucleated at the 2 cell stage than non-DUCs [DUC: 7%(2/29)vsNon-DUCs: 30%(91/305), p = 0.03]. DUCs were 7-fold more likely to be multinucleated at the 4 cell stage than non-DUCs [DUC: 7%(2/29)vs Non-DUCs:1%(3/302), p = 0.06].

Patient age was not associated with DUCs (DUCs: 36.9 ± 4 vs non-DUCs:37.1 ± 4, NS).

Limitations, reasons for caution: This was a retrospective-single clinic study. Causality is not determined.

Wider implications of the findings: Given the growing evidence that DUCs have compromised viability, it is important to understand the biology of how DUCs are connected to oocyte quality. Using AI to detect DUCs to avoid critical information being missed during embryo assessment can assist embryologists in maximising their efficacy of embryo selection.

Trial registration number: NA

Abstract citation ID: dead093.667

P-309 High-viscosity oil overlay provides less protection against media evaporation in extended dry incubation conditions compared with conventional light oil but a reduced heat loss

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Study question: Does high-viscosity oil (HVO) improve stability on osmolality, temperature, pH, Na^+ concentration, and mouse embryo development compared with conventional light oil (CLO)?

Summary answer: In dry incubation condition, osmolality and Na^+ concentration were significantly higher in embryo culture media overlaid with either oils, but HVO was worst than CLO.

What is known already: Suboptimal *in-vitro* culture conditions affect embryo quality and viability. Overlaying culture media with oil is used to maintain a stable optimal culture environment, including reduction of evaporation. Despite dry incubators are known to increase evaporation, they have become more common, especially with the widespread use of time-lapse imaging. The risk of excessive evaporation with the consequent impact on osmolality is even more significant with the promoted usage of undisturbed extended embryo culturing. Novel products (HVO) therefore been developed and recently commercialised with the aim of reducing such effect. Nonetheless, no independent data are available to determine their superiority over CLO.

Study design, size, duration: Culture media dishes overlaid with CLO or HVO were incubated in dry and humidified conditions while mimicking extended culture conditions in four independent experimental groups. Changes in osmolality, pH and Na+ concentrations over three days were measured. Lipid peroxidation and mouse embryo development were recorded to assess toxicity. Additionally, the ability of different oils to maintain the temperature in the culture drops when culture dishes were removed from the incubator was compared.

Participants/materials, setting, methods: Standard embryo culture media drops overlayered with HVO or CLO were incubated in two equally calibrated, identical incubators providing dry or humid conditions. Osmolality (Day1, D3, D5 measured by freezing-point depression osmometer), temperature drop rate at room temperature (T-type thermocouple sensor probe), pH and [Na^+] (hand-held blood gas analyser), mouse embryo assay (MEA, 75 embryos from naturally mated CD1 female mice (N = 5), and lipid peroxidation end-product levels (MDA, TBARS assay) were compared among groups.

Main results and the role of chance: The osmolality of the media incubated in humidified conditions did not show a significant change throughout the 5-day experiment, regardless of the type of oil used. However, in dry conditions, a significant daily increase in osmolality was evident; changes were more prominent under HVO (D1, 278.13 ± 0.82; D3, 286.00 ± 1.51; D5 289.86 ± 1.17 mOsm/kg H2O ± SEM. p ≤ 0.001). The osmolality of the media overlaid by the same type of oil raised between different incubators, and reached a significantly higher value on D5. [Na^+] values were consistent with the osmolality changes. The recovery time of the temperature on the heated stage to 37 °C was similar in CLO- and HLO-overlaid media (1:07 ± 1:02 min and 2:00 ± 0.7 min, respectively). The temperature drop rate after media were kept at ambient temperature was similar in the first 4 min. By the end, the temperature difference reached a significance between CLO and HVO overlays (32.4 ± 0.4 °C and 32.7 ± 0.4 °C respectively, p = 0.045). No significant differences were found in pH, lipid peroxidation or mouse embryo development between the study experimental groups.

Limitations, reasons for caution: The embryos for MEA were obtained without hyperstimulation, resulting in a small sample size for the study. The exact timing of fertilisation was impossible to detect after mice mating, which caused inconsistency in embryo grading.

Wider implications of the findings: Dry incubators may determine suboptimal embryo culture conditions. A newly developed heavy oil proved to be inferior to conventional light oil in maintaining osmolality stable. Further research should be carried out in engineering systems that limit culture media evaporation or support undisturbed embryo culture in humidified conditions.

Trial registration number: not applicable

Abstract citation ID: dead093.668

P-310 Bringing Transparency to Oocyte Assessment: the importance of including confounders when building Artificial Intelligence (AI) based support tools to quantify oocyte viability

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Study question: Which confounders (sperm quality, oocyte dysmorphism, culture time, images pre or post-ICSI, age) affect the ability of AI to predict blastulation based on oocyte images?

Summary answer: Sperm quality, oocyte dysmorphism, pre or post-ICSI image should be controlled for when building AI algorithms to predict blastulation based on oocyte images.

What is known already: Previous studies reporting on the use of AI to predict blastulation based on oocyte images have: (i) not accounted for confounders affecting blastulation (i.e. sperm quality, culture time), and (ii) used post-ICSI images; without assessing whether the ICSI procedure affects the oocyte image as assessed by AI. Therefore, there is a risk of mislabeling viable oocytes as non-viable due to external factors, which could cause uncontrolled bias and failure to generalize when used in clinical practice. The objective was to assess how these confounders affect efficacy of prediction of blastulation from oocyte images by an AI-based oocyte assessment tool: CHLOE-OQ(Fairtility).

Study design, size, duration: Cohort study. Images of 1281 oocytes (February to June 2022) were taken pre and post ICSI using the Embryoscope, and the embryos cultured until day 7. Oocyte donor source and age, oocyte dysmorphias and sperm quality were documented. CHLOE-OQ algorithm was trained, validated and tested in a diverse data set, accounting for pre and post ICSI image datasets, quality of oocytes, quality of sperm and patient age.

Participants/materials, setting, methods: The primary endpoint was blastulation. Sperm quality data was classified into 4 groups: (A)All (n = 1281), (B)donor sperm only (n = 51), (C)donor sperm and normospermic samples from men not diagnosed with male factor infertility (n = 557), (D)abnormal sperm samples and other diagnosed male factor cycles (n = 747). Eggs were classified by source (own/donor), and by dysmorphisms: enlarged perivitelline space, abnormal Zona pellucida, cytoplasmic abnormalities, dark, enlarged oocytes.

Main results and the role of chance: Post-ICSI images had higher mean CHLOE-OQ score than pre ICSI images (0.28 ± 0.1 vs 0.33 ± 0.1, $p < 0.001$, paired t-test). Discrepancies were particularly identified in oocytes that degenerated following ICSI, and scored 0 by CHLOE-OQ despite having higher scores pre-ICSI. Using Post-ICSI images (AUC = 0.66, 95% confidence interval, CI: 0.63-0.69, n = 1281) improved the efficacy of prediction of blastulation compared to pre-ICSI images (AUC = 0.57: 0.53-0.60, n = 1281, $p < 0.001$), suggesting that ICSI affected the quality of the oocyte, and how an oocyte responds to ICSI, as assessed by AI, contributes to prediction of blastulation.

Efficacy of prediction (AUC) was not affected by the quality of the sperm: (A-OVERALL 0.658 [CI(95%): 0.626-0.687]; B-Donor 0.586 [CI(95%): 0.449-0.728]; C-normospermic 0.645 [CI(95%): 0.600-0.688], D male factor 0.678 [CI(95%): 0.639-0.715]).

Oocyte features associated with low CHLOE-OQ scores were: enlarged perivitelline space, dysmorphic oocytes, abnormal Zona pellucida, cytoplasmic abnormalities and dark and enlarged oocytes. Whilst spherical oocytes with normal zona and perivitelline space were characterized as being more likely to form a blastocyst.

Limitations, reasons for caution: This single-clinic study is retrospective. A multi-center study is underway. External factors affecting blastulation must be accounted for to avoid mislabeling of good oocytes as non-viable. There is also a need to understand oocyte dysmorphias identified by the AI algorithm to ensure biological transparency in clinical decision making.

Wider implications of the findings: Taking into account clinical and gamete confounders when building AI algorithms is a necessary strategy to ensure AI algorithms are generalized when incorporated into clinical practice, whilst reducing bias and promoting transparency in clinical decision making. The risk of not considering confounders leads to mislabeling, bias and inaccurate predictions.

Trial registration number: not applicable

Abstract citation ID: dead093.669

P-311 Embryo morphological grade and day of vitrification can impact the outcome of a single euploid frozen embryo transfer (FET)

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Study question: How does the embryo morphological grade and day of vitrification affect FET outcomes from in vitro fertilization cycles utilizing preimplantation genetic testing for aneuploidy (PGT-A)?

Summary answer: The morphological grade of a transferred embryo affects FET outcomes in age groups differently, and Day-5 embryos have significantly better pregnancy outcomes than Day-6 embryos.

What is known already: The use of PGT-A has shown to increase implantation rates, lower miscarriage rates and promote counseling for a single embryo transfer. Although euploid embryos are the priority for transfer, it is important to examine embryo characteristics that may influence these outcomes within these euploid embryos to maximize the outcome for each FET.

Study design, size, duration: In this retrospective study, 1,554 FETs transferring one euploid Day-5 or Day-6 embryo between January 2020 and October 2022 were evaluated, with the exclusion of donor oocyte, donor embryo, gestational carrier and Day-7 embryo transfer cycles. The inner cell mass and trophectoderm were each categorized as good (G), fair (F) or poor (P) respectively at the time of embryo cryopreservation. A total of 282 GG, 1,053 FG, and 209 FF embryos were transferred.

Participants/materials, setting, methods: Embryo grade categories were used to compare Day-5 versus Day-6 transfer outcomes and by age groups. Separating groups as < 34, 34-37, and ≥38 eliminated age as a significant factor. Primary outcomes included rates of positive chemical pregnancy (CP), biochemical loss (BL), clinical uterine gestation (CIG), and fetal cardiac activity (FCA). Independent t-tests compared age, and aggregate data was analyzed by proportional z-tests. A p-value <0.05 was defined as statistically significant with a 95% confidence interval.

Main results and the role of chance: Age and BL were not significant between transfer days or age groups. Day-5 had higher CP (GG: 84% vs 65%, $p < 0.001$; FG: 75% vs 59%, $p = 0.006$; FF: 63% vs 44%, $p = 0.019$), CIG (GG: 73% vs 57%, $p = 0.006$; FG: 64% vs 48%, $p < 0.001$; FF: 55% vs 34%, $p = 0.007$), and FCA (GG: 66% vs 52%, $p = 0.022$, FG: 60% vs 40%, $p < 0.001$, FF: 55% vs 30%, $p < 0.001$). For patients <34, GG had higher CP compared to FG and FF (74% vs 64%, $p = 0.017$; 74% vs 46%, $p < 0.001$), CIG (66% vs 55%s, $p = 0.011$; 66% vs 38%, $p < 0.001$) and FCA (61% vs 50%, $p = 0.012$; 61% vs 38%, $p = 0.001$). FG had higher CP (64% vs 46%, $p = 0.007$) and CIG (55% vs 38%, $p = 0.008$) compared to FF. For patients 34-37, GG and FG had higher CP (78% vs 53, $p < 0.001$; 70% vs 53%, $p = 0.004$), CIG (66% vs 42%, $p = 0.002$; 56% vs 42%, $p = 0.029$), and FCA (58% vs 35%, $p = 0.002$; 50% vs 35%, $p = 0.011$) compared to FF. When compared to FF in patients ≥38, GG and FG had increased CP (79% vs 47%, $p < 0.001$; 64% vs 47%, $p = 0.012$) and CIG (65% vs 36%, $p = 0.004$; 53% vs 36%, $p = 0.010$). Only GG had higher FCA (56% vs 36%, $p = 0.049$).

Limitations, reasons for caution: Even with the exclusion criteria, the retrospective nature of the study may be viewed as a limitation, so the significance of the results should be evaluated further.

Wider implications of the findings: Based on these results, this study demonstrates the need to compare the characteristics of euploid embryos before selection to maximize the chance of pregnancy. Embryos vitrified on Day-5 or categorized as GG, especially in patients <34, or FG should be prioritized over Day-6 or FF embryos.

Trial registration number: not applicable

Abstract citation ID: dead093.670
P-312 Automated sperm selection software (SiD) is as good as human selection for ICSI

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Study question: Automated sperm selection or human eye-based sperm selection, does it make a difference on IVF lab outcomes?

Summary answer: Single sperm selection by the AI SiD was as proficient as highly experienced embryologists and embryo development may have improved for inexperienced embryologists.

What is known already: The implementation of artificial intelligence in reproductive medicine has proven effective for a variety of applications. One application for this technology is single sperm selection during ICSI. The computer assisted program SiD, was designed to grade sperm, in real-time, based on motility parameters transforming qualitative sperm assessments into a quantitative score. SiD's assistance can be used to select the most optimal sperm for ICSI. The application of sperm selection by AI is expected to bypass user bias, fatigue, limited training, while also optimising time. However, there are limited studies correlating the usage of AI for sperm selection with ICSI outcomes.

Study design, size, duration: Between August and November 2022, sibling MII oocytes were randomly divided in two groups; 1-ICSIgroup: ICSI performed with sperm selected by the embryologist based on motility and 2-SID-ICSIgroup: ICSI performed with sperm selected using SiD software. Priority was given to sperm classified as "Best" by SID software for ICSI. "good" sperm were considered as second choice. Morphology selection was performed before ICSI in both groups. Embryos were cultured during 6 days in same conditions.

Participants/materials, setting, methods: Sperm preparation was performed using a microfluidic chamber (Zymot™). Sperm selection was performed as previously described, and ICSI was performed following routine procedures. Biological outcomes were analysed in both groups and compared including fertilization rate, cleavage rate, embryo and blastocyst development rate, top quality embryo rate and blastocyst development rate. Biological outcomes in both groups were also compared based on embryologist seniority. Finally, embryo morphokinetics parameters were analysed in both groups through manual TLT annotations.

Main results and the role of chance: A total of 226 MII oocytes were included in "ICSI group" and 227 in "SID-ICSI group. A non significant trend towards better outcomes was observed in the SID-ICSI group for all biological outcomes including fertilization rate (81,5% *vs* 80,5%), cleavage rate (98,4% *vs* 97,3%), day 3 embryo development rate (77,8% *vs* 73,1%), top quality development rate at day 3 (50,8% vs 50,0%), blastocyst development rate at day 5 (49,7% *vs* 45,1%), good quality blastocyst development rate at day 5 (48,1% *vs* 46,2%), top quality blastocyst development rate at day 5 (25,4% vs 23,1%). Similarly, a non-significant increase was observed in all these outcomes when comparing both groups when sperm selection is performed by a junior embryologist. Conversely, no difference was seen in both groups when sperm selection is performed by a senior embryologist. Finally, no significant difference was observed in the SID-ICSI group for all fertilization events (tPB2, cytoplasmic wave, tPN1, tPN2, presence of cytoplasmic halo, tPNf, and disappearance of cytoplasmic halo) other cleavage timings (t2->t10, tM, tSC, tEC, tSB) and embryonic cell cycles (ECC1, ECC2, ECC3 s2, s3).

Limitations, reasons for caution: The Zymot™ microfluidic chamber was used for sperm preparation. This device optimises sperm selection, giving rise to an already optimised population of spermatozoa. Future investigations evaluating the effect of SiD automated sperm selection software on biological outcomes in samples prepared with a less effective technique would potentially yield conversing results.

Wider implications of the findings: This pilot study demonstrated that the usage of the automated sperm selection software SiD gives rise to similar biological outcomes and embryo morphokinetics, indicating its effectiveness in sperm selection. We expect this program would be useful in the presence of less experienced laboratory members helping by standardizing sperm selection.

Trial registration number: NA

POSTER VIEWING
ENDOMETRIOSIS AND ENDOMETRIAL DISORDERS

Abstract citation ID: dead093.671
P-313 Dysregulation of endometrial stromal serotonin homeostasis impairs decidualization in patients with recurrent implantation failure via phosphatidylcholine metabolism

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Study question: Does abnormal serotonin homeostasis contribute to impaired endometrial decidualization in patients with recurrent implantation failure (RIF)?

Summary answer: Abnormal serotonin homeostasis in patients with RIF, which is accompanied by decreased expression of monoamine oxidase (MAO), affects decidualization of endometrial stromal cells.

What is known already: Previous studies have found that the expression of MAO, which metabolizes serotonin, is reduced in the endometrium of patients with RIF, and serotonin can induce disruption of implantation in rats. However, whether abnormal serotonin homeostasis leads to impaired decidualization in patients with RIF and the mechanism remains unclear.

Study design, size, duration: Endometrial samples from 31 patients with RIF and 31 fertile patients were used to investigate the expression levels of MAOA, MAOB, and serotonin. We isolated human endometrial stromal cells to investigate the role of MAOA, MAOB and serotonin in inducing decidualization in vitro and further explored the mechanism using RNA-seq and LC/MS analyses.

Participants/materials, setting, methods: The levels of serotonin in the endometrium of patients with RIF were detected by ELISA and immunohistofluorescence, and the key genes of abnormal serotonin metabolism were analyzed combined with single-cell sequencing data. The effects of MAO on the decidualization of stromal cells were investigated using human endometrial stromal cells in vitro induced decidualization model and mouse artificially induced decidualization model. The potential mechanisms of MAO regulating decidualization were explored by RNA-seq and LC/MS analysis.

Main results and the role of chance: We found that women with RIF have abnormal serotonin metabolism in the endometrium and attenuated MAO in endometrial stromal cells. Endometrial decidualization was accompanied by increased MAO in vivo and in vitro. Attenuated MAO caused increased local serotonin content in the endometrium, impairing stromal cell decidualization. RNA-seq and LC/MS analyses showed that abnormal lipid metabolism, especially phosphatidylcholine metabolism, was involved in MAO-deficiency induced defective decidualization. Furthermore, decidualization defects were rescued by phosphatidylcholine supplementation.

Limitations, reasons for caution: This study found that impaired serotonin metabolic homeostasis and abnormally reduced MAO expression were one of the reasons for repeated implantation failure. However, the source and other potential function of serotonin in the endometrium remain to be further explored.

Wider implications of the findings: This study shows new insights into the mechanisms of serotonin homeostasis in human endometrial decidualization and provides new biomarkers or targets for the treatment of patients with RIF.

Trial registration number: not applicable

Abstract citation ID: dead093.672
P-314 PlasmaShotting: a new method of treating "thin" endometrium

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Study question: Is a new method of subendometrial platelet-rich plasma (PRP) injection (PlasmaShotting) effective in patients with "thin" endometrium?

Summary answer: PlasmaShotting showed higher efficiency in the group of patients with "thin" endometrium compared to conventional intrauterine infusion of platelet-rich plasma

What is known already: The problem of "thin" endometrium is one of the urgent challenges of modern reproductive medicine. In patients suffering from this condition, there is a decrease in the pregnancy rate and an increase in miscarriage rates.

"Thin" endometrium is less than 7 mm according to ultrasound. To correct this condition, many methods have been proposed: hormone replacement therapy with high doses of estrogens, aspirin, GnRH agonists, vitamin E, pentoxifylline, sildenafil, intrauterine infusion of colony-stimulating factors (G-CSF) and PRP, but none of the proposed methods has shown enough effectiveness.

Study design, size, duration: To evaluate the effectiveness of PlasmaShotting we organized a study that included 72 people. Inclusion criteria were age less than 40 years, endometrial thickness less than 6.5 mm on days 8-10 of hormone replacement therapy, and 3 or more unsuccessful embryo transfers of excellent quality embryos before.

Participants/materials, setting, methods: The patients were divided into 2 groups:

Group 1 (23 patients) underwent hysteroscopy with submucosal injections of platelet-rich plasma (PRP - PlasmaShotting) on days 10-12 of the cycle.
Group 2 (49 patients) underwent intrauterine infusion of platelet-rich plasma (PRP) on days 10-12 of the cycle

Main results and the role of chance: The clinical pregnancy rate in group 1 was 47.8% (n = 11), and in group 2 was 34.6% (n = 17).

The live births rate in group 1 was 34.7% (n = 8), and in group 2 was 20.4% (n = 10).

The miscarriage rate was in group 1 was 13.1% (n = 3), and in group 2 was 14.2% (n = 7)

Limitations, reasons for caution: The research on the effectiveness of PlasmaShotting should be continued

Wider implications of the findings: PlasmaShotting as a new method of treating "thin" endometrium can be used in patients who can't achieve pregnancy

Trial registration number: not applicable

Abstract citation ID: dead093.673
P-315 To study the efficacy of Transvaginal ultrasound-guided Embryo Transfer over Transabdominal ultrasound-guided Embryo Transfer in transfers with poor visibility due to obesity or marked retroversion

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Study question: Why is Transvaginal guided embryo transfer,understudied and underutilised in difficult cases of embryo transfers with compromised visibility of uterine canal in Transabdominal ultrasound ?

Summary answer: In patients with poor visibility of uterine canal using TAUS due Obesity, retroveted uterus,PID,pelvic adhesions, endometriosis, TVUS-ET gives significantly higher clinical Pregnancy rates.

What is known already: TAUS-ET is most popular method of Embryo transfer worldwide. Despite the benefits , this procedure also presents some important drawbacks. Firstly, TAUS-guided ET requires an assistant to operate the transducer while physician performs ET procedure. Moreover, patient needs to fill her urinary bladder to enable visualization of uterine canal, which causes discomfort and sometimes uterine cramping during ET, which in turn can negatively impact implantation rates.TVUS is the main technique used in all other gynecological investigations. Because of high frequency and close proximity to target area, TVUS transducers provide better resolution of the uterocervical angle and improved overall image quality.

Study design, size, duration: This retrospective study was conducted to compare efficacy of TVUS-guided ET to conventional TAUS-guided ET in difficult transfers, who underwent treatment at Triveni IVF, Darbhanga, India between November 2020 to December 2022. It was based on data extracted from records of all women who underwent difficult TAUS-ET procedure between Nov 2020 to Nov 2021.Data from records of matching number of women who underwent TVUS-ET procedure between Dec 2021 to Dec 2022 were used for comparison.

Participants/materials, setting, methods: All were ICSI and FET transfers.

Inclusion criteria: (1) All included between 25–35 years of age with difficult visualisation of uterine canal during TAUS-Mock ET in previous cycle (2) BMI $\geq$ 30; (3) Retroverted uterus due to any underlying pathology 4)All pts included had good ovarian reserve; (4) unexplained infertility >3 years. Exclusion criteria: (1) severe male factor infertility, (2) any intrauterine factor of infertility (3) ovarian endometrioma or PCOS and (5) multiple ICSI failure (>3 trials).

Main results and the role of chance: Total of 123 cycles,61 guided by TVUS (Group 1) and 62 TAUS (Group 2) were included. Our results showed significantly higher Clinical Pregnancy rate in TVUS group as compared to TAUS group, in difficult transfers (43.3% vs. 36.1 % p = 0.0045). VAS (visual analog scale) of the pain during ET was significantly less in TVUS group in comparison to TAUS group (17% vs 64% p = 0.0001), also the abdominal discomfort was significantly less in TVUS group in comparison to TAUS group (11% vs. 69 % p = 0.0001).

TVUS-ET group showed significantly higher CPR as it has better resolution due to proximity to target organs allowing proper visualization of catheter tip especially in cases with obesity and marked uterine retroversion.TVUS has superior role in cases with difficult transfer because it can easily analyze the cause of difficult transfers as endocervical crypts, marked anteverted uterus, cervical canal tortuosity.

Another advantage of TVUS is requiring emptied urinary bladder in contrast to TAUS. Full bladder is both time consuming and causes abdominal discomfort and uterine cramps leading to increased patient anxiety.As regards to abdominal discomfort and uterine cramps, there was extremely high statistical significance between the two groups in our study.

Limitations, reasons for caution: Despite the wide benefits,TVUS-ET has not been widely adopted, on account of difficulty in manipulating ET catheter and vaginal transducer simultaneously in very constricted area.Other comfortable approaches that may assist physicians such as inner catheter can be pushed by embryologist while physician holds TVS-probe and outer catheter in place.

Wider implications of the findings: The results suggested that TVUS-guided ET was better in difficult transfers with poor visibility of uterine canal in studied sample. This technique presented deserves consideration for wider use since it can offer better visualization, excellent comfort to patients during the procedure, and requires only one operator.

Trial registration number: Not applicable

Abstract citation ID: dead093.674
P-316 Involvement of collective cell migration in endometrial invasiveness in uterine adenomyosis and the role of M2 macrophages

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Study question: How do endometrial cells manage to invade surrounding myometrium to establish adenomyotic lesions and what is the role of macrophages in this process?

Summary answer: M2 macrophage-mediated invasiveness and resistance of endometrial cells to physiological cell death appear to contribute to the pathogenesis of adenomyosis.

What is known already: Despite the high prevalence and debilitating symptoms of uterine adenomyosis, its pathogenesis has not yet been elucidated. To date, the most acceptable theory to explain disease development suggests invasion of the myometrium by eutopic endometrial cells.

Recent data suggest aberrant infiltration of immune cells but not platelets in adenomyosis, eventually leading to enhanced cell motility via collective cell migration (CCM) and resistance to physiological cell death during disease pathogenesis.

Study design, size, duration: A retrospective immunohistochemistry-based study was conducted on formalin-fixed paraffin-embedded uterine tissue from 17 women (8 adenomyosis patients and 9 healthy subjects). Fresh endometrial biopsies were retrieved from 16 women (6 adenomyosis patients and 10 healthy subjects) for in vitro culture of epithelial and stromal cells, and co-culture with THP-1 monocyte-derived M2 macrophages.

Participants/materials, setting, methods: Double immunofluorescence was performed to detect macrophages of M1 and M2 phenotypes. Invasiveness of endometrial cells upon interaction with macrophages was assessed by in vitro invasion assays and quantitative PCR for genes involved in cell motility and epithelial-mesenchymal transition (EMT). Mechanisms of disease progression were investigated by immunohistochemistry against E-cadherin, N-cadherin and matrix metalloproteinase 9 (MMP9). Caspase 3 and microtubule-associated protein light chain 3 beta (LC3B) were studied as markers of apoptosis and autophagy respectively.

Main results and the role of chance: Only M2 macrophages were found to accumulate in adenomyosis, in higher numbers in both endometrium and ectopic lesions from adenomyosis patients than healthy controls. Co-culture with M2 macrophages significantly increased invasion capacity in endometrial epithelial and stromal cells from both adenomyosis patients and healthy controls. Epithelial cells from adenomyosis patients were nonetheless more invasive than their healthy counterparts in the presence of M2 macrophages. No gene expression differences pointing to EMT were noted, either between co-cultured and control cells, or between adenomyotic and healthy cells, refuting the implication of this mechanism in adenomyosis invasiveness. E- and N-cadherin protein expression did not differ significantly between eutopic endometrium from adenomyosis subjects and healthy tissue, but N-cadherin expression was stronger in ectopic lesions. In adenomyosis, both E- and N-cadherin were more extensively expressed in basal (also known as invasive) glands than functional glands, suggesting maintenance of intercellular junctions and subsequent CCM. Extracellular matrix-degrading enzyme MMP9 was more abundantly expressed in eutopic stroma from adenomyosis patients compared to healthy tissue. Lower levels of caspase 3 were detected in both endometrium and lesions from adenomyosis patients, while LC3B was found significantly decreased in adenomyotic stroma compared to that of healthy subjects.

Limitations, reasons for caution: A limitation of the study is the 2D nature of in vitro cultures, which cannot fully reconstitute in vivo cell arrangement and cell-cell interactions, nor allow to observe CCM in real time.

Wider implications of the findings: M2 macrophages accumulating in endometrium and ectopic lesions from adenomyosis patients boost invasion capacity of endometrial epithelial and stromal cells, as shown by in vitro co-culture experiments. These findings indicate that M2 macrophages play a key role in disease pathogenesis and could be targeted to develop novel therapeutic options.

Trial registration number: not applicable

Abstract citation ID: dead093.675

P-317 Associations between endometrial microbiome composition and cellular senescence

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Study question: Is there an association between the percentage of senescent cells and microbiome composition in human endometrium?

Summary answer: We found that cellular senescence is negatively associated with the relative abundance of certain bacterial taxa.

What is known already: Recent studies indicated that endometrial microbiome characteristics and certain levels of cellular senescence are essential for the establishment of normal endometrial receptivity and successful embryo implantation. The secretion of senescence-associated secretory phenotype (SASP) components is responsible for immune cell activation and might cause endometrial dysbiosis. On the other hand, some studies suggest that the presence of specific metabolic groups of bacteria and their metabolites could influence cellular stress response that might suppress the development of senescent cells. However, the direct relationship between the quantity of senescent cells and microbiome content in the endometrium has never been studied.

Study design, size, duration: This was a prospective observational cohort study performed between April 2021 and January 2023. Endometrial biopsies were collected from 30 women aged between 34 and 45 during the mid-luteal phase (LH + 7) in a natural cycle. Each biopsy was divided for genetic and immunohistochemical analyses. The exclusion criteria were history of recent inflammatory disease, chronic endometritis, recent antibiotic treatment, endocrinological disorders, autoimmune diseases, oncological diseases, moderate or severe endometriosis, adenomyosis, uterine hyperplasia, and endometrial polyps.

Participants/materials, setting, methods: We used immunohistochemical biomarker $p16^{ink4a}$ (MAD-000690QD–7, Master Diagnostica) to identify senescent cells. The percentages of positively stained cells in the endometrial stroma and in the luminal epithelium were calculated by HALO image analysis software (version 2.3, IndicaLabs). Endometrial microbiota composition was analyzed after DNA isolation from the endometrial samples using 16S rRNA (v4-v5 region) gene sequencing. Statistical analysis was performed by Spearman's correlation test using SPSS v.21 (IBM Corp., Armonk, NY, USA).

Main results and the role of chance: A total of 271 distinct bacterial species and 668 genera were identified across the studied 30 endometrial samples. The percentage of p16+ senescent endometrial stromal cells ranged from 0 to 1.7%, with a mean value of 0.37%, while the percentage of p16+ luminal epithelial cells ranged between 2.5% and 74.0%, with a mean value of 21.5%

Spearman analysis revealed significant negative correlation between the percentage of p16+ stromal cells and the relative abundance of *Porphyromonas sp.* ($R = -0.41$, $p = 0.03$) and *Sphingobacterium sp.* ($R = -0.37$, $p = 0.05$). The percentage of p16+ luminal senescent cells correlated negatively with the abundance of *Micrococcus luteus* ($R = -0.44$, $p = 0.02$), *Moraxella osloensis* ($R = -0.37$, $p = 0.05$), *Rubellimicrobium sp.* ($R = -0.46$, $p = 0.02$), *Stenotrophomonas sp.* ($R = -0.45$, $p = 0.03$), *Chryseobacterium sp.* ($R = -0.41$, $p = 0.04$), *Carnobacterium sp.* ($R = -0.40$, $p = 0.04$), *Deinococcus sp.* ($R = -0.38$, $p = 0.05$), and *Enhydrobacter sp.* ($R = -0.37$, $p = 0.05$).

Limitations, reasons for caution: The study was limited in sample size. The assessment includes only endometrial samples from the mid-luteal phase of the cycle.

Wider implications of the findings: We found that the level of senescence is negatively associated with the relative abundance of certain bacteria taxa in human endometrium, including some related to impaired reproductive

function and early embryonic development arrest. These findings suggest a possible specific interaction between senescent cells and microbiome that might influence endometrial receptivity.

Trial registration number: The current research was funded by National Science Fund, Ministry of Education, Bulgaria, Contract № KP-06-N53/14/16.11.2021

Abstract citation ID: dead093.676

P-318 Epidemiologic analysis of endometriosis awareness in Turkey

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Study question: What is the prevalence of endometriosis in Turkey, and what are the impacts of endometriosis-related symptoms on social, occupational, medical, and daily life activities?

Summary answer: Endometriosis is a debilitating disease that affects 18% of Turkish women of reproductive age. Social, occupational, and psychological functioning are all negatively impacted.

What is known already: Endometriosis is a chronic, inflammatory disease. It affects about 6–10 percent of women worldwide. It can alter the quality of life, mental health, relationships, and sexuality of the carriers. The delay in the diagnosis of endometriosis causes a loss of workforce, a decrease in productivity, and, most importantly, excessive finances. Previous studies from developed countries show endometriosis carriers have lower annual incomes and higher short or long-term workforce loss. Due to the difficulty of diagnosing most cases, the actual incidence of endometriosis in the population, including in developed countries, is unknown.

Study design, size, duration: This study used a web-based survey to identify the prevalence of symptoms and endometriosis-diagnosed women. For this purpose, the World Endometriosis Research Foundation (WERF) EndoCost tool was utilized and altered by the Turkish demographic and healthcare background. Between September 15, 2022, and November 2022, the survey was accessible. In this process, 16304 surveys were completed, with 15673 being counted.

Participants/materials, setting, methods: The survey's applicants were reached through social media platforms (Facebook and Instagram), which included social media accounts of influencers with followers from all over the country, reports of authors and women's support organizations, countywide student clubs of universities, and nurses working on family medicine in all parts of the country delivering to regions of responsibility. The ethical committee approval have been taken from an university hospital ethical commission (E-10840098-772.02-4247).

Main results and the role of chance: Our study showed that the prevalence of endometriosis in developing countries was not insignificant. This survey had 15673 participants, and 2880 (18.3%) were diagnosed with endometriosis. There was a significant difference in the impact of endometriosis-related symptoms on social, occupational, medical, and daily life between the participants with endometriosis and the non-endometriosis participants. The percentage of participants with endometriosis (1.3%) who reported quitting work/school due to pain was statistically higher than the non-endometriosis population (0.6%) ($P=0.001$). Also, 21.2% of endometriosis participants reported feeling socially isolated related to their condition ($P=0.001$). One thousand one hundred fifty-two individuals with endometriosis reported work/school difficulties (28.3%), and 224 respondents could not attend class/work due to endometriosis-related symptoms (7.4%). In addition, most respondents with endometriosis (46.0%) reported problems in a personal relationship, and interestingly, according to 1820 (63.2%) participants with endometriosis, people did not believe their pain or symptoms. There is also this to consider: our data shows that the average number of physician visits before diagnosis was four.

Limitations, reasons for caution: The study is limited by self-reported patient responses that were not confirmed by medical records or other complementary data.

Wider implications of the findings: Our findings can be used to increase awareness of the prevalence and challenges of endometriosis carriers, which may help develop future prevention programs. Since most participants with endometriosis experience financial difficulties due to their treatment, health policies can be designed to improve this issue.

Trial registration number: not applicable

Abstract citation ID: dead093.677

P-319 Transcriptomic analysis reveals deregulated molecular mechanisms related to miscarriage, preeclampsia, and pregnancy complications during gestational endometrial phase in adenomyosis

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Study question: Is there any molecular deregulation in the gestational endometrium of women with adenomyosis responsible for adenomyosis-related pregnancy disorders?

Summary answer: Women with adenomyosis show deregulated molecular mechanisms in their gestational phase endometrium involved in miscarriage, preeclampsia, and pregnancy complications, causing adenomyosis-related infertility.

What is known already: Women with adenomyosis are characterized by having defective decidualization, altered endometrial receptivity, impaired embryo-maternal communication, implantation failure and higher risk of preeclampsia and miscarriage. However, molecular mechanisms underlying these infertility-related conditions remain unknown, mainly due to the inaccessibility of obtaining and maintaining these endometrial tissues. We have previously established adenomyosis endometrial organoids and differentiated them into gestational phase, demonstrating the maintenance of disease-specific traits. For this reason, here we use the already established adenomyosis organoid platform to describe the molecular mechanisms deregulated during gestational phase involved in adenomyosis-associated pregnancy disorders, which could be possible infertility therapeutical targets.

Study design, size, duration: Endometrial organoids from eutopic endometrium of adenomyosis and non-disease (control) women ($n=15$/group) were established and differentiated into gestational phase (ADENO-GESTorg and CONTROL-GESTorg) by supplementation with ovarian and pregnancy hormones. An RNA-sequencing was performed. DESeq2 was used to identify differentially expressed genes (DEGs), FDR < 0.05. GO and KEGG analysis were performed to study DEGs implication at biologically functional level. QIAGEN Ingenuity Pathway Analysis (IPA) was used to further identify upregulated and downregulated pathways.

Participants/materials, setting, methods: Endometrial biopsies were obtained by hysteroscopy from adenomyosis and control patients ($n=15$/group) at IVI Valencia Clinics. Patients between 18≤45 years old and BMI≤28 kg/m^2 diagnosed with adenomyosis (adenomyosis group) or without adenomyosis nor other uterine pathologies (control group) were included in the study.

Main results and the role of chance: We identified 1999 DEGs (153 upregulated and 1846 downregulated) in ADENO- compared to CONTROL-GESTorg. Between upregulated genes, *CXCL14* restricts trophoblast invasion and outgrowth, *CYP24A1* is increased in spontaneous miscarriage and

preeclamptic placentas, and *PTAFR* induces preterm delivery in mice (log2 Fold Change [log2FC] = 1.6, 2.4, 0.8). Among downregulated genes, deregulated *PGR* expression has been related to severe preeclampsia and recurrent pregnancy loss, whereas defects on *ZWINT, ESCO2* and *MCM6* expression are associated with high incidence of aneuploidy, leading to miscarriage, infertility, and newborn disorders (log2FC = −2.21, -2.57, -2.53, -2.48). Functional analysis showed deregulated functions as blastocyst growth, in utero embryonic development, developmental maturation and response to oxidative stress, standing out for their possible involvement in pregnancy disorders. IPA predicted a significant inhibition of D-myo-inositol metabolism (z score [z] = −0.7) affecting oocyte and embryo quality, VEGF signaling (z = −1.5) disturbing trophoblast adaptation to hypoxia, ERK/MAPK signaling (z = −4.0) causing embryonic lethality due to placental alterations; and significant activation of PPARα/RXRα (z = 0.5) that gives rise to recurrent miscarriage and preeclampsia, p53 signaling (z = 1.1) related with preeclampsia-complicated pregnancies, and RHOGDI and PTEN signaling (z = 3.6, 3.4) both promoting preeclampsia.

Limitations, reasons for caution: Although organoid platforms faithfully recapitulate tissue of origin characteristics as well as disease-specific traits, they are still in vitro models that need to be translated into in vivo studies to further corroborate the results obtained.

Wider implications of the findings: Molecular mechanisms involved in impaired embryo development, pregnancy loss, preeclampsia and placental defects are deregulated in gestational phase endometrium of women with adenomyosis. These deregulated mechanisms could be therapeutic target to develop pharmacological treatments to ameliorate adenomyosis-related infertility.

Trial registration number: not applicable

Abstract citation ID: dead093.678
P-320 Change of bone mineral density after long-term use of dienogest with calcium and vitamin D supplementation after surgical treatment of endometrioma

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Study question: Does long-term use of dienogest reduce bone mineral density (BMD)? Is ovarian reserve after surgery a risk factor for BMD loss?

Summary answer: BMD was not significantly changed after long-term use of DNG for up to 8years. In patients with minimal changes, postoperative ovarian reserve were not different.

What is known already: Dienogest (DNG) is a promising first-line treatment option for the long-term management of debilitating endometriosis-associated symptoms. However, there're some debates about the bone loss after long-term treatment. Some studies reported the BMD reduction after 2-3 years, however, predictive risk factors for BMD reduction were not found. Hypergonadotropic amenorrhea is well known risk factors for osteoporosis. Research on whether postoperative ovarian function decline is associated with osteoporosis is still lacking.

Study design, size, duration: This retrospective study were performed with 6180 reproductive-aged women who underwent conservative surgery for endometriomas and received postoperative dienogest (2mg/day) to prevent recurrence in the single center from May 2013 to March 2022. Among them, Sixty nine women who took DNG for more than two years after surgery and followed up BMD were enrolled for this study.

Participants/materials, setting, methods: Sixty nine women taking DNG for more than 24 months up to 101 months after laparoscopic operation for endometriosis were retrospectively analyzed. Calcium and vitamins were taken as supplements. The changes of BMD were evaluated every year during DNG treatment by using dual energy X-ray absorptiometry. Pain and tumor recurrence were evaluated every three months after operation. For the risk evaluation, age, BMI, tumor size and bilaterality, FSH, estradiol and AMH levels were evaluated.

Main results and the role of chance: Mean age of enrolled women was 39.64 ± 7.59, and mean BMI was 21.79 ± 3.51 kg/m^2. Mean duration of medication was 41.33 ± 17.66 months (24-101 months). Tumor size was 5.35 ± 4.29cm. Preoperative AMH was 2.81 ± 2.07 ng/mL, postoperative AMH was 1.78 ± 2.11 ng/mL, reduction of AMH levels was 1.24 ± 1.26 ng/mL (0.0 - 4.15). Mean BMD level of femur after DNG treatment was 0.8264 ± 0.1201 g/cm^2, which was not different compared to baseline (0.8404 ± 0.1194 g/cm^2). BMD levels of lumbar spine were not different either after DNG treatment (baseline 0.9411 ± 0.1141 g/cm^2, post-treatment 0.9477 ± 0.1346 g/cm^2, separately). Only 15% of patients showed minimally decreased BMD at femur (-0.85%), mean femur BMD levels were not statistically different compared those of unchanged BMD group. Although 62.5% of patients showed a decrease in BMD at lumbar spine, mean BMD levels were not statistically different either. Postoperative FSH, estradiol and AMH levels were not significantly different between women who had reduced BMD at both site after long-term DNG use and women who did not.

Limitations, reasons for caution: As a retrospective study based on existing medical records, other factors that might affect the bone mineral density such as the diet, physical activity could not be assessed. Unfortunately, only a limited number of patients were included in the study as most patients received DNG for less than 2 years.

Wider implications of the findings: The current study contributes to enrich literature about the advantages of using DNG in a long term to prevent endometriosis recurrence.

Trial registration number: 2022-07-062

Abstract citation ID: dead093.679
P-321 increased expression of TGF-β1 contributes to the downregulation of progesterone receptor expression in the eutopic endometrium of infertile women with minimal/mild endometriosis

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Study question: Do immune cells regulate progesterone resistance and endometrial receptivity of eutopic endometrial stromal cells (ESCs) by transforming growth factor-β1 (TGF-β1)?

Summary answer: By activating TGF-β/Smad signaling in eutopic ESCs, elevated TGF-β1 from CD45+ immune cells could attenuate expression of progesterone receptor (PR), and further decrease endometrial receptivity.

What is known already: Endometriosis is a hormone-dependent disease related to impaired immunoregulation. We characterized the trascriptomic transformation of eutopic endometrium from patients with minimal/mild endometriosis and controls across the menstrual cycle. However, the regulatory mechanism of altered immune microenvironment on eutopic ESCs remains unclear.

Study design, size, duration: Single-cell RNA transcriptomes were profiled using 10x Genomics platform. Primary culture of eutopic ESCs was performed to explore the effects of TGF-β1 on the expression of Smad / PR, and the in vitro decidualization. Additionally, co-immunoprecipitation (Co-IP) was used to explore the direct interaction between Smad and PR.

Participants/materials, setting, methods: Endometrial tissue samples were obtained from twenty-four women who were laparoscopically confirmed minimal/mild endometriosis according to the revised American Fertility Society classification. Additionally, four patients who were laparoscopically endometriosis-free were enrolled as controls. All samples were collected in DMEM/F12 medium and processed in one of two ways, either for single-cell sequencing (n = 10) or primary cell culture (n = 14).

Main results and the role of chance: The results of single-cell RNA sequencing revealed that the percentage of CD45+ cells in eutopic endometrium was increase in both proliferative (26% vs 24.1%) and secretory (19.3%

vs 8.3%) stages. Overexpression of TGF-β1 of CD45+ cells was observed in eutopic endometrium of endometriosis in both proliferative and secretory stages. We found attenuate expression of PRB ($p = 0.026$) and PRA ($p = 0.678$) after using TGF-β1 in eutopic ESCs by western blot. Similarly, qRT-PCR results showed that the mRNA level of PR ($p<0.001$), PRB ($p = 0.003$) and HOXA10 ($p<0.001$) decreased significantly after TGF-β1 treatment, but that increased ($p<0.01$) after SB431542 treatment in the eutopic ESCs. Moreover, TGF-β1 has a negative effect on the in vitro decidualization of eutopic ESCs ($p = 0.003$). And the group with treatment of both TGF-β1 and SB435142 in eutopic ESCs showed significant decidual-like changes with increased prolactin level ($p = 0.01$). Co-IP showed no physical interaction between the PR and p-Smad3/Smad3 proteins.

Limitations, reasons for caution: This study focused on CD45+ immune cells and the cytokine, TGF-β1. Further in vivo and in vitro studies are needed to elucidate the specific types of immune cell and the mechanisms that behind endometrial receptivity decrease in endometriosis.

Wider implications of the findings: Our results demonstrate abnormal activation of CD45+ cells in eutopic endometrium with minimal/mild endometriosis. And overexpression of TGF-β1 was found by CD45+ cells. We found that TGF-β1 suppresses PR expression and decidualization in eutpoic ESCs. These results provide a novel insight into the mechanisms behind progesterone resistance in endometriosis.

Trial registration number: not applicable

Abstract citation ID: dead093.680
P-322 Single-cell transcriptome analysis reveals endometrial immune microenvironment in minimal/mild endometriosis

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Study question: This study aimed to systematically understand the endometrial leukocyte types, inflammatory environment, and impaired receptivity at single-cell resolution.

Summary answer: These results provide new insights into the endometrial immune microenvironment and impaired endometrial receptivity in infertile women with minimal/mild endometriosis.

What is known already: Endometriosis is a common inflammatory disorder in women of reproductive age due to an abnormal endometrial immune environment and is associated with infertility.

Study design, size, duration: We profiled single-cell RNA transcriptomes of 138,057 endometrial cells from endometriosis patients (n = 6) and control (n = 7), respectively, using 10x Genomics platform.

Participants/materials, setting, methods: We profiled single-cell RNA transcriptomes of 138,057 endometrial cells from endometriosis patients (n = 6) and control (n = 7), respectively, using 10x Genomics platform.

Main results and the role of chance: We found that one cluster of epithelial cells that expressed PAEP and CXCL14 was mostly from the control during the window of implantation (WOI). This epithelial cell type is absent in the eutopic endometrium during the secretory phase. The proportion of endometrial immune cells decreased in the secretory phase in the control group, whereas the cycle variation of total immune cells, NK cells, and T cells was absent in endometriosis. Endometrial immune cells secreted more IL-10 in the secretory phase than in the proliferative phase in the control group; the opposite trend was observed in endometriosis. Proinflammatory cytokines levels in the endometrial immune cells were higher in endometriosis than in the control group. Trajectory analysis revealed that the secretory phase epithelial cells decreased in endometriosis. Ligand–receptor analysis revealed that 11 ligand-receptor pairs were upregulated between endometrial immune and epithelial cells during WOI.

Limitations, reasons for caution: Our study had some limitations, such as the small sample size and lack of validation experiments. Further studies are needed to demonstrated the conclusions of our bioinformatic analysis, like in situ validation of receptor-ligand pairs analysis. Therefore, more evidence is needed to clearly state the pathogenesis of endometriosis-associated infertility.

Wider implications of the findings: This database will provide an essential resource for understanding the eutopic endometrial inflammatory environment and defective endometrial receptivity in patients with endometriosis-associated infertility.

Trial registration number: 82071625

Abstract citation ID: dead093.681
P-323 Associations between endometrial microbiome and the local immune cell composition during the mid-secretory phase

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Study question: Is there an association between the endometrial microbiome and the local immune cell composition during the mid-secretory phase?

Summary answer: The percentage of endometrial neutrophils was negatively related to the highest number of microorganisms, while B-cells and T-helpers were positively related to certain bacterial taxa.

What is known already: Endometrial receptivity is strongly influenced by the local immune cell composition and its inflammatory status. Meanwhile, many studies on the endometrial microbiome and its impact on receptivity are arising. Knowing that bacterial composition has an effect on the immune system, it is worth studying the potential associations between the microbiome and immune cell populations in the endometrium during the mid-secretory phase. This relationship could adjust the magnitude of inflammation which has a strong effect on embryo implantation process. Investigations on the endometrial bacterial communities' effect on the local immune cell composition could be applied in improving assisted reproductive technologies.

Study design, size, duration: Prospective observational study performed between April 2021 and January 2023 in a private in vitro hospital. Endometrial biopsies were collected from 30 women aged between 34 and 45 years during the mid-secretory phase, on day 7 after LH surge. Patients with history of recent inflammatory disease, chronic endometritis, recent antibiotic treatment, endocrinological disorders, autoimmune diseases, oncological diseases, moderate or severe endometriosis, adenomyosis, uterine hyperplasia, and endometrial polyps were not included.

Participants/materials, setting, methods: B cells, T cells, T helpers, NK cells, macrophages and neutrophils in the endometrial stroma were identified by immunohistochemical staining with antibodies against CD79α (IS62, Dako), CD3 (BRB063, Zytomed), CD4 (IS649, Dako), CD56 (A00121-0007, ScyTek), CD14 (E-AB-71017, Elabscience) and neutrophil elastase (950334, NOVUSBIO), respectively. The immune cell percentages were calculated by HALO image analysis software (version 2.3, IndicaLabs). Endometrial microbiota (EM) composition was analyzed after DNA isolation using 16S rRNA (v4-v5 region) gene sequencing.

Main results and the role of chance: Totally 271 bacterial species and 668 genera were identified in the endometrial samples. The median (range) immune cell percentages found were: 0.083% (0.49) B-cells, 0.974% (4.93) T-cells, 0.066% (0.44) T-helpers, 0.931% (5.36) NK-cells, 1.129% (3.11) macrophages and 0.207% (3.86) neutrophils.

Spearman analysis revealed significant correlations between T-helpers percentage and the relative abundance of *Cutibacterium acnes* (R = 0.475, p = 0.012) and its genus Cutibacterium (R = 0.477, p = 0.012) as well as between B-cells and the relative abundance of *Bacteroides vulgatus* (R = 0.532,

p = 0.003) and its genus Bacteroides (R = 0.457,p = 0.013). NK-cells presence was not related to any of the found bacterial species or genus (p > 0.05).

Neutrophils demonstrated significant correlations with the relative abundance of the highest number of species: *Prevotella melaninogenica* (R = −0.404, p = 0.03), *Butyrivibrio crossotus* (R = 0.433,p = 0.02), *Moraxella osloensis* (R = −0.433, p = 0.019), *Blautia sp.* (R = 0.427, p = 0.021), *Alicyclobacillus acidocaldarius* (R = −401,p = 0.031) also related with the T-cells percentage (R = −0.380, p = 0.042) and *Acinetobacter lwoffii* (R = −0.458, p = 0.008) also related with the macrophages (R = −0.454, p = 0.013). Among the studied species *Alloprevotella tannerae* was showing significant correlations with neutrophils (R = −0.0602, p = 0.001), macrophages (R = −0.416, p = 0.025) and T-cells (R = −0.464, p = 0.011).

At genus level, most of the bacteria were related to the neutrophils – Pseudoalteromonas (R = −0.540, p = 0.002), Aliterella (R = −0.617, p<0.001), Endhydrobacter (R = −0.433,p = 0.019) and AKIW781 (R = −0.493, p = 0.011). Aliterella and AKIW781 were also related to the macrophages (R = −0.473, p = 0.01 and R = −0.423, p = 0.031, respectively) and T-cells (R = −0.387, p = 0.038 and R = −0.519, p = 0.007, respectively).

Limitations, reasons for caution: The study was limited in sample size.

Wider implications of the findings: The results of this study indicated that there is a quantitative relation between the main endometrial immune cells and the endometrial core microbiome. The association between these two determinants of endometrial receptivity could help in understanding the fine tuning of the endometrium for accepting the embryo.

Trial registration number: The current research was funded by National Science Fund, Ministry of Education, Bulgaria, Contract № KP-06-N53/14/16.11.2021.

Abstract citation ID: dead093.682
P-324 The impact of intrafollicular iron on granulosa cells and follicular development in women with ovarian endometriomas

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Study question: Whether and how iron overload in ovaries affects follicular development in patients with ovarian endometriosis?

Summary answer: Iron overload microenvironment that affects the growth and development of granulosa cells and oocytes, leading to fewer oocytes retrieved and decreased ovarian reserve.

What is known already: The presence of an endometrioma with the high levels of free iron, shows an adverse effect on the surrounding ovarian tissue as reflected by a reduced number of developing follicles and oocytes retrieved in in vitro fertilization cycles. However there is few published data available focusing on the potential risk of free iron in folliculogenesis of patients with endometrioma. This study aimed to identify the impact of intrafollicular iron on granulosa cells (GCs) and follicular development in ovarian endometrioma patients.

Study design, size, duration: The clinical data of 50 patients with unilateral ovarian endometriosis who underwent in vitro fertilization, intracytoplasmic sperm injection, and embryo transfer in the Reproductive Medicine Center of Sun Yat-sen Memorial Hospital from October 2019 to September 2021 were prospectively collected. Meanwhile, 50 infertile patients without endometriosis were selected as the control group; they were matched by age, duration of infertility, and body mass index.

Participants/materials, setting, methods: Ffollicular fluid and granulosa cells were collected.The clinical data of the two groups of infertile patients were compared. The iron ion in follicular fluid was detected by colorimetry,-transferrin, eatrogen and the ferritin concentration in follicular fluid was detected by enzyme-linked immunosorbent assay. qPCR and Western blotting were used to detect the expression of iron homeostasis-related genes and proteins in luteinized granulosa cells. Oocyte retrieval rates, maturation and fertilization rates were analyzed.

Main results and the role of chance: There were no significant differences in baseline data, including age, duration of infertility, BMI, between the two groups. The AFC and AMH in the experimental group were significantly lower than that in the control group. The number of oocytes obtained in the experimental group was 8.35 ± 5.80 vs. 13.71 ± 5.62, P > 0.05. P = 0.003; and the average oocyte retrieval rate (68.56 ± 34.97% vs. 88.64 ± 23.11%, p = 0.032) was significantly lower than those in the control group. The concentration of an iron in the follicular fluid of the cystic ovary (63.63 ± 24.69 mg/L) was significantly higher than that of the healthy ovary (51.65 ± 13.80 mg/L) and the control ovary (39.23 ± 16.89 mg/L), P = 0.005. The levels of ferritin light chain and heavy chain were significantly higher but transferrin and estrogen were lower in the cystic ovary than in the healthy ovary and the control ovary. qPCR and Western blot results showed that the mRNA and protein expression of transferrin receptor 1, ferritin heavy chain, and ferritin light chain were significantly higher in the cystic ovary than in the healthy ovary and the control ovary.

Limitations, reasons for caution: For limitation of sampling, we could not obtain the information from human growing follicles, animal experiments would be involved in our subsequent study.

Wider implications of the findings: These results provide novel information suggesting that when surgical removal of the endometrioma is not an option, further investigation is needed to clarify whether and how the medical therapy may limit or slow the damage caused by endometrioma.

Trial registration number: not applicabl

Abstract citation ID: dead093.683
P-325 Evidence for dysregulation of oncogene pathways at the gene expression level in the epithelium of deep infiltrating endometriosis compared to adenomyosis

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Study question: How does the gene expression profile of epithelium cells differ between deep infiltrating endometriosis (DIE) and adenomyosis (FA)?

Summary answer: The gene expression of epithelial cells from DIE reveals a significant upregulation of the PI3K-pathway and downregulation of the RAS-pathway compared to epithelium from FA.

What is known already: Both DIE and FA, are histologically considered benign but have features of malignant diseases, such as a tendency for local tissue invasion. Recent next generation sequencing studies have detected driver mutations in cancer-associated genes such as PIK3CA, ARID1A, PPP2R1A and KRAS in the epithelium of DIE, as well as in KRAS in epithelium of FA. Furthermore, identical mutations in the KRAS gene were detected in coexisting adenomyotic and endometriotic lesions, supporting the theory of a common molecular mechanism. The gene expression differences between these two entities were therefore investigated in the present study.

Study design, size, duration: This is an experimental analysis of 7 adenomyosis and 19 DIE samples (histologically confirmed), that were formalin-fixed and paraffin-embedded (FFPE) collected between 2003 and 2018. These were provided by the tissue bank of the National Centre for Tumour Diseases (Heidelberg, Germany), in accordance with the Ethics Committee of the University of Heidelberg (approval number S-362/2017). In addition, the medical and epidemiological data of the corresponding patients were retrospectively analyzed based on the clinical data collected.

Participants/materials, setting, methods: For this study, the epithelia of FFPE samples were microdissected in a laser-guided fashion. After RNA extraction, the expression of 770 genes was analyzed using the nCounter Technology with the Human PanCancer Pathways Panel (Nanostring). All genes with an adjusted false discovery rate of p < 0.05 and a fold change of < 0.66 or > 1.5 were considered differentially expressed and subjected to functional annotation and clustering using DAVID bioinformatics resources.

Main results and the role of chance: Our analysis revealed a total of 162 differentially expressed genes, that were either significantly increased (n = 116) or decreased (n = 46) in epithelium of DIE compared to that of FA (with log2 fold changes of < 0.66 or > 1.5 and an adjusted p-value of < 0.05). Gene ontology and KEGG pathway analysis for genes with increased expression in DIE compared with FA revealed significant upregulation of genes belonging to the PI3K-pathway and the focal adhesion pathway, as well as pathways related to viral infection, endocrine resistance, and malignancy. The upregulated pathways in adenomyosis mainly included the RAS-pathway.

Limitations, reasons for caution: The average age of DIE patients was lower, but many of them were hormonally inactive due to GnRH analogues, which mitigates any age-related differences. Furthermore, only relative expression was studied between the groups without comparison to normal endometrium. Overall, the sample size is relatively small and further studies are needed.

Wider implications of the findings: The expression of different signaling pathways in the two entities could, for example, explain the different sensitivity of the two diseases to certain therapeutic approaches. While DIE responds well to therapy with the progestogen dienogest, progesterone resistance is often described in adenomyosis patients.

Trial registration number: not applicable

Abstract citation ID: dead093.684

P-326 No higher incidence of chronic endometritis in infertile women with polycystic ovary syndrome: a propensity score-matched study

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Study question: Do infertile women with polycystic ovary syndrome(PCOS) have a higher incidence of chronic endometritis(CE) and need to screened for CE before assisted reproductive technology(ART)?

Summary answer: Under the diagnosis by CD138 immunohistochemical staining, no significant difference was found in the comparison of CE incidence between PCOS patients and non-PCOS patients.

What is known already: PCOS is the most common endocrinopathy among women of reproductive age and causes increased risks of embryo implantation failure and pregnancy loss. The chronic low-grade inflammation of the endometrium in PCOS women should not be ignored. CE is a local inflammation of the endometrium and also harms endometrial receptivity. Some specialists suggest that women at high risk of CE (such as endometrial polyps, repeated implantation failure, etc.) should be screened for CE before before ART. However, the risk of CE under the diagnosis by CD138 immunohistochemical staining for endometrial specimen in PCOS women has not been previously evaluated.

Study design, size, duration: We conducted a retrospective analysis of all infertile patients undergoing their first hysteroscopic operations during 2017-2022 from one tertiary hospital. All patients underwent hysteroscopy in the proliferative phase and endometrial biopsy for immunohistochemistry staining for CD138 was carried out. Cesarean section, intrauterine adhesion, recurrent spontaneous abortion or recurrent implantation failure and other diseases potentially related to chronic endometritis were strictly excluded. The CE incidence between PCOS patients and non-PCOS patients was compared.

Participants/materials, setting, methods: Endometrial biopsy and immunohistochemistry staining for CD138 was carried out among all patients. Demographic information were recorded. A propensity score matching (PSM) model was used to match the independent variables to balance the influence of confounding factors. Subgroup analysis according to different body mass index were also taken among PCOS patients and non-PCOS patients. Furthermore, a multivariate logistic model was used to explore the risk factors affecting CE incidence.

Main results and the role of chance: A total of 205 cases were allocated to the PCOS group, while 4021 cases were allocated to the non-PCOS group in the final analysis. After PSM, 197 PCOS patients were matched with 197 patients in the non-PCOS group. Significant differences were only found in the comparisons of PCOS-associated characteristics ($P < 0.05$) and the other baseline characteristics of the two groups were not significantly different($P > 0.05$). No higher CE incidence in infertile women with PCOS was found either in total analysis or after PSM ($P = 0.969$, OR 1.01, 95% CI: 0.75-1.36; $P = 0.385$, OR 1.21, 95% CI: 0.75-1.36; respectively). Similar results were discovered in the subgroup of BMI≥28 kg/m2, BMI 24 -28kg/m2, and BMI<24 kg/m2 ($P = 0.301$, OR 1.64, 95% CI: 0.64-4.19; $P = 0.671$, OR 1.13, 95% CI: 0.65-1.95; $P = 0.427$, OR 0.85, 95% CI: 0.58-1.27; respectively). Interestingly, a rising trend of CE incidence was found with the BMI increased in the PCOS group, even though no significant differences were found (BMI<24 kg/m2: 28.80%; BMI 24 -28kg/m2: 36.66%; BMI≥28 kg/m2: 45.00%; respectively). Multivariate logistic regression showed that age, infertility duration, infertility type, PCOS, and obesity were not the independent risk factors affecting CE incidence.

Limitations, reasons for caution: Information bias cannot be ruled out because of the retrospective design of this study. Hence, we used PSM and regression analysis to minimize the impact of confounding variable. Besides, the sample size of patients with PCOS was limited. Therefore, a larger sample size will be used in our future study.

Wider implications of the findings: We firstly investigate the relationship between PCOS and CE diagnosed by CD138. The current evidence does not support PCOS women should screened for CE before ART. In the future, a larger sample of studies are needed to investigate the relationship between PCOS and CE, especially for obese PCOS patients.

Trial registration number: not applicable

Abstract citation ID: dead093.685

P-327 Intrauterine fluid in patients with cesarean section defects in association with ivf outcomes

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Study question: This study evaluate the effect of cesarean section scar defects associated with intrauterine fluid during endometrial preparation on pregnancy outcomes.

Summary answer: Patients with Cesarean section scar defect and intrauterine fluid during endometrial preparation had a substantially lower pregnancy outcome compared to those without uterine fluid.

What is known already: The Cesarean section rate in developed countries has increased continuously, leaving many short-term and long-term consequences. Intrauterine fluid, which reported incidence may reach 40% in patients who have had a previous caesarean section, may play an important role in reducing endometrial receptivity and further impair pregnancy outcomes.

Study design, size, duration: This is a retrospective cohort study performed on patients who had undergone frozen embryo transfer at an Assisted Reproductive Technology Center from January 2019 to December 2019.

Participants/materials, setting, methods: 2,350 patients without intrauterine fluid and 74 patients with Cesarean section scar defect who had intrauterine fluid during endometrial preparation but none at progesterone supplementation were analyzed. Multiple logistic regression was performed for the probability of the pregnancy outcome of embryo transfer and measured the association with the status of intrauterine fluid, age, endometrial thickness, number of previous transfer, number of embryos, type of infertility, and difficult embryo transfer at the alpha level of 0.05%.

Main results and the role of chance: The outcomes among 2,424 patients undergoing cryopreserved embryo transfer were 59.45% for pregnancy rate, 8.7% for biochemical pregnancy rate, 50.33% for clinical pregnancy rate, and 43.15% for ongoing pregnancy rate. The analysis of multiple logistic regression indicated that the presence of intrauterine fluid during endometrial preparation did not affect the pregnancy rate. However, in patients with intrauterine fluid, there was an increased rate of biochemical pregnancy (OR 2.46, 95%CI

1.35-4.50) and a reduced rate of ongoing pregnancy (OR 0.54, 95%CI 0.32-0.89).

Limitations, reasons for caution: The major limitations of the present study are the retrospective design and small number of patients with uterine fluid. Additionally, the composition of the uterine fluid (eg, blood, mucus, purulent, or transparent) cannot be classified by an ultrasound exam.

Wider implications of the findings: Intrauterine fluid may be an innovative and controllable factor that should be considered in patients who have a history of caesarean section in order to improve pregnancy outcomes.

Trial registration number: Not Applicable

Abstract citation ID: dead093.686

P-328 Endometrial receptivity in women with endometriosis undergoing Assisted Reproductive Technology (ART) after frozen embryo transfer: a matched pair case-control study

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Study question: Does endometriosis affect endometrial receptivity in frozen/thawed embryo transfer (FET) cycles?

Summary answer: Stage III/IV endometriosis-patients undergoing ART submitted to FET cycles have implantation, pregnancy and live-birth rates, as well as pregnancy outcomes, similar to control women.

What is known already: The increased infertility rate observed in women with endometriosis is supposed to be linked, among other factors, to molecular alterations already documented in the endometrial tissue of these affected women. These alterations might influence the interaction between the embryo and the endometrium, leading to a compromised implantation; however, data from the literature are controversial and do not always corroborate the hypothesis of a lower implantation rate in women with endometriosis.

Study design, size, duration: This is a retrospective matched case-control study including 101 women diagnosed with stage III/IV endometriosis. They were matched in a 1:1 ratio with patients undergoing frozen embryo transfers after ART treatments for other infertility-related indications at the Fondazione IRCCS Ca'Granda Ospedale Maggiore Policlinico di Milano, between 2015 and 2022.

Participants/materials, setting, methods: Patients (n = 202) were submitted to ovarian stimulation according to standard protocols and only single embryo transfer cases and the 1st ART cycle with freeze-all protocol were included. To match, it was considered the women's age (± 1 year), and the number (=) and quality (± 1 top *vs* low) of blastocysts obtained in each cycle, considering the Istanbul consensus as the embryo standard assessment protocol. The main outcome evaluated in this study was implantation rate.

Main results and the role of chance: Baseline characteristics did not significantly vary between the two study groups, except for AMH levels, which was considerably lower in the endometriosis group compared to control (4.0 [2.7 - 5.4] *vs* 4.6 [3.7 - 6.5], respectively; p = 0.03). Considering the treatment outcomes, the number of oocytes inseminated/injected in each cycle was similar between endometriosis and control group (8 [7 - 10] *vs* 9 [7 - 11], respectively; p = 0.26). In line with the matching performed, the number of blastocysts generated did not differ between women with and without endometriosis (3 [2 - 4] *vs* 3 [2 - 4], respectively; p = 1.00), as well as the number of top-quality blastocysts (2 [1 - 3] *vs* 2 [1 - 3], respectively; p = 0.76). In regard to our main outcome evaluated, implantation rate in women with endometriosis and their controls were similar (36% *vs* 40%, respectively; p = 0.52). There was also no difference between the endometriosis group and controls considering cumulative pregnancy outcomes, such as pregnancy rate (58% vs 65%, respectively; p = 0.39), cumulative live birth rate (51% *vs* 59%, respectively; p = 0.26), pregnancy complications (6% *vs* 14%, respectively; p = 0.34), and pregnancy loss (16% *vs* 19%, respectively; p = 0.70).

Limitations, reasons for caution: As it is a retrospective study, our reports might be subjected to misclassification bias. Also, some of the women in the control group may have asymptomatic undiagnosed endometriosis; however, considering the incidence of this disease, it should not compromise our evaluation.

Wider implications of the findings: Our present findings give support to the idea that endometriosis does not influence the implantation rate in women with endometriosis undergoing ART submitted to FET. Considering fertility, this finding raises doubts about the clinical relevance of the molecular aberrations detected in the endometrium of women affected by endometriosis.

Trial registration number: not applicable

Abstract citation ID: dead093.687

P-329 Oocyte donation outcomes in endometriosis patients with multiple IVF failures

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Study question: What are the reproductive outcomes and the prognostic factors of live birth rates in endometriosis patients referred to oocyte donation after multiple in vitro fertilization(IVF)-failures?

Summary answer: In this specific population, oocyte donation appears to be a viable option to optimize the reproductive outcomes, as the cumulative live birth rate reaches 63.2%.

What is known already: Assisted Reproductive Technology(ART) is currently recognized as one of the main therapeutic options to manage endometriosis-related infertility, often resulting in satisfactory outcome. A burning issue in this field remains how to deal with patients undergoing recurrent ART failures. As there appear to be two main factors contributing to the failures, i.e., a deleterious effect of endometriosis on embryo implantation and altered oocyte and embryo quality, two competing therapeutic strategies can be considered:(i) endometriosis surgery and (ii) oocyte donation. However, no data has been published to date in the specific population of endometriosis patients with previous IVF failures, undergoing oocyte donation.

Study design, size, duration: Observational cohort study including fifty-seven women with endometriosis-related infertility and multiple IVF failures (≥ 2 failed IVF cycles) referred to oocyte donation who consulted at our institution between January 2013 and June 2022.

Participants/materials, setting, methods: Endometriosis was diagnosed based on published imaging criteria using transvaginal sonography and magnetic resonance imaging and confirmed histologically in women who had a history of surgery for endometriosis. The main outcomes measured were the clinical pregnancy rate (CPR) and the cumulative live birth rate (CLBR). We compared the characteristics of women who had a live birth versus those who did not using univariate and multivariate analysis to identify determinant factors of fertility outcome.

Main results and the role of chance: Fifty-seven patients underwent 90 oocyte donation cycles, after 244 failed autologous IVF cycles. The mean age of the population was 36.8 ± 3.3 years, with a mean infertility duration of 3.6 ± 2.2 years, and a mean number of autologous IVF/ICSI cycles of 4.4 ± 2.3 cycles per patient. Three patients (5.3%) had superficial endometriosis, 2 patients (3.5%) had ovarian endometriomas, and 52 patients (91.2%) had deep infiltrating endometriosis, among which 30 patients (57.7%) had bowel lesions. Thirty patients (52.6%) had associated adenomyosis. Overall, the CPR and the CLBR per patient were 49/57 (85.9%) and 36/57 (63.2%), respectively. After multivariate analysis, only being nulligravida (p = 0.002) remained an independent negative predictive factor of the live birth rate.

Limitations, reasons for caution: In 35.1% of patients there was no surgical/histological proof of endometriosis. However, for all the study participants, endometriosis was diagnosed and staged using patient interviews, clinical examination, and imaging techniques to assess for endometriosis phenotypes, which is in line with the structured diagnostic process acknowledged as being appropriate for endometriosis.

Wider implications of the findings: In this specific population, poor embryo quality may be the main limiting factor of ART success rates, rather than impaired endometrial receptivity. These data are reason for clinicians not to systematically refer infertile endometriosis patients with recurrent IVF failures to surgery.

Trial registration number: not applicable

Abstract citation ID: dead093.688

P-330 Is 17 alpha hydroxyprogesterone caproate an adequate alternative to improve reproductive outcomes in artificially prepared frozen embryo transfer cycles (FET)?

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Study question: Is 17 alpha-hydroxyprogesterone caproate an adequate option to improve reproductive outcomes in patients with low serum progesterone (P4) level the day of frozen embryo transfer?

Summary answer: Patients with serum P4<10ng/ml may benefit with the addition of intramuscular 17alpha-hydroxyprogesterone caproate injections, and have similar ongoing pregnancy rates than patients with serum P4≥10ng/ml.

What is known already: Indications of embryo cryopreservation have expanded in the last years. Recently published papers highlight that low serum progesterone levels before frozen embryo transfers decrease pregnancy and live birth rates and increase miscarriage rates, even in cases of euploid embryos transfers. Other studies have published a beneficial effect with the addition of daily subcutaneous progesterone injections in patients with low serum progesterone levels around the time of embryo transfers. In Argentina subcutaneous progesterone has been available since October 2022, not before. 17alpha-hydroxyprogesterone caproate is a synthetic progestin used for luteal phase support and approved for the prevention of preterm birth.

Study design, size, duration: This prospective cohort study was performed between May 2021 and August 2022 in 114 patients undergoing 138 FET cycles after an artificial endometrial preparation with estradiol valerate 4mg daily and micronized vaginal progesterone 200mg, three times daily.

Participants/materials, setting, methods: Patients <50 years old with triple-layer endometrium ≥7mm underwent frozen embryo transfer. Progesterone was measured immediately before the embryo transfer. Group 1 (N = 47): P4>10ng/ml; Group 2 (N = 46): P4<10ng/ml with addition of 17 alpha-hydroxyprogesterone caproate after embryo transfer; Group 3 (N = 46): P4<10ng/ml without addition of 17 alpha-hydroxyprogesterone. Primary endpoint was to compare ongoing pregnancy rate beyond week 12 between the three groups. Secondary endpoints were pregnancy rate and miscarriage rate.

Main results and the role of chance: Parameters were comparable between the groups in terms of age, body mass index, estradiol, P4 levels at the beginning of endometrial preparation and number of embryos transferred. Ongoing pregnancy rate was: Group 1 = 38.3% (18/47); Group 2 = 39.1% (18/46); Group 3 = 28.3% (13/46) (p = 0,337). Pregnancy rate was: Group 1 = 48,9% (23/47); Group 2 = 50% (23/46); Group 3 = 50% (23/46) (p>0,05). Miscarriage rate was: Group 1 = 21.73% (5/23), Group 2 = 26.1% (6/23) and Group 3 = 43.47% (10/23) (p = 0,208). Although there were no significant differences between the groups, both, Group 1 and Group 2, had higher ongoing pregnancy rates and lower miscarriage rates than group 3. This is probably due to the limited sample size.

Limitations, reasons for caution: The main limitation of our study is the sample size and the fact that most of the embryos were not genetically evaluated before being transferred, which would eliminate a variable that could interfere with the results obtained.

Only micronized progesterone and 17alpha-hydroxyprogesterone caproate were studied.

Wider implications of the findings: The addition of exogenous 17alpha-hydroxyprogesterone caproate on the embryo transfer day may improve ongoing pregnancy rates in cases of low serum progesterone levels, being a simple and safe strategy to individualize luteal phase support when no other form of progesterone administration is available.

Trial registration number: NOT APPLICABLE

Abstract citation ID: dead093.689

P-331 Transcriptomic profiling of ovaries with experimental endometrioma in mice reveals the underlying mechanisms of abnormal oocyte quality and functions in endometrioma-associated infertility

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Study question: What are the underlying molecular mechanism and significant pathways mediated the endometrioma-associated infertility?

Summary answer: Significantly altered genes that participated in folliculogenesis, fibrosis, oxidative stress and inflammation are pivotal in the pathogenesis of infertility in endometrioma.

What is known already: Endometrioma (OMA) is the most common subtype of endometriosis, of which the endometriotic lesions implant in the ovary. Women with OMA are usually coupled with disrupted folliculogenesis, hampered ovulation, impaired oocyte quality, and undesired fertility outcomes. However, the underlying mechanisms of the disrupted folliculogenesis, hampered ovulation and impaired oocyte quality in OMA-associated infertility are still unclear. Next-generation sequencing (NGS) has been widely applied in whole-genome sequencing, RNA sequencing, and epigenomics. It is a promising technology that may provide full picture of the pathogenesis of infertility associated with OMA.

Study design, size, duration: An experimental mouse model of OMA was established in 4 weeks to study the effects of OMA on fertility outcomes and the underlying mechanism. Eight mice were included in experimental and sham control groups each. Half of the mice were then mated with male compeer and fertility outcomes were assessed after delivery. The other half were euthanized without mating to collect whole ovarian tissues for whole genome bulk RNA sequencing and then immunohistochemistry (IHC) validation.

Participants/materials, setting, methods: Minced uterine tissues from donor mice were inserted into ovarian bursa in recipient mice. After overnight mating, the pregnant mice were confirmed by positive vaginal plug. Live fetuses, abortion rate, and stillbirth were counted after delivery. Ovaries were either dissected to extract total RNA for sequencing, or paraffin-fixed and embedded for IHC, or superovulated to collect oocytes for counting and staining.

Main results and the role of chance: Successful establishment of endometriotic lesions in ovarian tissues was confirmed morphologically and histologically in recipient mice. The pregnancy rate was defined as the ratio of the number of pregnant mice to the total number of mated females. Compared to control group, a significantly decreased pregnancy rate was found in the OMA group. The average number and size of pups in the OMA group was significantly reduced. Meanwhile, abortion and stillbirth rates were significant higher in OMA group. Oocytes isolated from OMA ovaries had lower number than controls. Large proportion of the oocytes had significantly higher abnormal spindle rates, indicating mitotic disruption. A large proportion of the oocytes also had impaired cortical granule migration, indicating affected organelle organization. Furthermore, expression of follicle development markers (i.e. Forkhead box O3, anti-müllerian hormone, follicle-stimulating hormone receptor) were significantly decreased in OMA group. RNA sequencing

identified several differentially expressed genes. The Gene Oncology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis demonstrated the dysregulation of signaling pathways associated with folliculogenesis, fibrosis, oxidative stress and inflammation, whichmight trigger OMA and lead to abnormal oocyte quality and functions in endometrioma-associated infertility.

Limitations, reasons for caution: Although ethical issue in human, studies based on this animal model may not reflect the exact situation in human. Moreover, future research is needed to understand the biological functions of differentially expressed pathways in the pathogenesis of OMA-related infertility. Furthermore, the manifestations in individual cells of ovaries are still unclear.

Wider implications of the findings: The results demonstrated that endometrioma remarkably deteriorated fecundity through several potential pathways. The underlying deteriorating effects and mechanisms might help to understand the biological and molecular mechanism and to develop novel therapeutic targets to improve fertility outcomes in women with endometrioma.

Trial registration number: not applicable

Abstract citation ID: dead093.690

P-332 Standard-dose Vitamin D supplementation as an effective and safe non-hormonal treatment for the prevention of uterine fibroid growth in reproductive-age women: A pilot study

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Study question: Could low-dose vitamin D supplementation prevent uterine fibroid growth in reproductive-age women with hypovitaminosis D?

Summary answer: Six-month low-dose Vitamin D supplementation (≈ 1500 IU/day) in patients with hypovitaminosis D significantly decrease uterine fibroid growth compared to patients not receiving supplementation.

What is known already: Uterine fibroids (UF) are the most common tumor of the reproductive tract, affecting up to 77% of reproductive-age women. Due to the association between hypovitaminosis D and UF, several studies have evaluated the effect of a high Vitamin D (VitD) dose (3500-8500 IU/day) on UF size, proposing VitD supplementation as a therapeutic option. However, the standard dose recommended for VitD deficiency (800-2000 IU/day) has not been assessed. We aimed to evaluate if the restoration of normal serum levels of 25(OH)D by standard VitD dose in patients with UF and hypovitaminosis D can avoid the growth of UF in reproductive-age women.

Study design, size, duration: Ambispective observational study carried out at La Fe University Hospital. In the study group, patients (n = 6) were prospectively recruited from 2019 to 2022. Clinical data from the control group (n = 14) was collected retrospectively from 2016 to 2018. The study was approved by the autonomous government ethics committee (CAEPO; Spain) (ISS-COL-2028-01).

Participants/materials, setting, methods: Twenty women under 45 years old with uterine fibroids and hypovitaminosis D [25(OH)D levels < 30 ng/mL] were included. The study group received VitD for 6 months (25000 IU/2 weeks). Study outcomes: UF size determined by ultrasound, serum levels of 25(OH)D, Calcium, AST, ALT, Bilirubin and Creatinine were determined at 0, 3 and 6 months. Data from control group was obtained retrospectively from patients diagnosed with UF and hypovitaminosis D without VitD supplementation.

Main results and the role of chance: At the beginning of the treatment, no significant differences were found between patients included in the control and VitD group regarding age (40.57 ± 2.76 vs. 38.73 ± 4.92), BMI (23.6 5 ± 2.96 vs. 25.36 ± 4.25), VitD levels (18.57 ± 7.47 vs. 23.37 ± 37.29) and UF size (67.07 ± 20.68 vs. 56.17 ± 12.56). After treatment, VitD levels significantly increased in the study group (23.37 ± 37.29 vs. 37.02 ± 6.35, p = 0.001). Regarding size, the control group showed a significant UF growth of 17.3 ± 14.31% (p = 0.0005) after 6 months, while in patients treated with VitD UF size slightly decreased (-5.84 ± 2.14 %, p = 0.15). In addition, when comparing % of UF growth in both groups at the end of the treatment, UF growth was significantly lower in patients treated with VitD compared to control (-5.84% vs. 17.3%, p = 0.0002). There were no significant changes in serum parameters analyzed in the study group after 6 months of VitD supplementation: Calcium (9.15 vs. 9.20 mg/dL), AST (15.83 vs. 13 U/L), ALT (17.83 vs. 16 U/L), Bilirubin (0.42 vs. 0.48 mg/dL) and Creatinine (0.69 vs. 0.67 mg/dL).

Limitations, reasons for caution: This is an observational study and thus possible confounders cannot be completely excluded. More data are needed to draw firm conclusions and it will be critical to increase the sample size to check if the results observed in this work remain in the general population.

Wider implications of the findings: To our knowledge, this is the first study evaluating the effect of standard VitD dose on UF growth in reproductive-age women with hypovitaminosis D. Our results suggest that low-dose VitD could be a safe non-hormonal therapeutic option in the management of UF in patients willing to preserve their fertility.

Trial registration number: NCT03991078

Abstract citation ID: dead093.691

P-333 Thin endometrium, randomised clinical trial using plasma rich in growth factors

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Study question: Does the use of platelet growth factors instilled into thin endometrium in oestrogen-replacement treatment cycles improve endometrial thickness?

Summary answer: The use of growth factors in thin endometrium improves endometrial growth

What is known already: Thin endometrium impairs implantation rates and IVF outcomes. To date there is no effective treatment to improve the proliferation.

PRGF-Endoret is used in multiple clinical indications. The autologous collection of growth factors (PRGF), applied to the area to be treated, produces a controlled release of multiple molecules with regenerative capacity in the focus of the lesion.

Study design, size, duration: Prospective randomised clinical trial to determine the effects of PRGF-ENDORET therapy in patients with thin endometrium undergoing oestrogen treatment for embryo transfer.

Twenty-two patients were included from March 2018 to May 2021.

At the end of the trial, a complementary retrospective study was conducted analysing all the treatments of these patients without live birth(21) before and after participating in the study until December 2022. 15 embryo-transfer in study group and 27 in control group.

Participants/materials, setting, methods: Patients of IVI Bilbao clinic with endometrium less than or equal to 5mm after 10 days of treatment.

PRGF was prepared according to the PRGF-Endoret technique using the medical device developed by BTI Biotechnology Institute.

The study group received three instillations of PRGF Endoret using a kitazato IUI cannula.

The first instillation was performed with in situ activated coagulum and the 2nd and 3rd instillations with PRGF-rich supernatants that were previously stored at -20 °C.

Main results and the role of chance: The clinical trial showed a significant increase in endometrial thickness 1.3 ± 0.67 mm compared to the control group of 0.58 ± 0.51 mm. Prior to treatment the mean endometrial thickness was 4.29 ± 0.88 in the control group and 4.44 ± 0.40 in the study group. After randomisation the mean was 4.87 ± 0.76 in the control group and 5.74 ± 0.87 in the study group.

2 pregnancies only in the study group and 1 live birth.

The retrospective study shows 0.7mm increase in mean endometrial thickness in the post-PRGF cycles in the study group patients. Of the 15 transfers that were performed a posteriori, there were implantation rate of 40% and a 20% live birth rate, 40% biochemical miscarriage and 20% clinical miscarriage, with a significant increase of 0.94mm compared to the endometrium of the instillation cycle.

In the control group of patients who received a posteriori factors there was also a significant increase in thickness of 1.6mm compared to the study cycle with an implantation rate of 58.33% and a 33% live birth rate, 58.33 biochemical miscarriage and 25% clinical miscarriage.

Comparing all the cycles of these patients before and after PRGF, no statistical significance was found, although there was an increase of 0.29mm.

Limitations, reasons for caution: The number of patients included was lower than initially planned, but once the statistical power was adjusted, its safety and efficacy was demonstrated.

More studies should be done to demonstrate when to instil, how many times, when transfer, but it is clear that it is safe and effective.

Wider implications of the findings: The use of factors has been shown to increase endometrial thickness. It could be thought to open the door to favouring endometrial receptivity and therefore live birth.

Trial registration number: 2016-001716-38

Abstract citation ID: dead093.692
P-334 Transcriptomic patterns in early-secretory and mid-secretory endometrium in a natural menstrual cycle immediately before in vitro fertilization and embryo transfer

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Study question: To evaluate endometrial transcriptomic patterns in early-secretory phase (ESP) and mid-secretory phase (MSP) in a natural menstrual cycle before IVF-ET

Summary answer: Endometial ranscriptomic patterns in ESP and MSP immediately before IVF-ET appear to differ according to IVF-ET outcome, which may be implicated in endometrial receptivity.

What is known already: Biologic processes and molecular participants in the transition from ESP to MSP endometrium underscore the complex events in preparing for embryo implantation. Previously reported genes with altered expression during endometrial transition from the ES phase to the MS phase were frequently involved in ion binding, cell cycle regulation, transport of signaling proteins, or immune modulation.

Although several studies have compared gene expression in the receptive phase and pre-receptive phases in an attempt to identify a molecular signature characteristic of a receptive endometrium, a consensus has not been reached regarding the genes accounting for transcriptomic differences between phases.

Study design, size, duration: Differentially expressed genes (DEGs) in the MSP, compared to the ESP, were identified using NanoString nCounter data in both the pregnant and non-pregnant groups.

Participants/materials, setting, methods: A total of 30 patients whose endometrial tissues were obtained in a natural menstrual cycle immediately before IVF-ET were included. Endometrial dating was histologically confirmed as ESP (cycle day 16 to 18) or MSP (cycle day 19 to 21) according to Noyes criteria. The patients were divided into two groups depending on IVF-ET outcome: pregnant (n = 14; 7 in ESP and 7 in MSP) or non-pregnant (n = 16; 8 in ESP and 8 in MSP).

Main results and the role of chance: A total of 14 DEGs were identified when comparing the ES phase and MS phase endometrium in the pregnant group. A total of 12 DEGs were identified when comparing the ES phase and MS phase endometrium in the non-pregnant group Nine genes were upregulated in the MS phase endometrium, compared to the ES phase endometrium, in both the pregnant and non-pregnant groups: adrenoceptor alpha 2A (ADRA2A), interleukin 1 receptor-associated kinase 2 (IRAK2), a disintegrin and metalloproteinase with thrombospondin repeats 15 (ADAMTS15), serpin family E member 1 (SERPINE1), integrin subunit beta 3 (ITGB3), transmembrane protein 252 (TMEM252), huntingtin associated protein 1 (HAP1), C2 calcium-dependent domain containing 4A (C2CD4A), and integrin subunit alpha 2 (ITGA2). Four genes were upregulated in the MS phase endometrium, compared to the ES phase endometrium, only in the pregnant group: TMEM37, galactosidase beta 1 like 2 (GLB1L2), Rho family GTPase 3 (RND3), and cytochrome P450 family 24 subfamily A member 1 (CYP24A1). One gene (ADAMTS8) was downregulated and one gene (monoamine oxidase A [MAOA]) was upregulated in the MS phase endometrium, compared to the ES phase endometrium, only in the non-pregnant group.

Limitations, reasons for caution: Firstly, since our sample size was relatively small, our results should be considered preliminary. Secondly, because endometrial tissue samples wee not obtained in IVF-ET cycle, they may not reflect changes in an actual pregnancy cycle.Thirdly, there may have been embryonic effects on implantation that were not accounted for.

Wider implications of the findings: TMEM37, GLB1L2, RND3, and CYP24A1 were upregulated only in the pregnant group; ADAMTS8 was downregulated while MAOA was upregulated only in the non-pregnant group.These novel DEGs, which have not been previously studied, may have functional significance during the WOI and serve as potential biomarkers of endometrial receptivity.

Trial registration number: not applicable

Abstract citation ID: dead093.693
P-335 Nervous system drugs are predicted as potential repurposing candidates for treatment in endometrial failure

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Study question: Is there a most represented group of approved drugs that can revert the impaired endometrial expression pattern associated with endometrial failure to improve reproductive outcomes?

Summary answer: Transcriptomics-based drug repurposing strategy identifies drugs currently used for nervous system diseases as repurposing candidates for endometrial therapies in infertility.

What is known already: Endometrium is a dynamic tissue with a key role in human reproduction whose alterations lead to infertility problems. Transcriptomic studies have been performed to understand molecular bases of endometrial failure, but effective treatments remain unknown. Transcriptomics-based drug repurposing strategy identifies approved drugs with a reversed disease gene expression profile, where genes up-regulated in a condition are down-regulated with the drug therapy and vice-versa, having a predicted therapeutic effect. Encouraging results for conditions such as preterm birth or cancer have already been shown using this methodology. In this study, we applied this approach to obtain treatment predictions for endometrial failure.

Study design, size, duration: A prospective multicenter study was performed between January 2019 and December 2021. A total of 192 women (18-50 years old) undergoing IVF with good-quality embryos were included and an endometrial sample in mid-secretory phase was collected to study their gene expression. Through artificial intelligence algorithms, we identified transcriptomic patterns with different endometrial prognoses. Expression changes among good or poor prognosis profiles were used to identify potential approved therapies to treat infertility leveraging drug expression databases.

Participants/materials, setting, methods: Gene signatures associated with poor prognosis were identified (FDR<0.05) and queried against the Connectivity Map database, containing gene expression profiles for 1,309 drugs in 5 different cell lines. Statistical analyses were performed to evaluate the drugs selected and obtain a score based on the drug expression reversal of the disease signatures. Significant drugs (adj. p-value) inversely associated with the gene signatures were highlighted. Finally, approved drugs were classified according to Anatomical Therapeutic Chemical codes.

Main results and the role of chance: We identified four transcriptomic profiles significantly (p-value<0.05) associated with different reproductive outcomes in the first embryo transfer after biopsy collection. Two poor prognosis profiles, one of them more related to clinical miscarriage, P1 (implantation rate = 30.4%, live birth rate = 42.9%, clinical miscarriage rate = 50%), and another to biochemical miscarriage, P2 (implantation rate = 20.0%, live birth rate = 33.3%, biochemical miscarriage rate = 66.7%) were compared with the best prognosis profile (implantation rate = 64.6%, live birth rate = 95.2%, biochemical miscarriage rate = 0.0%, clinical miscarriage rate = 4.8%), obtaining a total of 439 and 4,984 differential expressed genes, respectively for P1 and P2. After applying the drug repurposing methodology, we selected 50 and 32 significant approved drugs (adj. p-value<0.05) for P1 and P2. A total of 14 and 11 different drugs categories were obtained for each poor prognosis profile highlighting Nervous System (26%) and Alimentary tract and metabolism (12%) for P1, and Nervous System (23%), Antineoplastic and Immune modulating agents (15%) and Antiparasitic products (15%) for P2. Moreover, when both nervous system categories were compared, a total of 15 drugs were found in common, making possible the use of a unique drug for both profiles.

Limitations, reasons for caution: Since the Connectivity Map database does not include endometrial tissue, the action of these drugs should be validated experimentally in endometrial cell culture. Afterwards, to prioritize the best drug, side effects and approved doses reported in drug databases should be taken into consideration in further analyses.

Wider implications of the findings: The use of drug repurposing generates hypotheses for finding suitable drugs for endometrial failure, not requiring preclinical trials and going directly to Phase II clinical trials. These potential treatments will improve reproductive outcomes. Nervous system drugs seem to have a considerable effect on endometrium revealing possible causes of endometrial failure.

Trial registration number: Not Applicable

Abstract citation ID: dead093.694

P-336 Presence of stage III/IV endometriosis associated with systemic inflammation poses as individual risk factor for coronary heart disease in Indian women: Inklings for the search

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Study question: Can investigating endometriosis—cardiovascular interaction identify women with stage III/IV endometriosis and elucidate pathophysiology of the female heart at a broader level?

Summary answer: Endothelial dysfunction can occur in absence of structural atherosclerotic changes in young Indian women with endometriosis urging lifelong multidisciplinary care to manage long-term health impact.

What is known already: Chronic inflammation, enhanced oxidative stress, endothelial dysfunction, and cellular proliferation are hallmarks of both atherosclerosis and endometriosis with unveiled cellular and molecular overlaps between the duos. Further, treatment/s of endometriosis, like, hysterectomy or oophorectomy and/or analgesics confer increased risk of coronary heart disease (CHD) to women with endometriosis. However, CHD in women with endometriosis remains understudied, under-recognized, and underdiagnosed. Incidentally, early atherosclerosis cannot be explained exclusively by traditional cardiovascular risk factors. Aim of the present study was to correlate subclinical atherosclerosis with metabolic parameters and markers of endothelial inflammation in Indian women with stage III/IV endometriosis.

Study design, size, duration: This observational prospective cohort study constituted 1407 consented women diagnosed with laparoscopically confirmed endometriosis (Group A; n = 718) with age matched control (Group B; n = 689) (counterpart/s of male infertility) from October 2021 to September 2022. Sub-clinical atherosclerosis was investigated before laparoscopy by ultrasound evaluation of carotid intima-media thickness (cIMT) and flow-mediated dilation (FMD). Serum samples were stored at -80 °C for evaluation of biochemical and inflammatory parameters. European Society of Cardiology guidelines were followed for standard definition/s.

Participants/materials, setting, methods: Traditional (obesity, hypertension) and metabolic (dyslipidaemia, diabetes, hyperhomocysteinemia) cardiovascular risk factor/s were assessed during evaluation and chemiluminescence respectively. Serum levels of interleukin (IL)-6, IL-8, IL-10, TNF-a, VEGF, and VCAM-1 were determined by enzyme-linked-immunosorbent assay/s. Univariate comparisons and bivariate correlations were conducted by Student's t-test, and Spearman correlation respectively. Adjusted relative risks (aRR) with 95% confidence intervals (CI) were calculated by Cox-proportional hazard/s model among Group A vs Group B. Statistical significance was set at $p < 0.05$.

Main results and the role of chance: 62.95% and 37.04% women had stage III and stage IV endometriosis respectively as defined according to European Society for Human Reproduction and Embryology 2014 guideline. aRR (adjusted for demographic, obesity, diabetes mellitus, reproductive history, and migraine), documented a higher risk of hypertension (aRR 1.43; 95%CI 1.33–1.54) in women with endometriosis. On stratification of group A in less than and more than 40 years, laboratory parameters were similar except for significantly higher ($p < 0.01$) serum values of homocysteine, low density lipoprotein and cholesterol, in both age range of group A. FMD, indirect marker of endothelial dysfunction, was found to be lower in group A compared to controls (mean difference: −7.54, 95% CI: -10.32− -4.73; $p < 0.001$, however, cIMT was similar between both cohort/s. aRR of endometriosis in relation with hypercholesterolemia (1.07; 95%CI 1.01–1.25; $p < 0.001$), hypertension (1.22; 95%CI 1.14–1.30; $p < 0.001$), and hyperhomocysteinemia (1.04; 95%CI 0.96–1.12; $p < 0.08$) decreased with increasing age (>40 years). Systemic inflammation markers, showed an inverse relationship ($p < 0.001$) between values of FMD and serum levels of IL-6 ($r = -0.34$), TNF-a ($r = -0.85$), and VCAM-1 ($r = -0.28$) in group A. Other correlations were not statistically significant.

Limitations, reasons for caution: This observational study is not supported by patients with typical cardiovascular risk factors and hence limits generalizability of evidence to other ethnicities. Further, no information on hormonal treatments, such as dianogest (a synthetic progesterone) and/or leuprolide (gonadotropin-releasing hormone analog) are available to assess extent of association between endometriosis and CHD.

Wider implications of the findings: Higher propensity of subclinical atherosclerosis in younger women with laparoscopically-confirmed endometriosis represent higher systemic inflammation cueing to increased risk for CHD. This suggests the need for risk awareness and subsequent screening for CHD and healthy lifestyle promotion among gynaecologist/s and public health specialists.

Trial registration number: Not applicable

Abstract citation ID: dead093.695

P-337 Does Endometriosis Have More Detrimental Effect on Ovarian Reserve in the Presence of Autoimmunity? A Prospective Observational Study

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Study question: Is the coexistence of autoimmunity more harmful to ovarian reserve in endometriosis patients?

Summary answer: The presence of autoimmunity does have additional harm to ovarian reserve in patients with endometriosis.

What is known already: It is known that endometriosis has a negative impact on ovarian reserve via different pathways. The presence of inflammation, increased reactive oxygen substances, and iron deposits in ovarian endometrioma have detrimental effects on the ovarian reserve. Although endometriosis was reported to have co-incidence with immunologic disorders up to 50%, there is not enough publication to assess the impact of autoimmunity on ovarian reserve in endometriosis.

Study design, size, duration: A prospective observational study including 100 women with endometriosis was performed between January 2022 and January 2023 in the endometriosis center of a tertiary university hospital.

Participants/materials, setting, methods: Patients who were diagnosed with endometriosis, under age 40, without confirmed systemic disease, enrolled in the study. All patients were assessed by physical examination and routine ultrasound check. Endometriosis diagnosis was based on that assessment or verified previous surgery. The blood samples were collected from all patients. Serum anti-mullerian hormone (AMH) and immunologic panel tests (anti-nuclear antibody profile, lupus anticoagulant antibody, thyroid antibodies) were analyzed.

Main results and the role of chance: A total of 99 patients were enrolled in the analysis (one patient was excluded because of missing parameters). Patients were divided into two groups depending on the presence of any autoimmune antibody. There were 58 patients with negative test (autoimmune (-) group) and 41 patients with at least one positive antibody (autoimmune (+) group). The demographic parameters, including age, fertility situation, abuse, pain scores, endometrioma diameter, and laterality, were similar between the groups. The anti-mullerian hormone levels were significantly lower in the autoimmune (+) group, (Respectively with interquartile ranges; 2.2 ng/ml (0.6-5.3) vs. 0.9 ng/ml (0.19-3.6), $p = 0.01$) When the results were adjusted according to the age and surgical history, the significant negative effect of autoimmunity on the ovarian reserve disappeared ($p = 0.156$).

Limitations, reasons for caution: The lack of sufficient studies in this area makes our results important. The small sample size and lack of subgroup analysis depending on different autoimmune diseases limit the study's strength.

Wider implications of the findings: This observational study revealed that almost half of the endometriosis patients have autoimmunity. However, the results supported the existing data; the impact of autoimmunity on ovarian reserve in endometriosis is a novel research field, and the present study suggests it should be further investigated in endometriosis patients.

Trial registration number: Not Applicable

Abstract citation ID: dead093.696

P-338 Preliminary results of the DINE Study (Dienogest vs. Norethindrone Acetate in Endometriosis Treatment)

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Study question: How do the two-progestin type NETA and dienogest affect on pain and recurrence in patients with endometriosis?

Summary answer: NETA and dienogest are progestin derivatives that have similar effects on pain and recurrence in treating endometriosis and canbe safely preferred for treatment.

What is known already: Although surgical treatment significantly improves pain symptoms in endometriosis, it may be associated with significant urinary, intestinal and vascular complications; In addition, postoperative pain and recurrence rates of endometriotic lesions are high. Medical treatment gains importance, and progestins are mainly used in treatment. NETA is one of the most researched progestins for the treatment. It has a good safety profile and efficacy in curing endometriosis-related pain, making NETA a viable medical option among first-line treatments.

Study design, size, duration: The study was designed as a single-center, prospective, randomized study. Patients were randomly assigned to one of the two treatment protocols, with the single digit 'NETA' and double 'dienogest' according to the last digit of their protocol number. Patient recruitment started in February 2022. The patients were evaluated at 6-month visits for one year.

Participants/materials, setting, methods: Seventy patients aged 18-40 years were included. Patients with contraindicated progestins and pelvic inflammatory disease were excluded from the study. Demographic data, medical and surgical treatment histories were recorded. Patients were evaluated with detailed physical examination, ultrasound, and laboratory tests. They were questioned because of their chronic pain symptoms, they were asked to give a score between 1 and 10 with the numerical scoring system and recorded. They were re-evaluated at 6 and 12 months.

Main results and the role of chance: 40 dienogest and 30 NETA patients were included in the study. There were eight patients (one headache, one orthostatic hypotension, five metrorrhagia, one pregnancy request) in the NETA group and 11 patients (1 headache, one decreased sexual desire, one surgery, eight metrorrhagia) in the dienogest group. In the final analysis, 48 patients were included in the study. There was no significant difference between the two groups regarding initial laboratory, ultrasonographic and demographic parameters. ($p > 0.5$) There was no significant difference between the two groups in dropout rates at six months. ($p = 0.65$)

Dysmenorrhea, dyspareunia, dyschezia, and chronic pelvic pain scores were similar both at the beginning of treatment and at the 6th-month follow-up, and no significant difference was observed in both group. ($p > 0.5$) Significant improvement was observed in all scores in both groups.($p < 0.001$) In the 6th month, the most common side effects in the dienogest group were emotional lability (26%), and metrorrhagia (15%), while the NETA group were metrorrhagia (40%) and weight gain (20%). No significant effect was observed on the lipid profile and bone mineral density at the 6th-month control.

Limitations, reasons for caution: Our study is the first study in the literature to compare NETA and dienogest in the medical treatment of endometriosis. The strongest aspect is that it is a randomized control study. The small number of study population and the high dropout rates are the limitations.

Wider implications of the findings: NETA and dienogest are two progestin derivatives that are effective on pain and recurrence in the medical treatment of endometriosis and can be safely preferred for treatment. An analysis of our long-term data and further studies are needed to evaluate which progestin preparation is more effective and safe in treatment.

Trial registration number: NCT05476172

Abstract citation ID: dead093.697

P-339 Activities and changes in the number of natural killer cells in endometriosis: systematic review and meta-analysis

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Study question: What are the differences in peripheral, uterine, and peritoneal NK (uNK, pNK, pfNK) cell counts/percentages, and activities in women with endometriosis compared controls

Summary answer: The mean percentage level of uNK, pNK, and peritoneal NK (pfNK) cells has no significant difference in women with endometriosis compared controls

What is known already: NK cells *play an important role in the pathogenesis of endometriosis*

Study design, size, duration: This systematic review and meta-analysis included 36 experimental studies (case control and cross sectional). Narrative review is also conducted on the NK cell activity, cytokine expression, regulations and receptors.

Participants/materials, setting, methods: Women suffering with endometriosis confirmed with laparoscopy and/or pathology was the cases whereas, women having different pelvic pathology such as myoma, ovarian cyst, healthy woman and verified not having endometriosis with laparoscopy and/or pathology was considered as controls for this study.

Databases (PubMed, Web of Science, Scopus, Google scholar, and EMBASE through OVID) were used to search for the available studies. RevMan 5.4 and STATA 14 were used to analyze the data.

Main results and the role of chance: The mean percentage level of uNK, pNK, and peritoneal NK (pfNK) cells has no significant difference (standard mean difference SMD 0.13, 95%CI -0.35, 0.62; $P = 0.59$, I^2 74%; total 393 women, 6 studies), (SMD 0.27, 95%CI -0.07, 0.61; $P = 0.12$, I^2 80%; total 825 women, 11 studies), and (SMD 0.31, 95%CI -0.68, 01.29; $P = 0.59$, I^2 94%; total 404 women,7 studies) in endometriosis patients compared with controls respectively. The pooled NK (uNK, pNK, pfNK) cell cytotoxicity/activity level is significantly higher in controls compared with women with endometriosis (MD 5.43, 95%CI 2.29, 8.57; $P < 0.007$, I^2 50%; total 323 women, 7 studies). The NK cytotoxicity/activity level in early stages of endometriosis has no significant difference compared with advanced stages of endometriosis (MD 2.29, 95%CI –1.98-6.57; $P = 0.29$, I^2 87%; total 263 women, 7 studies). There are variations in studies conducted in NK cell activities in endometriosis: broadly categorized as NK cell cytotoxicity, cytokine expression and NK cell regulation and receptors (Inhibition and activation). The cytotoxic activity of NK cells decreased in women with endometriosis and correlated with the severity of the disease, however the cytokine expression and inhibitor receptors are equivocal across studies.

Limitations, reasons for caution: We unable to conduct sub-group analysis to investigate the cytotoxic activity of NK cell in women with endometriosis and its stages in different samples such as pNK, uNK, and pfNK due to the lack of studies for the analysis.

Wider implications of the findings: This study revealed that the activity of NK cells in women with endometriosis is significantly lower than controls.Though the mean difference of NK cell count and percentage has no significant difference, the cytotoxicity activity of NK cell is higher in controls compared with women with endometriosis

Trial registration number: CRD42022384060

Abstract citation ID: dead093.698
P-340 Endometrial microbiome - is every dysbiosis an inflammation?

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Study question: The endometrial microbiome, in addition to other parameters of inflammation (CD138 presence, NK bright fraction presence), may be a complementary marker of ongoing local inflammation.

Summary answer: Presented results of the microbiome in comparison with the CD 138 marker may indicate inflammation in the endometrium, which is caused by dysbiosis.

What is known already: The study of the microbiome and the assessment of its impact on female reproductive system on embryo implantation, pregnancy maintenance and the success of the in vitro fertilization (IVF) procedure is an increasingly popular research topic. The protective role of *Lactobacillus* (other than *L.iners*) is known, and probably its low number may be related to the impossibility of embryo implantation. Dysfunctional mycobiome may be the cause of chronic inflammation of the endometrium, including endometriosis and recurrent implantation failures. Importantly, the dysbiotic profile of the endometrial microflora often does not cause any clinical symptoms.

Study design, size, duration: The clinical study included 182 women in child-bearing age, who were patients of the Gyncentrum Clinic (Poland), and who were qualified for the IVF procedure. In this group of patients, 201 samples combined were tested, obtaining 19 negative results due to the low quality of swabs, and the small amount of microbiota impossible to efficiently isolate. In the study, samples were analysed from January to December 2022 collection.

Participants/materials, setting, methods: To analyze the assumed parameters, patients treated at the Gyncentrum Clinic underwent a routine hysteroscopy procedure, during which endometrial swabs (determination of the molecular microbiome using the Next Generation Sequencing NGS method) and tissue fragments (determination of CD138 immunohistochemically and NK fractions by flow cytometry) were collected. Nucleic acids were isolated from the swabs for the preparation of libraries, based on the Illumina-16S Metagenomic Sequencing Library Preparation.

Main results and the role of chance: The microbiome study and other inflammation parameters in the group of our patients allowed us to observe some trends. Due to the microbiological endometrium profile, the patients were divided into 3 groups: 1) normal microflora (domination of *Lactobacillus* other than *L.iners* and/or *Bifidobacterium*; n = 135), 2) moderate dysbiosis (presence of *Lactobacillus* and/or *Bifidobacterium* and/or *L. iners*-max. 50% of all sample- and also other potentially harmful species e.g. *Enterobacteriaceae* group, *Streptococcus* group, *Veilonella*; n = 28); 3) dysbiosis (with/ without low number of *Lactobacillus* and *Bifidobacterium*, strong dominance of harmful species listed above or *L.iners* dominance; n = 19). We observed that in the group of endometrial dysbiosis patients, an increased CD138 index ($\geqslant$1/10 high power fields HPF) is more often noted - in the group of dysbiotic patients with CD138 = 0/10 HPF 7.21%, in the group of dysbiotic patients with increased CD 138 15.49%. We didn't notice any trends comparing the results of CD138 to the NK bright fraction (CD56++/CD16-) tested in endometrial specimens, or the relationship of this fraction to the microbiome. Observed trend indicates a certain relationship in the presence of abnormal microflora and inflammation expressed by CD138, but it requires confirmation on a larger study group and obtain information about the success of pregnancy.

Limitations, reasons for caution: The conducted research require broader analysis on a larger group of patients. It is necessary to obtain full information about the success of pregnancy in all analyzed patients. Identification of taxa using the NGS method is not the same as the identification of microorganisms living in the studied microenvironment.

Wider implications of the findings: Presented results should be treated as a starting point for further analyses, important in the context of research based on infertility, focusing on relationships between the microbiome and the IVF effectiveness. It seems interesting to extend the molecular study of the microbiome to its other elements - viruses, fungi, protozoa.

Trial registration number: 161/KBL/OIL/2021

Abstract citation ID: dead093.699
P-341 Endometrial compaction and its impact on reproductive outcome

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Study question: Is there a relationship between endometrial compaction and clinical pregnancy rate in frozen embryo transfer (FET) cycles?

Summary answer: Clinical pregnancy rate (CPR) were similar in both patients that demonstrated endometrial compaction or no compaction in FETs cycles.

What is known already: There has been increasing interest in the correlation between endometrial compaction and clinical outcomes but there has been conflicting evidence from prior investigations.

Study design, size, duration: Retrospective Observational data Analysis of (n-350) patients undergoing Embryo Transfer during January 2022 to December 2022 at our clinic.

Participants/materials, setting, methods: This study was performed at a single, academically affiliated fertility center in which patients who had an FET using programmed/modified natural cycle protocol were included. Endometrial thickness at time start of progesterone(T1)(TVS) & at time of Embryo transfer (T2)(TAS)was measured, and percentage on ET compaction in both HRT cycle and modified natural cycle was studied. The primary outcome (CPR) was based on proportion of compaction (percentage difference in EMT between T1 and T2).

Main results and the role of chance: Of the 350 participants included, 64%, 38% and10%, of women exhibited >0%, >5% and >10% endometrial compaction, respectively. Endometrial compaction was not predictive of Clinical Pregnancy Rate (CPR) at any of the defined cutoffs .Primary outcomes included CPR (defined as the presence of at least one gestational sac with a fetal pole with cardiac activity on transvaginal ultrasound between 6 and 9 weeks gestation), biochemical pregnancy rate and spontaneous abortion rate. Patients were discharged to their obstetrician when a clinical pregnancy was confirmed between 6 and 9 weeks gestation.

Limitations, reasons for caution: There was the potential for measurement error in the recorded EMTs. The T2 measurement was performed transabdominally, which may cause potential measurement error, as it is generally accepted that transvaginal measurements of EMT are more accurate, though, any bias is expected to be non-differential

Wider implications of the findings: Assessing endometrial compaction may lead to unnecessary cycle cancellation. However, further studies are needed to determine if routine screening for endometrial compaction would improve clinical outcomes.

Trial registration number: not applicable

Abstract citation ID: dead093.700

P-342 Uterine contractile patterns in adenomyosis patients normalize under hormonal contraception use: the WAVES study

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Study question: Comparison of uterine contractility (UC) in adenomyosis patients (AP) with and without hormonal contraception (HC) compared to controls with HC, measured by transvaginal ultrasound (TVUS).

Summary answer: AP with HC show better contraction coordination compared to untreated AP. AP with HC show comparable UC compared to controls with HC.

What is known already: Adenomyosis is a disease of the uterus that can cause dysmenorrhoea, menorrhagia, dyspareunia and subfertility. These symptoms could be explained by the different contraction patterns in women with adenomyosis compared to healthy controls. Therapeutic use of hormonal contraception reduces the symptoms experienced by women with adenomyosis. This could be explained by the normalization of contraction patterns, which has not yet been objectively quantified due to the absence of a suitable measurement tool. A novel speckle-tracking and strain analysis by 2D TVUS recordings has recently been used to assess differences in contraction coordination, contraction frequency, velocity and direction in healthy women.

Study design, size, duration: This study is part of an ongoing multi-centre prospective observational cohort study investigating UC on TVUS. Our study includes the TVUS recordings of 23 women with adenomyosis without hormonal contraception treatment, 15 women with adenomyosis undergoing hormonal contraception treatment, and 17 women with healthy uteri undergoing hormonal contraception treatment. Patients were included in 3 centres from 2017 to 2023 (Catharina Hospital Eindhoven, Fertility Clinic Thessaloniki and University Federico Napels).

Participants/materials, setting, methods: 23 women with sonographic suspicion of adenomyosis without HC, 15 women with adenomyosis undergoing HC treatment and 17 women with healthy uteri with HC were included. HC included oral combined HC, progesterone only pill and hormonal IUD. UC frequency, amplitude, velocity and coordination were assessed by applying a dedicated speckle-tracking and strain analysis to 2-4-minute TVUS recordings in midsagittal section. AP with HC were compared to AP without contraception and to healthy controls with HC.

Main results and the role of chance: Age, BMI, parity and uterus volume were significantly higher in the women with adenomyosis compared to the healthy controls ($p < 0.05$). The adenomyosis group with contraception showed more contraction coordination compared to the adenomyosis group without hormonal contraception treatment (0.23 ± 0.10 vs. 0.29 ± 0.11, $p = 0.041$). There was a tendency towards higher contraction frequency (1.53 ± 0.21 vs. 1.42, $p = 0.153$) and lower amplitude (0.56 ± 0.04 vs. 0.65 ± 0.04, $p = 0.159$) in the adenomyosis group with hormonal contraception treatment compared to the adenomyosis group without hormonal contraception treatment. There were no significant differences in uterine contractility between the adenomyosis group with hormonal contraception treatment compared to the healthy group with hormonal contraception treatment.

Limitations, reasons for caution: No sub-analysis was done to assess effects of additional adenomyosis and contraception characteristics due to this being an ongoing study. Women with extensive adenomyosis were not included due to impossibility to perform analysis of ultrasound recordings. AP were older, had higher BMI and larger uterus volumes than healthy controls.

Wider implications of the findings: The normalization of UC under therapeutic use of HC compared to untreated AP and the lack of differences in UC between AP and healthy controls with HC, confirms the therapeutic effect on adenomyotic symptoms. This presents a new therapeutic efficacy marker for adenomyosis.

Trial registration number: NL52466.100.15

Abstract citation ID: dead093.701

P-343 The role of Yes-Associated protein (YAP) activity in uterus adenogenesis process

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Study question: Could Yes-Associated protein(YAP) be effective in the adenogenesis process that occurs in the mouse uterus in the postnatal period?

Summary answer: It was observed that the YAP and phospho-YAP levels decreased in mice with inhibited gland development during adenogenesis process when compared to the control groups.

What is known already: The mouse uterus is composed of undifferentiated mesenchyme surrounded by a single layer of epithelium at birth. On the 5th Postnatal Day (PD), the luminal epithelium begins to bud, and the process called adenogenesis, which is termed as gland development in the uterus, begins. The gland development is evident on the PD 10th day. The uterus shows adult tissue characteristics by PD 15th-20th days. Although it has been stated that various signaling pathways are active in this process, Hippo signaling pathway role in the adenogenesis process have not been clarified yet.

Study design, size, duration: The uterus with normal gland development and gland development inhibited by Progesterone were evaluated for YAP and phospho-YAP, which is one of the main components of Hippo signaling pathway, by immunohistochemistry, western blotting, and q-RT-PCR methods. For experimental groups, 50 μg/g Progesterone was dissolved in 0.1 mL Sesame oil and for control groups, only 0.1 mL of Sesame oil given subcutaneously on PN 2nd-10th days. Animals were sacrificed on the PN 5th-10th and 15th days.

Participants/materials, setting, methods: In total of 30 newborn Balb/c female mice were used. They were divided as control (n = 15) and experimental(n = 15) groups. After the injections were finished, mice from both groups at PN5th(n = 5), PN10th(n = 5), and PN15th(n = 5) days were cervical dissociated, and uteri were collected. For immunohistochemical analyses, tissues were taken into buffered formalin, paraffin blocks were made, and the sections were taken. Protein and RNA isolations were performed from uteri for western blotting and q-RT-PCR analyses, respectively.

Main results and the role of chance: When YAP expression was evaluated by immunohistochemistry, western blotting and q-RT-PCR methods, there was no difference between the groups on the PN 5th day. But there was an active signaling was observed in the control groups on the PN10th day, which is very important for gland formation, and on the PN 15th day, that is effective in the maturation process. On the PN10th and 15th days, it was determined that YAP and phospho-YAP signal expression were decreased significantly in the experimental groups when compared with the control groups in immunohistochemically and protein levels. In addition, these findings were supported by mRNA analysis with YAP.

Limitations, reasons for caution: The study was performed *in vivo* mouse model. Further studies can be done with a transgenic animal model.

Wider implications of the findings: Considering the contribution of uterine gland development to the implantation process, errors in the development of these glands may cause infertility. This study can bring a new perspective to researching the uterus adenogenesis period and causes of infertility and treatment approaches.

Trial registration number: not applicable

Abstract citation ID: dead093.702

P-344 Prevalence and maternal risk factors for neonatal uterine bleeding (NUB): a possible origin of endometriosis?

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Study question: What is the prevalence and the cause of neonatal uterine bleeding, also known as "genital crisis"? Is it associated with maternal lifestyle and obstetric history?

Summary answer: The prevalence of NUB has been estimated as 29% and it seems to be correlated to some aspects of the maternal lifestyle

What is known already: NUB is considered a physiological phenomenon characterized by a menstrual-like bleeding occurring during the first days of life in female newborns, with an estimated incidence of 3-5%. It seems to be related to the reduction of steroid hormones in the baby after the separation from the maternal placenta, although, actually, some theories hypothesize NUB as a consequence of the exposure to molecular disruptors during intrauterine life. However, data regarding NUB still remain uncertain. Interestingly, recent theories assume a correlation between NUB and endometriosis onset, considering it as a consequence of the retrograde menstruation of stem cells during the genital crisis

Study design, size, duration: This is a prospective cohort study performed in a population of 105 mothers-to-be who delivered their babies at Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico of Milan between March 2022 and December 2022. Inclusion criteria were: gestation of a single female fetus, age between 18 and 42, being in healthy condition and undergoing a low-risk pregnancy. Women with endometriosis had also been included

Participants/materials, setting, methods: At the time of recruitment few days before delivery, patients were asked to fill a questionnaire regarding general clinical characteristics (BMI, smoking habits, diet). After two weeks from delivery, a follow up was performed asking them information about pregnancy outcomes (time to pregnancy, complications and type of delivery), a possible vaginal bleeding for the baby and its duration, the type of lactation (breastfeeding, formula or both) and clinical neonatal information.

Main results and the role of chance: NUB frequency was unexpectedly high. Twenty-nine percent of the babies experienced NUB with a duration of 3 days and insurgence 2 days post-partum. Among the variables analyzed, genital crisis resulted higher in newborns from women with significantly higher BMI compared to women with a lower BMI (BMI 23 vs 21.5 respectively, $p<0.05$). Smoking during pregnancy was associated with a higher incidence of newborns with NUB (20% vs 4% in controls; $p < 0.05$). According to the type of lactation, in newborns with genital crisis, 43% of them received maternal breastfeeding while the same type of feeding has been reported in 69% of controls ($p<0.05$). Lastly, soy consumption by the mothers was observed only in 41% of cases of genital crisis while among mothers whose newborns did not experience NUB, 58% declared presence of soy in their diet ($p<0.05$).

Limitations, reasons for caution: The population size is relatively small. Also, as prospective cohort study, loss of individuals at follow up and recall bias may occur

Wider implications of the findings: Collecting more information on the hormonal determinants of NUB could be useful for future studies on intrauterine life and reproductive disorders. If a link between NUB and endometriosis is confirmed, NUB might lead to the understanding of the onset of this pathological condition

Trial registration number: not applicable

Abstract citation ID: dead093.703

P-345 GM-CSF drives endometrial repair via p-STAT3 mediating angiogenesis

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Study question: Does granulocyte macrophage colony-stimulating factor (GM-CSF) play a role in endometrial angiogenesis and can it be used as a new application for treating endometrial regeneration?

Summary answer: GM-CSF improves endometrial repair via promoting endometrial angiogenesis and may provide a novel insight and therapeutics for endometrial injury.

What is known already: GM-CSF is a cytokine normally expressed in the female reproductive tract and plays key roles in embryo implantation and subsequent development. Our previous study found that intraperitoneally (i.p.)injection of GM-CSF can significantly improve endometrial repair by promoting endometrial glandular cells proliferation and stromal cells migration, increasing the thickness of endometrium and embryo implantation in mice. However, whether GM-CSF is involved in endometrial angiogenesis is unknown.

Study design, size, duration: To observe whether angiogenesis is promoted by GM-CSF, the expression of angiogenic factor CD31 was evaluated after injection of GM-CSF into endometrial injured mice model. The effect of GM-CSF on vascular g regeneration was also explored by zebrafish embryo model of intersegmental vascular injury. Human umbilical vein vascular endothelial cells (HUVECs) were cultured in vitro, and the role of GM-CSF on HUVECs were examined through EdU, Tube formation, Scratch repair and Transwell assays.

Participants/materials, setting, methods: 20μl 90% ethanol was used to establish endometrial injured mice model. After modeling, compared with i.p. injection of GM-CSF and saline, observing whether GM-CSF had the effect of repairing angiogenesis of injured endometrium, by evaluating expression of CD31. Real-time PCR, Western Blot were used to verify differentially expressed levels of mRNA and protein. Western Blot was used to confirm protein location. ChIP was used to analyze stat3 regulation of GM-CSF.

Main results and the role of chance: GM-CSF promoted expression of angiogenic factor CD31 in the mice model of endometrial injury. GM-CSF can repair and regenerate the zebrafish embryo model of intersegmental vascular injury caused by Sorafenib. GM-CSF can promote angiogenesis by HUVECs proliferation and migration, and significantly increase the formation of vascular-like network structures In GM-CSF treatment group, the mRNA expression of *VEGF*, *MMP2*, *Ang1*, *Ang2* and *Tie2* mRNA in HUVECs cells increased significantly; GM-CSF can activate the phosphorylation expression levels of p-FAK, p-Src, p-ERK 1/2, p-STAT3, p-p38 MAPK, pc-Jun, p-CREB, p-Akt, and p-eNOS. And it can increase the expression of downstream VEGF and MMP2 proteins. GM-CSF treatment for 30 min can promote phosphorylation and translocation of STAT3 from cytoplasm to nucleus, thereby regulating the expression of VEGF and MMP2 downstream. While FAK inhibitors (PF573228) and STAT3 knockdown can abrogate GM-CSF effect on HUVECs.

Limitations, reasons for caution: The results at the cellular and animal level can't be completely mimic human endometrial injury. In future, well-designed clinical trials are needed to investigate the efficacy and safety of GM-CSF for treatment of human endometrial vascular injury.

Wider implications of the findings: GM-CSF can promote the vascular regeneration in mice model of endometrial injury through p-STAT3. Our findings provide new ideas for clinical treatment of endometrial injury.

Trial registration number: Not applicable

Abstract citation ID: dead093.704

P-346 Migration-associated microRNAs are dysregulated in endometriosis: potential diagnostic biomarkers

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Study question: What is the role of dysregulated endometrial microRNAs (miRNAs) in the development of endometriosis?

Summary answer: Dysregulated miRNAs in the endometrium of women with endometriosis can also be found in endometriomas and may affect the migratory ability of endometriotic cells.

What is known already: The molecular mechanisms underlying the pathogenesis of endometriosis are poorly understood. One of the hypotheses is that changes in cell properties observed in endometriotic lesions could be initiated already in the endometrium. Dysregulation of miRNAs in the endometrium of women with endometriosis has been reported in previous microarray-based studies. However, little overlap has been seen between published miRNA expression data. Moreover, the potential role of dysregulated miRNAs in the endometrium of women with endometriosis and whether these changes are also present in the endometrioma are largely unknown.

Study design, size, duration: In this experimental case-control study miRNA gene expression in proliferative phase endometrium was compared between 15 women with laparoscopically confirmed endometriosis (stage III–IV) and 17 age-matched controls who were laparoscopically confirmed to be free of endometriosis. Selected miRNAs were further compared between paired proliferative-phase endometrium and endometrioma from a new cohort of women with endometriosis (stage III–IV) and studied *in vitro* to understand their effect on cell migration.

Participants/materials, setting, methods: Samples were collected during laparoscopic operations performed at Tartu University Hospital, Estonia. Dysregulated endometrial miRNAs were detected with small RNA sequencing and differential gene expression analysis. Target genes and biological pathways were predicted to understand their potential role in the disease. Selected miRNAs were further studied for their expression in endometriomas using qRT-PCR and *in-vitro* for their proliferation and migration ability using a transwell-migration assay after miRNA mimic transfection of the 12Z endometriotic cell line.

Main results and the role of chance: In total, we identified 9 upregulated and 5 downregulated ($-2 <$fold change > 2, FDR < 0.05) miRNAs in the endometrium of women with endometriosis compared to controls. In-silico analyses showed that predicted target genes of the dysregulated miRNAs were significantly enriched in migration-related KEGG pathways such as adherens junctions, focal adhesion, MAPK-, PI3-AKT-, and TGF-beta signaling. The most down-regulated miRNA, miR-193b-5p, and the most up-regulated miRNA, miR-374b-5p, were selected for further characterization. Validation of their expression in endometriomas showed a significant up-regulation of both miRNAs compared to paired endometrium (Fold change > 2, FDR $<$ 0.05). Since it has been reported that altered cell migratory ability could be involved in the pathogenesis of endometriosis and our dysregulated miRNA were associated with migration-related pathways, we explored if miR-193b-5p mimic transfection affects the migration capacity of 12Z cells. A 2-fold decrease in cell migration (p-value 0.0021) was observed after 12Z cell mimic transfection. No change in proliferation was demonstrated.

Limitations, reasons for caution: Although our findings both in-silico and in-vitro suggest a link between dysregulated miRNAs and cell migration, further *in-vitro* studies in primary cells and *in-vivo* studies in animal models are needed to reveal the specific pathways that these miRNAs regulate to explain the observed functional changes in the context of endometriosis.

Wider implications of the findings: This study gives molecular insight into the pathogenesis of endometriosis, a poorly understood disease, by demonstrating changes in miRNA expression in both the endometrium and endometrioma that potentially can be linked to a changed cell migratory ability. Furthermore, identified miRNAs could be further evaluated as diagnostic biomarkers in larger studies.

Trial registration number: Not applicable

Abstract citation ID: dead093.705

P-347 the causal effects of serum lipids and apolipoproteins on endometriosis: a two-sample mendelian randomization analysis

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Study question: Is the risk of endometriosis associated with genetically predicted levels of different blood lipid and apolipoprotein traits?

Summary answer: Our Mendelian randomization (MR) analysis indicated that causal associations existed between some serum lipid and apolipoprotein levels and endometriosis occurrence in European ancestry.

What is known already: Endometriosis is a chronic gynecological disease with a high prevalence among reproductive-aged women. Recently, many observational, epidemiological studies have investigated the causal effect of different serum lipid and apolipoprotein parameters on the occurrence and progression of endometriosis. However, the results of these studies remained controversial. Moreover, no different subtypes of endometriosis were investigated in previous observational studies, thus detailed information about the associations was insufficient.

Study design, size, duration: We performed a two-sample MR analysis in the largest available genetic datasets. To correctly employ the MR method, the following assumptions are required to be met: (a) the genetic variants must be strongly associated with the exposures; (b) the single-nucleotide polymorphisms (SNPs) are not correlated with the confounders that are related to the exposure and the outcome; (c) the SNPs can influence the outcome solely through the exposure and no other biological pathways are concerned.

Participants/materials, setting, methods: Summary-level data for genetic variants of different serum lipid and apolipoprotein traits were derived from the UK Biobank and EBI database. Instrumental variables (IVs) associated with endometriosis and all the subtypes were acquired from the FinnGen biobank. The MR method is an accurate way to determine the underlying causality between the exposure and the outcome, which can minimize the effect of potential confounders and avoid reverse causality.

Main results and the role of chance: Our results demonstrated that five serum lipid-related traits were causally linked to lower odds of endometriosis, including very low-density lipoprotein (VLDL) cholesterol (OR: 1.231, 95% CI: 1.035-1.463, p = 0.019), cholesterol in chylomicron (CM) and extremely large VLDL (OR: 1.290, 95% CI: 1.013-1.644, p = 0.039), total cholesterol (TC) (OR: 1.250, 95% CI: 1.001-1.562, p = 0.049), apolipoprotein B (OR: 1.273, 95% CI: 1.022-1.587, p = 0.031), the ratio of apolipoprotein B to apolipoprotein A1 (OR: 1.163, 95% CI: 1.011-1.337, p = 0.035), while high-density lipoprotein cholesterol (HDL) (OR: 0.853, 95% CI: 0.754-0.963, p = 0.011) and apolipoprotein A1 (OR: 0.874, 95% CI: 0.770-0.993, p = 0.038) showed the opposite. Subgroup analyses about subtypes of endometriosis suggested the causal association of several serum lipid and apolipoprotein levels with peritoneal endometriosis. Neither pleiotropy nor heterogeneity was found in our study. No evidence was detected on the causal relations of low-density lipoprotein cholesterol (LDL) (OR: 1.014, 95% CI: 0.859-1.197, p = 0.868 and OR: 1.029, 95% CI: 0.906-1.169, p = 0.659, respectively) and triglyceride (TG) (OR: 1.138, 95% CI: 0.978-1.324, p = 0.095) with endometriosis.

Limitations, reasons for caution: Several limitations do exist in this study. We could not control the uniformity of sex between the exposure and the outcome, due to a lack of suitable summary-level data. Furthermore, it is possible that some of the null findings were due to limited statistical power from current genome-wide association study.

Wider implications of the findings: Our study found strong evidence for the possibility that aberrant lipid metabolism is causally involved in the pathogenesis of endometriosis, especially for endometriosis of pelvic peritoneum, and shed new insights on targeting serum lipid and apolipoprotein levels as a potential novel strategy for the prevention and treatment of endometriosis.

Trial registration number: not applicable

Abstract citation ID: dead093.706

P-348 Correlation between cesarean scar defect and chronic endometritis and its effect on the pregnancy outcomes of in vitro fertilization

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Study question: Do patients with symptomatic cesarean scar defect (CSD) have a higher risk of chronic endometritis (CE) and worse early pregnancy outcomes after hysteroscopic reconstruction for CSD?

Summary answer: Symptomatic CSD has a positive correlation with CE. Antibiotic administration is a promising option for patients with CE to obtain better early pregnancy outcomes.

What is known already: Cesarean scar defect (CSD), a niche-shaped myometrium defect, can be noticed after cesarean sections (CS). Studies about the impact of CSD on fertility are limited. The uterine isthmic region is a sperm reservoir, and CSD may negatively affect natural fertility. CE could reduce endometrial receptivity and thus leads to a lower clinical pregnancy rate. Secondary infertile patients with a history of CS are quite common in the department of reproductive medicine. However, there was still limited data to determine whether CSD correlates with CE, especially in cases suffering from symptomatic CSD with postmenstrual spotting.

Study design, size, duration: This is a retrospective study including 118 patients with a leading symptom of postmenstrual spotting and 224 asymptomatic patients from January 1, 2018 to May 31, 2021.

Participants/materials, setting, methods: Group A was with a leading symptom of postmenstrual spotting, and group B was asymptomatic. Office operative hysteroscopy was performed to correct the CSD for symptomatic patients in group A, and thermoablation of the endometrium in CSD was performed. Out-patient hysteroscopy was performed for asymptomatic patients in group B, and only the morphology of the endometrium was evaluated. Both groups have CD138 immunohistochemistry staining of their endometrium.

Main results and the role of chance: A higher incidence of CE was found in group A compared with group B (50/118 *vs.* 62/224, *P*=0.006, OR = 1.921, 95%CI=1.203-3.068). There were no significant differences in the comparisons of chemical pregnancy rate (26/86 *vs.* 75/216, *P*=0.455, OR = 0.815, 95%CI=0.475-1.396), ectopic pregnancy rate (1/86 *vs.* 1/216, *P*=0.489, OR = 2.529, 95%CI=0.156-40.901), miscarriage rate (6/86 *vs.* 22/216, *P*=0.368, OR = 0.661, 95%CI=0.258-1.692), preterm birth rate (2/86 *vs.* 4/216, *P*=1.000, OR = 1.262, 95%CI=0.227-7.020) or full-term pregnancy rate (12/86 *vs.* 35/216, *P*=0.626, OR = 0.839, 95%CI=0.413-1.704) between the two groups.

Limitations, reasons for caution: First, different doctors might have inconsistent judgments on the definition of postmenstrual spotting. Second, three different operators performed the hysteroscopy. It might contribute to the heterogeneity of the study findings.

Wider implications of the findings: Canalling and thermoablation during hysteroscopy are effective treatments for symptomatic CSD. CSD accompanied by CE should be given more attention before embryo transfer.

Trial registration number: No. 2020A1515110791

Abstract citation ID: dead093.707
P-349 Modelling the impact of acute and chronic decidual senescence on endometrial stemness in 3D assembloids

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Study question: Is de-differentiation of committed endometrial stromal and epithelial cells in response to decidual senescence involved in regulating stemness of cycling human endometrium?

Summary answer: Transient decidual senescence promotes endometrial tissue rejuvenation by reprogramming stromal and epithelial cells into progenitor stem-like cells whereas chronic senescence causes stem cell depletion.

What is known already: In wound healing, acute but not prolonged senescence, a cellular state characterised by permanent cell cycle arrest and production of a complex secretome rich in inflammatory mediators, ECM proteins and proteinases, growth factors and angiogenic modulators, has been shown to promote de-differentiation of committed cells into stem-like progenitors, thereby enhancing tissue regeneration upon immune clearance of senescent cells. Menstrual repair depends on endometrial progenitor cells but whether they represent stromal and epithelial cells that have de-differentiated in response to acute premenstrual decidual senescence is not known.

Study design, size, duration: Endometrial 'instant' assembloids, consisting of gland organoids and primary stromal cells in collagen hydrogels, were established from freshly isolated cells from mid-luteal endometrial biopsies. The assembloids were then subjected over 36 days to 4 cycles of decidualization, a process associated with acute inflammatory senescence, followed by hormonal withdrawal. To induce chronic senescence, assembloids were decidualized continuously for 14 days. Stemness of assembloids was assessed in undifferentiated and decidualized cultures at the end of each 'cycle'.

Participants/materials, setting, methods: 'Instant' assembloids, which closely recapitulate native endometrium, were established in collagen hydrogels from 10 midluteal biopsies and subjected to cyclical or prolonged decidualization. Decidualization was monitored by RT-qPCR analysis, using epithelial and stromal cells isolated at regular timepoints. The level of stemness of stromal and epithelial cells was measured by colony-forming unit (CFU) and organoid formation efficacy (OFE) assays, respectively.

Main results and the role of chance: Repeated cycles of hormonal stimulation and withdrawal resulted in cyclical decidualization of instant assembloids, as characterised by the induction of decidual stromal (*PRL*, *SCARA5* and *DIO2*) and epithelial (*PAEP* and *SPP1)* marker genes when compared to parallel undifferentiated assembloids. Cyclicity enabled cells to maintain a healthy state and preserved the structural integrity of the assembloids. Further, cyclical decidualization of assembloids enhanced CFU activity and OFE activity of stromal and epithelial cells, respectively, indicative of active de-differentiation of committed cells. By contrast, chronic senescence, as induced by a prolonged decidualization, resulted in stem cell depletion in both glandular and stromal compartments and progressive loss of structural integrity of assembloids. We observed a reduced expression of the decidual marker gene *PRL* and an enhanced expression of *IGFBP1* representing the increased stress state of cells. Taken together, induction of acute decidual senescence resulted in a robust de-differentiation response and increased abundance of stromal and epithelial progenitor cells, whereas prolonged senescence caused stem cell exhaustion in both cellular compartments.

Limitations, reasons for caution: Although the cellular responses observed in our 'instant' assembloid model were robust, caution is warranted when extrapolating from *in vitro* observations. Further, although the 'instant' assembloid model enables co-culturing of endometrial endothelial and immune cells, current hydrogels greatly limit their migratory capacity. The mechanisms of cellular de-differentiation are incompletely understood.

Wider implications of the findings: Our findings indicate that the level of premenstrual decidual senescence in the superficial layer regulates stemness in the basal layer, thus ensuring inter-cycle endometrial homeostasis. Conversely, prolonged decidual senescence associated with clinical miscarriages may plausibly increase the risk of further pregnancy loss by depleting stemness of the regenerative basal layer.

Trial registration number: N/A

Abstract citation ID: dead093.708
P-350 Evaluation of EndoSERA Autologous Platelet Derived Growth Factors for improving clinical pregnancy and live birth rate in FET cycles for women with refractory thin endometrium

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Study question: Can Autologous Platelet Derived Growth Factors (hereafter EndoSERA) be used as promising coadjuvant therapy in assisted reproductive techniques to improve endometrial thickness and implantation rate?

Summary answer: Use of EndoSERA significantly increased endometrial growth, clinical pregnancy and Live Birth Rate In Patients With refractory thin endometrium during FET cycles.

What is known already: Inadequate endometrial thickness and receptivity are major causes for RIF and surrogacy is reasonable option when endometrium remains unresponsive to conventional treatments. Recent studies demonstrated platelet-rich plasma (PRP) improves pregnancy outcomes in thin endometrium and RIF patients. The mechanisms of PRP have not been completely elucidated, but laboratory studies have shown that the high concentration of growth factors in PRP can potentially speed up the healing process. Need for an optimized method to ensure right concentration of platelets and growth factors to maximize the therapeutic outcomes formed the basis for developing EndoSERA-implantation friendly platelet derived growth factors concentrate.

Study design, size, duration: In this prospective interventional self-controlled study, 55 women in the age group of 25-45 years from July 2018 to July 2022 with $>$ 3 failed FET due to refractory thin endometrium, negative hysteroscopic screening for endometrial pathology, and negative bacteriologic screening and failed to get pregnant with multiple immune therapy regimens like intralipid infusions, granulocyte colony-stimulating factor infusions, steroid therapy, endometrial scratching were selected to undergo Endo-SERA treatment.

Participants/materials, setting, methods: After obtaining informed consent subjects were treated with intrauterine infusion of EndoSERA 3 times (Day7 and Day 12 of their menstrual cycle day and 2 days before ET). 54 patients underwent FET. Intrauterine infusion of 0.8 ml of EndoSERA was infused into uterine cavity in addition to standard HRT protocols. Clinical pregnancy was determined by positive serum β-HCG, 2weeks after ET and presence of fetal heart beat in trans-vaginal ultrasound 5weeks after ET.

Main results and the role of chance: Post EndoSERA administration endometrial thickness(ET) was significantly thicker (7.86 $\pm$ 0.22 vs 6.22 $\pm$ 0.31 mm; P $<$.05) in 98% of patients and optimal response to EndoSERA was considered to reach ET $\geq$ 7mm after 2nd EndoSERA dose administration. Only one patient's cycle got canceled due to ET $<$ 7mm. Out of 55 women, 42 became pregnant (76%) and 12 patients did not conceive (22%). 39 women (70.9%) had a clinical pregnancy and 5 women (9.1%) miscarried before 6-12 wks and 3 women (5%) had biochemical pregnancy. 33 women (60%) had delivered healthy full-term babies and one patient (2%) is in her 24th week of uneventful gestation. There were no adverse effects reported by the patients who were treated with EndoSERA. There are studies highlighting the need for an angiogenic and anti-inflammatory environment for successful implantation. EndoSERA is standardized to contain 6-9 folds higher amounts of growth factors than peripheral blood majorly implantation friendly and anti-inflammatory cytokines which restore impaired uterine environments and optimized for cyclical requirements of proliferation, secretion & Implantation phases of Endometrium. It has also been shown to be safe,

reproducible, and effective in mimicking the natural processes of tissue repair and regeneration.

Limitations, reasons for caution: This prospective self-controlled study with small sample size lacks a randomized control group. While the beneficial effects observed in this study are improved than published data, larger study with patients recruited based on inclusion criteria of refractory thin endometrium is proposed for recommending EndoSERA as routine adjuvant during IVF procedures.

Wider implications of the findings: EndoSERA improved implantation, pregnancy, and live birth rates (LBR) in refractory thin endometrium patients which indicates clearly endometrial thickness and receptivity improvement and cumulative 62% has motivated us to plan randomized controlled studies to confirm the results and provide opportunity for women with refractory thin endometrium to conceive without surrogacy.

Trial registration number: Not Applicable

Abstract citation ID: dead093.709

P-351 The impact of ovarian endometriosis on oocyte competence in IVF/ICSI cycles: A systematic review and meta-analysis

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Study question: Is oocyte competence affected in patients with ovarian endometriosis in IVF/ICSI cycles?

Summary answer: In IVF cycles ovarian endometrioma negatively impacts oocyte quality in terms of oocytes and mature (MII) oocytes retrieved but not the fertilization and blastulation rates.

What is known already: Is still controversial whether the presence of a non-surgically treated ovarian endometrioma alone may adversely affect the oocyte quality in IVF/ICSI treatments.

As oocyte quality is well reflected by the ability to complete maturation and undergo successful fertilization, the best clinical markers of oocyte competence are represented by number of MII oocytes retrieved and fertilization rates.

Even though women with endometriomas had fewer oocytes and MII oocytes retrieved than women without, no further differences in reproductive outcomes have been found. The most recent meta-analysis, however, has serious limitations, such as clinical heterogeneity of the studies and a small sample size.

Study design, size, duration: A systematic review and meta-analysis of studies evaluating clinical markers of oocyte competence in women with ovarian endometriosis were conducted. Electronic searches were performed in PubMed, Cochrane database, and ClinicalTrials.gov up to December 2022. Randomized controlled trials and observational studies were eligible for inclusion. The risk of bias was assessed using the Newcastle–Ottawa Quality Assessment Scale. The quality of evidence was evaluated using the Grading of Recommendations Assessment, Development and Evaluation (GRADE) approach.

Participants/materials, setting, methods: Studies reporting ART outcomes among women with endometrioma were included. The main outcomes were clinical markers of oocyte competence including the number of oocytes retrieved, MII oocytes retrieved, ovarian sensitivity index (OSI), fertilization and blastulation rates. Mean differences (MD) and odds ratios (ORs) with 95% confidence intervals (CI) were calculated using the random and fixed effects model.

Main results and the role of chance: Of 876 unique records identified, 30 studies met inclusion criteria, and 21 studies, totaling 5962 participants, were included in the meta-analysis. The results showed that significantly fewer oocytes retrieved (mean difference (MD) = -1.67; 95% confidence interval (CI), -2.46, -0.88; p = <0.0001; I2=92%; low quality) and MII oocytes (MD = -2.45; 95% CI, -3.23, -1.67; p = <0.0001; I2=86%; moderate quality) were observed in women with endometriomas compared with the control. However, the OSI (MD = -1.47; 95% CI, -3.74, 0.81; p = 0.21; I2=97%; very low quality), fertilization rate (Odds ratio (OR) = 1.04; 95% CI, 0.78, 1.39; p = 0.79; I2=93%; low quality) and blastulation rate (OR = 0.90; 95% CI, 0.66, 1.22; p = 0.51; I2=95%; low quality) were not significantly different between the groups.

Limitations, reasons for caution: Despite removing patients with previous surgeries and diverse stages of endometriosis, main outcomes showed significant heterogeneity. The unilaterality/bilaterality, size, and the general extent of endometriomas could cause this heterogeneity. The quality of evidence generated from our findings is low, mainly due to the factors mentioned above.

Wider implications of the findings: Our findings suggest that endometriomas do not hinder fertility chances, as fertilization and blastulation rates were not compromised. Due to the risk of ovarian damage, these results argue against endometrioma excision. Further clinical trials with adequately-powered sample sizes should focus on blastulation and euploidy rates to validate our findings.

Trial registration number: Not applicable

Abstract citation ID: dead093.710

P-352 Evaluation of CD25 and CD69 activation markers expression on peripheral blood cells subpopulations in endometriosis patients

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Study question: We aim to determine the percentages of CD25-positive and CD69-positive lymphocytes in peripheral blood of endometriosis (EMS) patients.

Summary answer: Higher percentages of lymphocytes expressing CD69 and CD25 markers in EMS and their correlation with the severity of the disease, indicate persistent activation of lymphocytes

What is known already: Exposure to antigens causes activation markers to appear on the surface of lymphocytes. Among them, we can distinguish early and late activation markers. The earliest one is CD69, which is expressed upon activation by the TCR receptor. CD69 plays a role in the proliferation and survival of activated T lymphocytes. CD25 is a moderate late activation marker, considered the most important marker of cellular response activation. The significance of CD69 and CD25 expression by T cells in endometriosis has not yet been determined. Increased CD69 expression on various lymphocyte subsets in peritoneal fluid in EMS has been reported.

Study design, size, duration: Within this prospective study between January 2016 and August 2018 immune diagnostics of the number of CD25-positive and CD69-positive lymphocytes in peripheral blood of EMS patients (firstly diagnosed during laparoscopy and confirmed histopatologically) and non-EMS controls were performed using flow cytometry analysis. Total number of 74 subjects were included in the study. All patients signed written informed consent before their enrollment in the study.

Participants/materials, setting, methods: We enrolled 54 subjects with previously untreated endometriosis and 20 healthy age-matched controls. Peripheral blood was collected from control patients to assess the immunophenotype of lymphocytes and measure expression of activation markers Cd25 and CD69 on several subtypes B and T lymphocytes. Diagnosis and assessment of the stage of the EMS was established during laparoscopy using

rASRM score. Differences were considered statistically significant with a $p < 0.05$.

Main results and the role of chance: Significantly higher expression of the CD25 and CD69 antigen was found in both CD3+ T, CD4+ T and CD8+ T cells as well as CD19+ B cells. In each of the studied groups, a significance level of $p < 0.001$ was obtained. There was a weak positive correlation between the percentage of CD4+CD25+ T cells and the stage of endometriosis ($R = 0.357$; $p = 0.008$) and negative correlations between the percentage of endometriosis and the percentage of CD3+CD69+ T cells ($R = -0.554$; $p < 0.001$), T CD4+CD69+ ($R = -0.554$, $p < 0.001$) and T CD8+CD69+ ($R = -0.553$, $p < 0.001$). The expression of activation markers, CD25 and CD69 antigens in patients suffering from endometriosis with accompanying clinical symptoms was also evaluated. n the group of patients with endometriosis accompanied by infertility or pelvic pain, no statistical differences in the expression of CD25 and CD69 antigens were observed. The only clinical condition coexisting with statistically different expression of activation markers is adhesion disease accompanying endometriosis - a statistically significantly lower percentages of T CD3+CD69+ ($p = 0.002$), T CD4+CD69+ ($p = 0.002$) and T CD8+CD69+ ($p = 0.002$).

Limitations, reasons for caution: Small sample size is an important limitation of this study. In addition, evaluating immunological analysis only in serum samples does not let us drive any conclusions on the local changes of endometriosis lesions.

Wider implications of the findings: Clinical interpretation of changed expression of CD69 and CD25 in EMS could be useful in evaluating mechanisms of cellular activation, peripheral tolerance and immune imbalance involved in pathogenesis of endometriosis. Further studies to understand mechanisms underlying correlation between stage of the disease and expression of CD69 should be considered.

Trial registration number: not applicable

Abstract citation ID: dead093.711
P-353 possible correlation between the second-to-four digit ratio (2d:4d) and endometriosis: a case-control study

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Study question: This study investigates a possible association between the second-to-four digit ratio and endometriosis.

Summary answer: There is an association between a higher 2D:4D ratio and the presence of endometriosis.

What is known already: Endometriosis is characterised by the presence of endometrium epithelium and/or stroma outside the uterus. Recent findings suggest that endocrine disruptors during intrauterine life could determine the onset of the disease.The ratio between the length of the index finger (2D) and the ring finger (4D) is a sexually dimorphic feature. Moreover, it has been proposed as a marker of prenatal hormonal exposure since high level of androgens results in a lower 2D:4D ratio, whereas a prenatal oestrogenic environment results in a higher one. Therefore, a longer 2D:4D ratio could be considered as a silent sign of the disease.

Study design, size, duration: This study was conducted at the Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico. Participants were recruited from July 2021 to October 2022. Cases included women with a past surgical diagnosis of endometriosis or with a current nonsurgical diagnosis of the disease. Women attending the outpatient clinics for periodic visits, contraception, severe male infertility, and cervical cancer screening programme and without a previous clinical or surgical diagnosis of endometriosis were enrolled as the control group.

Participants/materials, setting, methods: Women with endometriosis were divided into two groups: deep infiltrating endometriosis (DIE) and ovarian endometrioma (OMA) depending on the lesion localization. Among controls, endometriosis was excluded based on gynaecological history, pelvic transvaginal ultrasound, gynaecological bimanual examination and vaginal inspection.The digit lengths were measured on the right hand's ventral surface because previous studies suggested that the right hand is more sensitive to androgens. In addition, other clinical details were collected on standardised forms.

Main results and the role of chance: A total of 424 participants (endometriosis n = 212; controls n = 212) were recruited for this study. The group of cases included 114 women with ovarian endometriomas and 98 patients with deep infiltrating endometriosis.Although the median age in the endometriosis group was 37 years, significantly higher than controls ($p < 0.01$), other variables (such as BMI, ethnicity, smoking habit, age at menarche, parity, previous miscarriages and previous IVF) did not differ between the study groups.The right hand 2D:4D digit ratio resulted significantly higher in women with endometriosis compared to controls with 2D:4D ratio of 1.00 [0.97 - 1.03] and 0.99 [0.96 - 1.02] respectively ($p = 0.002$).The significant association remained when exclusively focussing on women with ovarian endometriomas ($p = 0.002$). In contrast, the association was no more significant when the analysis was restricted to women with deep infiltrating forms ($p = 0.07$).These findings support the potential role of intrauterine exposure of estrogen in the pathogenesis of the disease. A low ratio of testosterone-to-estradiol during fetal life may play a crucial role in endometriosis onset and progression.

Limitations, reasons for caution: The selection of controls may represent a source of bias since endometriosis was ruled out based on gynaecological and ultrasonography examination. Therefore, it could not be excluded having inadvertently included some cases among controls.

Wider implications of the findings: In conclusion, there is an association between a higher 2D:4D ratio and the presence of endometriosis. Our results support the hypothesis claiming potential influences of intrauterine hormonal and endocrine disruptors exposure during fetal life on the onset of the disease.

Trial registration number: not applicable

Abstract citation ID: dead093.712
P-354 Endometriosis is negatively associated with morphokinetic indicators of embryo developmental competence.

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Study question: Is endometriosis associated with poorer embryo quality as assessed by fertilization and cleavage morpkokinetics?

Summary answer: Endometriosis is negatively associated with embryo quality as assessed by fertilization and cleavage morpkokinetics.

What is known already: The negative association between endometriosis and fertility is well-known. However, whether it predominantly derives from decreased oocyte quality or compromised endometrial receptivity is still an open question. Studies on the impact of endometriosis on oocyte/embryo developmental competence have generated discrepant results. Our group has generated strong evidence that fertilization and cleavage morphokinetics accurately reflects embryo quality. Recently, we have developed a scoring system (A-D) based on early morphokinetic parameters capable of guiding the selection of embryos with higher competence to achieve a live birth, being "A" the embryos with the highest morphokinetic score and "D" the embryos with the poorest.

Study design, size, duration: Retrospective non-interventional study, including patients who underwent IVF/ICSI cycles at the Biogenesi Reproductive Medicine Centre, Monza, Italy from 2018 to 2022. The

morphokinetic profiles of embryos produced in 283 cycles of patients with endometriosis (diagnosed by laparoscopic examination as stage III-IV, moderate/severe; ASRM criteria) were compared with those of embryos produced in 1336 cycles of patients presenting tubal or unexplained infertility factor (control).

Participants/materials, setting, methods: The study includes 1290 embryos from patients with endometriosis and 6197 control embryos. Embryo culture was performed in a time-lapse incubator. Embryos were retrospectively classified with 4 scores (A, B, C and D) derived from early morphokinetic parameters (tPNf, t2 and t4).

Patient/cycle characteristics and outcomes and morphokinetic parameters were compared using Fisher's (percentages) or Wilcoxon sum rank (continuous variables) tests. Differences in the distribution of morphokinetic scores were assessed with the Chi-square test.

Main results and the role of chance: Maternal age (36.3 ± 3.9 vs 37.5 ± 4.1 years; $p < 0.0001$), number of oocytes retrieved (7.4 ± 5.3 vs 9.6 ± 5.5; $p < 0.0001$) and BMI (22.0 ± 3.2 vs 22.6 ± 3.7; $p = 0.039$) were lower in patients with endometriosis as compared to controls. Embryos from patients with endometriosis reached tPNFa (h) (6.6 ± 1.6 vs 6.4 ± 1.6; $p = 0.013$), tPNf (h) (24.5 ± 3.6 vs 24.2 ± 3.6; $p = 0.002$) and t2 (h) (27.2 ± 3.7 vs 26.9 ± 3.8; $p = 0.002$) later than control embryos. In addition, the distribution of morphokinetic scores differed between groups; patients with endometriosis produced a lower percentage of A embryos (34.7% vs 41.0%) and a higher percentage of D embryos as compared to control patients (43.5% vs 39.6% respectively; $p < 0.001$). A multivariate analysis revealed that the negative association between endometriosis and achievement of the morphokinetic score A is independent of maternal age, paternal age, presence of male infertility factor and maternal BMI (OR 0.75; 95% IC 0.65-0.86; $p < 0.0001$).

Limitations, reasons for caution: The study is limited by its retrospective nature and other potentially interfering variables not included in the analysis.

Wider implications of the findings: Our findings indicate that endometriosis is associated with reduced embryo developmental competence as assessed by early morphokinetics. Therefore, while suggesting that endometriosis can indeed affect oocyte quality, our data shed light on the mechanisms linking this pathology with subfertility.

Trial registration number: Not Applicable

Abstract citation ID: dead093.713
P-355 Thin endometrium is associated to higher risk of having a displaced window of implantation (WOI)

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Study question: Is thin endometrium (<6 mm) associated to an abnormal receptivity status?

Summary answer: Thin endometrium (<6 mm) is significantly associated with a displaced WOI.

What is known already: The measurement of endometrial thickness by 2D vaginal ultrasound (US) is a routine practice in Assisted Reproduction Techniques (ART). Thin endometrium (<6 mm) is associated with poor reproductive outcome; however, the potential reason is unknown.

Study design, size, duration: Retrospective cohorts study including 25,888 patients in which endometrial lining was measured by 2D US the day before progesterone supplementation in HRT cycles for frozen embryo transfers and Endometrial Receptivity Analysis (ERA) was performed after 5 days of progesterone administration.

Endometrial thickness was classified as < 6 mm, 6-12 mm, or > 12 mm; ERA results were considered as receptive or displaced WOI (Pre-receptive, post-receptive, proliferative, and late-receptive) that needs a personalized embryo transfer (pET).

Participants/materials, setting, methods: Endometrial thickness was measured from one endometrial layer to the other on a longitudinal transvaginal scan at the site of maximum thickness evidenced by 2D US. For ERA analysis, RNA was extracted and sequenced by NGS. Then, the ERA computational predictor obtained the diagnosis as standard or displaced WOI.

Main results and the role of chance: RA results reveal that women with thin endometrium (<6mm) present significantly higher incidence of displaced WOI (47.49%) than 6-12mm (38.20%) ($p = 0.0038$), and >12mm (39.75%) ($p = 0.026$). No other differences were found.

Endometrial thickness (mm)	Standard WOI (%)	Displaced WOI (%)	TOTAL
<6	157 (52.51%)*	142 (47.49%)*	299
6-12	14552 (61.80%)	8994 (38.20%)	23546
>12	1231 (60.25%)	812 (39.75%)	2043
TOTAL	15940	9948	25888

*Significant different against other two groups.
Global Chi-square test for association P = 0.002.

Limitations, reasons for caution: This is a retrospective study, having innate limitations of its nature.

Wider implications of the findings: Thin endometrium (<6mm) is associated to a higher displaced WOI compared to normal lining or hypertrophic endometrium. Normal endometrial thickness (6-12 mm) considered as "receptive" does not preclude a transcriptomic receptivity status in 38.20% of the cases. This finding should be considered when embryo transfer is planned.

Trial registration number: Not applicable

Abstract citation ID: dead093.714
P-356 establishment of a novel 3D spheroid culture system of uterine leiomyoma cells

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Study question: To establish three-dimensional (3D) spheroid culture system of uterine leiomyoma (ULM) cells that responds to estrogen(E) and progesterone(P).

Summary answer: We established the 3D spheroid culture system of ULM cells, in which the ULM-spheroid grows in response to progesterone.

What is known already: ULMs proliferate in response to female hormones in the patients-derived xenograft model *in vivo*. However, it is controversial whether ULM cells proliferate in response to female hormones in *in vitro* monolayer culture system. We reported that ULM cells formed nodule-like aggregates of cells when the cells were grown in 3D culture using collagen gel. However, 3D spheroid culture system of ULM cells is not established yet. Recently, somatic mutations in the MED12 gene were detected in almost 70% of ULMs. It is also unclear whether responsiveness of ULM cells to female hormones differs between MED12-mutation positive and negative ULMs.

Study design, size, duration: Fourteen and 13 ULMs obtained from 18 premenopausal women were used for monolayer culture and 3D spheroid culture, respectively. The isolated ULM cells were confirmed to be smooth muscle cells with 80% purity and to have progesterone receptors by immunocytochemistry. The cells were preincubated for 2 days and then used for the following experiments below. MED12-mutation status was analyzed by Sanger

sequencing. Monolayer and spheroid cultures were done on MED12-mutation positive and negative ULM cells.

Participants/materials, setting, methods: For the monolayer culture system, cells were incubated with E+P, E alone, P alone, and control (without E+P) for 7 days. For 3D culture system, cells were incubated in the low attachment well dish to induce floating cell culture, and after 24 h, we identified the floating cell aggregates as spheroid of ULM cells. Then, the ULM-spheroids were incubated with E+P, E alone, P alone, E+P+selective progesterone receptor modulator (SPRM), and control for 7 days.

Main results and the role of chance: Responsiveness to female hormones was assessed by counting cells for monolayer culture, and by measuring the cross-sectional area of the spheroid for 3D culture system. ULM cells did not proliferate by any female hormones in the monolayer culture system. In the 3D spheroid culture system, the ULM-spheroid cells showed expression of alpha-smooth muscle actin and vimentin, indicating that most of the spheroid cells are smooth muscle cells. In MED12-mutation negative ULMs, the mean of the cross-sectional area of the spheroid of E+P , P alone, E alone, E+P+SPRM and control were 0.375mm^2, 0.343mm^2, 0.237mm^2, 0.252mm^2 and 0.193mm^2, respectively, while in MED12-mutation positive ULMs, the area were 0.349mm^2, 0.338mm^2, 0.268mm^2, 0.264mm^2. and 0.215mm^2. The morphology of the spheroid of the E alone, E+P+SPRM and control groups showed the loss of smooth muscle cells. These results suggest that the growth of the ULM-spheroid is maintained by progesterone, and that the responsiveness of the spheroid to progesterone does not differ between MED-mutation negative and positive ULM cells. Our study showed that ULM cells of the 3D spheroid culture system acquire function to respond to progesterone.

Limitations, reasons for caution: Further studies are needed to investigate whether the 3D spheroid culture system established in this study accurately reflects the responsiveness to female hormones of the *in vivo*.

Wider implications of the findings: Our study showed that the 3D spheroid culture system of ULM cells has the responsiveness to progesterone whereas the monolayer culture system does not. The 3D spheroid culture system of ULM cells established in this study would be useful as a screening system for therapeutic agents.

Trial registration number: non-clinical trials

Abstract citation ID: dead093.715
P-357 Downregulation (DR) with combined gonadotropin-releasing hormone agonist (GnRHa) and aromatase inhibitor (AI) optimizes the frozen-thawed embryo transfer (FET) results in women with adenomyosis

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Study question: Does combined GnRHa & AI therapy for downregulation in women with adenomyosis optimize the FET success rates / outcomes on par with women without adenomyosis?

Summary answer: GnRHa combined with AI used for downregulation in women with adenomyosis optimized the outcomes of frozen–thawed embryo transfer cycle on par with women without adenomyosis.

What is known already: Women with adenomyosis have lower rates of successful implantation via altered molecular expressions in the endometrium due to local hyperestrogenism & also an increased risk of early pregnancy loss (Munro et al 2019). DR improves the clinical pregnancy rate by reducing the endometrial inflammatory reaction and/or myometrial contractility and their impact on uterine receptivity in women with adenomyosis undergoing FET (Sania Latif et al 2021). Combined treatment for uterine adenomyosis with Anastrazole plus GnRHa showed better results than dienogest treatment with a higher reduction of symptoms & higher pregnancy rates (M Sbracia & F Scarpellini, 2018).

Study design, size, duration: A Retrospective cohort study conducted at a tertiary care fertility unit. Data for 326 women with/without adenomyosis undergoing frozen–thawed embryo transfer after IVF was was retrieved from the hospital's database and analyzed for a period between September 2021 to November 2022.

Participants/materials, setting, methods: Women with adenomyosis (n = 107) received Anastrozole 1mg/day for 2months plus 3doses of Inj. Goserelin 3.6mg subcutaneously at 28days interval between 2successive doses. Hormone replacement therapy (HRT) was started 2weeks after the 3rddose of Inj. Goserelin & FET was performed after an optimum endometrial thickness (EMT) was achieved. For women without adenomyosis (n = 219) HRT was started on cycle Day-2 & FET was performed after achieving an optimum EMT. Serum beta-hCG was performed on Day15 after FET.

Main results and the role of chance: Statistical analysis was performed using SPSS20version. Normally distributed continuous variables were compared using a student t-test, and categorical variables were compared by $\chi 2$ and Fisher's exact test, where appropriate. To reduce selection bias, propensity score matching was used, and propensity matching yielded 99 pairs.

Baseline characters like age (p-value=0.36), BMI (p-value=0.12), duration (p-value=0.28), type (p-value=1) & cause (p-value=0.3) of infertility, endometrial thickness (p-value=0.37), day of embryo transfer (p-value=0.57) were comparable for the two groups. FET results in terms of positive pregnancy test (Serum beta-hCG > 50mIU/ml) were found to be 68.21% for the DR- FET group and 67.71% for the HRT group which were comparable(p-value=0.17). This suggests that downregulation in women with adenomyosis helps achieve success rates similar to women without the disease.

Pregnancy outcomes like miscarriage (15 vs 13.13 %, p-value=1.00), biochemical pregnancy (3.03 vs 2.02 %, p-value=1.00) and ectopic pregnancy (1.01 vs 0 %, p-value=0.49) rates analyzed between the DR FET and HRT groups showed no statistically significant difference. Clinical pregnancy rates were almost similar for the 2 groups (55.71 Vs 57.46%, p-value=0.65).

Thus, we conclude that downregulation with combined GnRHa and AI optimizes the frozen-thawed embryo transfer results in women with adenomyosis on par with women without adenomyosis.

Limitations, reasons for caution: This is a retrospective study and hence randomized comparison was not possible. Women were followed up for 12 weeks of pregnancy, hence live birth rates were not analyzed in our study.

Wider implications of the findings: Combining the two treatment modalities (GnRHa + AI) which work at different sites optimizes IVF success rates & pregnancy outcomes. Hence we suggest that well-designed prospective randomized studies are needed to further analyze the synergistic role of this drug combination for downregulation in women with varied severity of adenomyosis.

Trial registration number: Not applicable

Abstract citation ID: dead093.716
P-358 Impact of localization of diffuse adenomyosis on reproductive outcomes and pregnancy complications: A prospective cohort study of 585 patients after frozen embryo replacement cycle

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Study question: Does localization of diffuse adenomyosis impact reproductive outcomes after the frozen embryo transfer (FET)?

Summary answer: Diffuse adenomyotic lesions involving the junctional zone (JZ) have a greater negative impact on reproductive outcomes than diffuse adenomyosis of the outer myometrium (OM).

What is known already: Adenomyosis is associated with higher miscarriage rate, significantly lower live-birth (LB) rate, and increased risk for pregnancy-related complications. However, the impact of adenomyosis on IVF outcomes remains unclear. Adenomyosis is often treated as a homogenous disease and studies on the effects of different types of adenomyosis on reproductive outcomes have yielded inconsistent results, due to a lack of standardized diagnostic criteria and a lack of agreement on the phenotypic classification of the disease based on their severity or location. There is a lack of studies

considering the effects of the severity or location of adenomyosis on reproductive outcomes after FET.

Study design, size, duration: This prospective cohort study was conducted at a tertiary-care hospital between January 2019 and December 2022. A total of 585 infertile women undergoing the first FET cycle were recruited. The study population included 368 women with diffuse adenomyosis where 201 women had diffuse adenomyosis of JZ and, 167 women had diffuse adenomyosis of OM. 217 women with male infertility were taken as controls.

Participants/materials, setting, methods: Adenomyosis was diagnosed with 2D-TVS using MUSA criteria where patients with two or more features and diffuse adenomyosis were included. Patients with diffuse adenomyosis were further divided based on the localization of adenomyotic lesions in OM or JZ. All the patients underwent FET-cycle. Pregnancy outcomes and complications were compared between different groups-those with diffuse adenomyosis of JZ, those with diffuse adenomyosis of OM, and controls. Adenomyosis patients as one group were also compared with controls.

Main results and the role of chance: The pregnancy rate was significantly lower in women with diffuse adenomyosis of JZ (26.37%) compared to diffuse adenomyosis of OM (47.9%) (OR:0.39, 95% CI 0.25-0.60; $P < 0.0001$). Similarly, the clinical pregnancy rate was also lower in diffuse adenomyosis with JZ involvement (23.38%) compared to women with diffuse adenomyosis of OM (42.51%) (OR:0.41, 95% CI 0.26-0.65; $P = 0.0001$). However, the biochemical pregnancy and miscarriage rates were comparable between the two adenomyosis groups. The LB rate was significantly lower in patients with JZ-involvement (16.42%) compared to women with OM-involvement (25.75%) (OR:0.57, 95% CI 0.34-0.94; $P = 0.029$). When all the adenomyosis patients were compared with the controls as one group, pregnancy rates were similar, but the miscarriage rate was significantly higher, and the LB rate was significantly lower in adenomyosis ($P < 0.05$). When the individual groups with adenomyosis were compared with controls, clinical pregnancy, and LB were comparable with the controls ($P > 0.05$) in women with OM involvement. However, when JZ was involved, the differences were significant ($P < 0.05$), highlighting the adverse impact of JZ involvement on IVF outcome. Pregnancy complications were comparable between the adenomyosis groups; however, there was a significantly higher incidence of gestational hypertension, IUGR, and pre-term labor for adenomyosis patients compared to the control ($P < 0.05$).

Limitations, reasons for caution: In the absence of a universally accepted diagnostic modality and classification system for adenomyosis, this study used ultrasound due to its lower cost and easy availability. The sample size of this study is limited to 368 patients from a single centre, larger multicentric studies are needed to make definitive conclusions.

Wider implications of the findings: Localizing adenomyotic lesions before starting IVF, may help in planning treatment strategies and provide adequate counseling on reproductive outcomes specific to each type. Pregnant women with adenomyosis should be managed carefully as high-risk pregnancies, considering the possible serious obstetric complications.

Trial registration number: CTRI/2019/01/016919

Abstract citation ID: dead093.717

P-359 The prevalence of benign coexisting gynaecological conditions with endometriosis: a systematic review and meta-analysis

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Study question: What is the prevalence of other benign gynaecological conditions in women with endometriosis. Are women with endometriosis at a higher risk of these conditions?

Summary answer: Women with endometriosis are at a higher risk of coexisting adenomyosis and endometrial polyps.

What is known already: Endometriosis is a chronic and debilitating condition with a prevalence of ~10% in women of reproductive age. Over recent years, associations between endometriosis and other gynaecological and non-gynaecological conditions have been speculated but data on the prevalence of benign gynaecological conditions coexisting with endometriosis is limited. Furthermore, whether women with endometriosis are at an increased risk of other gynaecological conditions is unclear, particularly, when some of those conditions share a hormonally driven pathway to endometriosis. Understanding the risk of coexistence with endometriosis is crucial in establishing an association, providing insights into disease pathophysiology, informing clinical surveillance, and improving patient care.

Study design, size, duration: The review was prospectively registered in PROSPERO (id: CRD42022307527). MEDLINE and Embase was searched from inception to June 2022 with no restrictions. Experimental and population-based observational studies were included. Grading of Recommendations, Assessment, Development and Evaluation (GRADE) criteria was used to rate the quality of evidence with the risk of bias in non-randomized studies of interventions (ROBINS-I) tool incorporated. A random effects model was used to pool the odds ratio (OR) data.

Participants/materials, setting, methods: Coexisting gynaecological conditions included were adenomyosis, fibroids, endometrial polyps (EP), polycystic ovarian syndrome (PCOS), benign ovarian cysts (BOC) or pelvic inflammatory disease (PID). All conditions were diagnosed by surgery, imaging or ICD coded medical records. Comparison groups were women with and without endometriosis. Outcome was prevalence expressed as a fraction. All prevalence estimates were either drawn from original papers or calculated. Meta-analysis was carried out if at least two studies were available.

Main results and the role of chance: 7137 studies were screened, and fifty-five studies reported on the prevalence of a coexisting gynaecological condition with endometriosis. Of these, 21 studies compared the prevalence in women without endometriosis and was included in the meta-analysis. The prevalence of coexisting adenomyosis (n = 34 studies; 11.0-91.9%.), fibroids (n = 16; 1.9-67.3%), EP (n = 14;1.6-68.4%.), PCOS (n = 3; 4.35-73.6%), BOC (n = 2;10.9-15.1%) and PID (n = 3;1.29-4.42) showed considerable variation between studies. Compared to women without endometriosis, women with endometriosis had a significantly higher prevalence of adenomyosis (OR 3.65, 95% CI 1.94-6.88, $P < 0.001$) and EPs (OR 4.04, 95% CI 2.85-5.74, $P < 0.001$) but not fibroids (OR 1.16, 95% CI 0.76-1.78, $P = 0.5$). There was insufficient comparative data for PCOS, BOC and PID. Overall, the quality of evidence for the prevalence estimate rated as low or very low on the GRADE criterion scale. The major factors associated with downgrading an outcome for quality was limitations in the study design and execution (risk of bias) and imprecision. The factors associated with increasing the quality of evidence was large effect sizes or sample sizes.

Limitations, reasons for caution: The wide variance in prevalences may be due to the differences in study design, study population, mode of diagnosis and criteria used to define the coexisting condition. The quality of evidence for prevalence effect estimates were low to very low quality, due to inherent biases associated with observational studies.

Wider implications of the findings: The wide variation in the prevalence of benign gynaecological conditions suggests over or under diagnosis. In our review endometriosis is associated with a significantly higher risk of adenomyosis and endometrial polyps. Clinicians may need to be mindful of this co-existence, but future high-quality studies are required to make robust conclusions.

Trial registration number: not applicable

Abstract citation ID: dead093.718

P-360 The effect of dietary interventions, or no intervention, on Pain and Quality of Life in women diagnosed with Endometriosis

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Study question: Explore the influence of a dietary intervention, the Low FODMAP diet or endometriosis diet, on endometriosis-related pain and thereby Quality of Life.

Summary answer: After adhering to a dietary intervention for six months, women reported less pain and better Quality of Life (QoL).

What is known already: Standard endometriosis treatment, hormonal therapy, surgery and/or pain management, can be insufficient in treating endometriosis or may be accompanied with unacceptable side effects. Therefore, there is an increasing interest among endometriosis patients in the application of self-management strategies. The Low FODMAP diet has previously been studied and found effective in reducing pain symptoms in endometriosis patients. Guidelines for the Low FODMAP diet have been developed. Limited studies are available on the efficacy of the endometriosis diet. A survey study recently found that it was effective in improving QoL. Currently no guidelines on the implementation of the endometriosis diet exist.

Study design, size, duration: We performed a prospective pilot study. Women could choose between adherence to a diet (Low FODMAP diet or endometriosis diet) or no diet. It was aimed to include 60 participants, 20 participants per group. When adhering to a dietary intervention, women received extensive guidance by a student dietician for three months. After three months, women were asked to continue the diet independently for another three months. The follow-up period covered six months for all participants.

Participants/materials, setting, methods: Between April 2021 and December 2022, we included women diagnosed with endometriosis, surgically and/or by imaging, and reported VAS pain scores $\geq$ 3cm (dysmenorrhea, deep dyspareunia, chronic pelvic pain). Primary endpoint focused on pain reduction (VAS, scale 0-10cm) in the symptoms *dysmenorrhea, deep dyspareunia, chronic pelvic pain, dysuria, tiredness* and *bloating*. Secondary endpoints focused on QoL measured using the Endometriosis Health Profile (EHP-30) and Gastro-intestinal health measured using the Gastro-Intestinal Quality of Life Index (GIQLI).

Main results and the role of chance: Sixty-two women (22 Low FODMAP diet, 21 Endometriosis diet, 19 control) participated. Women adhering to a diet reported less pain in all symptoms but *dysmenorrhea* (range $p < 0.001$ to $p = 0.012$) and better EHP-30 scores in the domains *pain, powerlessness, emotional wellbeing, self-image, work life* and *sexual intercourse* (range $p < 0.001$ to $p = 0.023$) after six months dietary adherence. When differentiating between the diets, women adhering to the Low FODMAP diet reported less *dysuria* and *bloating* ($p = 0.015$; $p < 0.001$, resp.) and better scores in the domains *pain, powerlessness* and *work life* ($p = 0.007$; $p = 0.002$; $p = 0.035$, resp.). Women adhering to the endometriosis diet reported less *bloating* and *tiredness* ($p = <0.001$; $p < 0.001$, resp.) and better scores in the domains *powerlessness, emotional wellbeing* and *self-image* ($p = 0.014$; $p = 0.022$; $p < 0.001$, resp.) after six months dietary adherence. When comparing it to the control group, women adhering to a dietary intervention reported significant less *bloating* ($p = 0.049$), and better scores in the domains *social support, medical profession* and *infertility* (range $p = 0.002$ to $p = 0.035$) at six month' follow-up.

There was a high level of satisfaction with the dietary guidance. Three out of 43 women discontinued adherence to their diet prematurely. Ultimately, 35 out of 43 women wanted to continue their diet (partially) after six months.

Limitations, reasons for caution: No sample size was calculated since efficacy data in literature was lacking. There was no randomization to optimize dietary adherence, possibly resulting in selection bias. Women found it hard to express their pain in VAS; their pain was still present but less frequent.

Wider implications of the findings: Women adhering to a diet reported lower pain scores and better QoL after six months. It could therefore be recommended to endometriosis patients with therapy-resistant pain, wishing to apply self-management strategies. However, caution is implied because data on long-term effects (>6 months) is lacking and drawing up guidelines is needed.

Trial registration number: W20_534 # 20.593

Abstract citation ID: dead093.719
P-361 Is HIF-2α involved in pre-menstrual conditioning of the endometrium to ensure optimal repair and prevent heavy menstrual bleeding?

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Study question: Does HIF-2α play a key role in pre-menstrual conditioning of the non-pregnant endometrium to optimise menstrual breakdown/repair and prevent heavy menstrual bleeding (HMB)?

Summary answer: HIF-2 was higher in secretory endometrium of women with HMB versus controls, but manipulation of HIF-2α in the mouse model did not impact endometrial breakdown/repair.

What is known already: Hypoxia inducible factor (HIF) is the master regulator of the cellular response to hypoxia and has two common alpha isoforms (HIF-1α/HIF-2α) with overlapping but distinct target genes. We revealed that menstrual endometrial HIF-1α is necessary for normal menstrual endometrial repair. We detected HIF-2α in human endometrium exclusively during the secretory phase. The role of HIF-2α in the non-pregnant endometrium remains undetermined but was important for successful uterine implantation and lung vascular remodelling in mice. We hypothesised that endometrial HIF-2 is required for optimising the endometrial vasculature pre-menstrually to ensure appropriate vasoconstriction and endometrial repair during menstruation.

Study design, size, duration: HIF-2 was examined in human endometrial tissue and a mouse model of simulated menstruation. Hif2α +/- mice provided genetic reduction of HIF-2 during simulated menses versus wild-type littermates. HIF-2α was also pharmacologically inhibited during the secretory phase (PT2385 vs vehicle controls). To increase HIF-2, Hif1α +/- mice were treated with a HIF stabiliser (DMOG) during the secretory phase to increase HIF-2α followed by decreased HIF-1α at menses –mimicking human endometrial findings in women with HMB.

Participants/materials, setting, methods: Human secretory endometrium was collected (ethically approved and consented) from women with objectively measured HMB (n = 5) and controls (n = 4) and HIF-2 detected by Western blot. Mouse uterus was collected at the time of progesterone withdrawal (T0, decidualisation) and 8h (T8, endometrial breakdown) and 24h (T24, endometrial repair) after progesterone withdrawal. Histological endometrial repair and menstrual blood loss (MBL) were quantified. Decidualisation markers were examined by RT-qPCR. Endometrial vessel maturity and hypoxia were assessed using immunohistochemistry/immunofluorescence.

Main results and the role of chance: Human secretory endometrial HIF-2α protein levels were higher in those with HMB compared to controls ($P < 0.05$). Genetic reduction of HIF-2α in our mouse model of menses (Hif2α +/-) revealed no significant differences in endometrial breakdown (T8) or repair grade (T24) and no difference in MBL at T8 versus controls. There were no significant differences in expression of decidualisation markers (Prlr/Prl3c1) and vessel maturity at T0 (CD31/α-SMA immunofluorescence staining), or the presence of hypoxia at T8 (pimonidazole immunohistochemistry staining). Pharmacological inhibition of HIF-2 at the time of decidualisation also showed no significant difference in endometrial repair at T24 versus controls. Increasing pre-menstrual HIF-2 via pharmacological stabilisation of HIF-2α in Hif1α+/- mice similarly did not significantly affect endometrial breakdown (T8) or repair (T24) or MBL at T8 versus vehicle treated controls, in contrast to our findings in women with HMB.

Limitations, reasons for caution: Histological scoring of endometrial breakdown/repair in the menstruation mouse model may not be sensitive

enough to detect subtle endometrial changes with increased HIF-2α. As HIF-1α delays endometrial repair, an additional timepoint 36h after progesterone withdrawal may better capture any delay in endometrial repair as a result of increased secretory HIF-2.

Wider implications of the findings: HIF-2α may play a greater role in implantation than pre-menstrual conditioning of the endometrium to optimise menstrual breakdown/repair and limit menstrual blood loss. Although endometrial breakdown was not significantly affected by HIF-2, the impact on repair warrants further investigation to fully delineate the role of HIF-2 in the non-pregnant endometrium.

Trial registration number: Not applicable

Abstract citation ID: dead093.720

P-362 Pregnancy outcomes of frozen-thawed single euploid blastocyst transfers after endometrial preparation with natural cycle, letrozole use, or programmed cycle

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Study question: Is natural cycle the best protocol for endometrial preparation in frozen-thawed single euploid blastocyst transfers?

Summary answer: Clinical and ongoing pregnancy were comparable between endometrial preparation methods although programmed cycle was associated with a higher risk of miscarriage compared to natural cycle.

What is known already: Utilization of frozen-thawed single euploid embryo transfers based on PGT-A have been substantially increased recently, but there are still no firm conclusions on the optimal protocol in frozen-thawed embryo transfers (FETs). Even so, significant concerns have been raised about maternal and fetal safety associated with the programmed cycles and several favorable outcomes of natural cycles and letrozole use in FETs have been suggested in recent years. Pregnancy outcomes after endometrial preparation with natural cycle, letrozole use, programmed cycles have not been compared in euploid blastocyst FETs.

Study design, size, duration: This was a retrospective cohort study at a single center including FET cycles from January 2019 to October 2021.

Participants/materials, setting, methods: A total of 708 frozen-thawed single euploid blastocyst transfer cycles were analyzed. Endometrial preparation was performed using natural cycle with hCG trigger (n = 60), ovulation induction with letrozole (n = 32), or programmed cycle (n = 616) at the discretion of each attending physician. Pregnancy outcomes including clinical pregnancy, ongoing pregnancy, and miscarriage were calculated using multivariable logistic regression.

Main results and the role of chance: The three groups were similar for age, BMI, basal FSH, TSH, AMH levels, PGT-A indications, presence of ovulatory dysfunction, endometrial thickness on the starting day of progesterone supplementation, and the proportion of good quality embryo transferred. The crude clinical pregnancy rate, ongoing pregnancy rate, and miscarriage rate were not different among the groups (55.0% vs. 68.9% vs. 61.9%, p = 0.40; 51.7% vs. 59.4% vs. 50.0%, p = 0.58; 6.1% vs. 13.6% vs. 20.4%, p = 0.11, respectively). After adjusting for confounders, clinical pregnancy and ongoing pregnancy outcomes of natural cycle were similar to that of letrozole cycle and programmed cycle. However, programmed cycle was associated with significantly higher odds of miscarriage compared to natural cycle (aOR 4.33, 95% CI 1.01-18.58).

Limitations, reasons for caution: This was not an intention-to-treat study due to its retrospective design. Another limitation of this study includes a small sample size and risk of patient selection bias for indication of PGT-A.

Wider implications of the findings: Natural cycle and ovulation induction with letrozole may be safely and effectively carried out for endometrial preparation in patients undergoing single euploid FETs.

Trial registration number: not applicable

Abstract citation ID: dead093.721

P-363 Ovarian endometriosis deteriorates embryo development in ART cycles: An embryonic time-lapse study

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Study question: Does ovarian endometriosis deteriorate embryo quality in terms of morphokinetic parameters determined by time-lapse images?

Summary answer: Embryos from ovarian endometriosis patients accelerate their early developmental events but decelerate after t5. Completion of late developmental events were significantly delayed in endometriosis group.

What is known already: Endometriosis may affect the oocyte quality and consequently affect the embryo quality in terms of embryo morphology and implantation ability. Morphokinetic evaluation of embryos provided more developmental details and might be utilised as a tool to distinguish the embryo developmental ability. Only scarce studies with limited case numbers have evaluated the association between endometriosis and developments of embryo in terms morphokinetic observation. Hardly any consensus has ever been drawn.

Study design, size, duration: The morphokinetic parameters of embryos were compared between patients with or without ovarian endometriosis. A total of 52 ovarian endometriosis cases and 208 controls without endometriosis, receiving their assisted reproductive techniques from April 2020 through June 2022, were included for analysis. Propensity Score Matching with age, BMI and AMH was performed in a ratio of 1:4 in order to eliminate the confounding factors.

Participants/materials, setting, methods: We analyzed a total of 737 top quality embryos obtained from 348 oocyte retrieval cycles performed at Taipei Fertility Center. 127 embryos were obtained from women affected by ovarian endometriosis and 617 were obtained from unaffected controls. All embryos were cultured in a time-lapse incubator chamber and completed blastulation.

Main results and the role of chance: Morphokinetic data showed that embryo development is distorted in embryos obtained from women with endometriosis. The timing of early developmental events, including tPNf (22.7 +/- 2.9 hr vs 23.5 +/- 4.1 hr, p = 0.03) and t2 (25.2 +/- 3.1 hr vs 26.1 +/- 4.4 hr, p = 0.03) were significantly shortened in the endometriosis group. However, the timing of late developmental events, including tM (89.7 +/- 9.8 hr vs 86.6 +/- 11.4 hr, p = 0.006) and tB (111.0 +/- 11.0 hr vs 108.3 +/- 12.5 hr, p = 0.03) were significantly prolonged in the endometriosis group. The timing of t5 by morphokinetic observation was found to be equal both in ovarian endometriosis group and control group (50.4 +/- 8.7 hr vs 50.4 +/- 8.4 hr, p = 0.99). The average of KID score of embryos from ovarian endometriosis group was lower than that from normal control, but it did not reached statistical significance (4.07 +/- 2.11 vs 4.49 +/- 2.70, p = 0.27). The initial serum hCG levels in pregnant women from both endometriosis and non-endometriosis participants showed that hCG levels were significantly higher in non-endometriosis cases (628.8 +/- 967.7 mIU/ml vs 1581.5 +/- 1885.1 mIU/ml, p = 0.01).

Limitations, reasons for caution: This is a retrospective observational study with propensity score matching method to eliminate confounding factors such as age, BMI and AMH.

Wider implications of the findings: The effects of prolonged morphokinetic parameters on the embryo quality need further study to clarify; however, they may serve as markers in selecting embryos with implantation potential.

Trial registration number: Not applicable

Abstract citation ID: dead093.722

P-364 Obstetric and birth outcomes in women with endometriosis: findings from a UK population-based cohort

Abstract withdrawn by the authors

Abstract citation ID: dead093.723

P-365 The composition of the endometrial microbiota, as determined by MicroBioMap®, significantly affects clinical outcomes in assisted reproduction treatments

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Study question: How does the presence of specific pathogenic or health-promoting microbial species in the endometrium relate to female fertility in assisted reproduction treatments (ART)?

Summary answer: Detection of endometrial pathogens using MicroBioMap® is associated with poor ART outcomes, whereas sterile endometria show the highest pregnancy and live birth rates.

What is known already: Vaginal eubiosis is characterised by low microbial diversity and dominance of *Lactobacillus*, which displays bioregulatory functions and prevents the growth of pathogenic microorganisms. Vaginal dysbiosis is directly linked to infertility and poor pregnancy outcomes. However, the endometrial microbiota composition and its role in fertility are more controversial. Robust reports show that chronic endometritis (CE) is highly prevalent among infertile patients, whose outcomes improve after antibiotic treatment. However, the role of *Lactobacillus* in this compartment, whether it also displays bioregulatory functions, or if it is essential and beneficial for women undergoing ART, is still unclear.

Study design, size, duration: This is a case-control retrospective study analysing the endometrial microbiota in the window of implantation (WOI) and the ART outcomes of 456 women undergoing IVF cycles in egg donation regimes with/without PGT-A (177 and 163 respectively), or using their own eggs in cycles with/without PGT-A (91 and 25 respectively), between March 2016 and April 2021 in IVF-Life Alicante (Spain). ART results after single embryo transfer (sET) of patients with different endometrial microbiota profiles were compared.

Participants/materials, setting, methods: Presence of 18 microbial CE pathogens and 4 *Lactobacillus* species was determined by high-throughput qPCR (MicroBioMap®) in 456 endometrial samples obtained during the WOI (determined by ER Map® receptivity test) of a hormone replacement therapy cycle. Clinical outcomes (clinical pregnancy, CP; live birth, LB) of sETs performed during the WOI (guided by ER Map®) were compared in relation to the endometrial microbiota profile. In PGT-A cycles, embryo ploidy was determined by NGS (VeriSeq-MiSeq) before sET.

Main results and the role of chance: According to the microbial profile detected by MicroBioMap®, patients were categorised as i) *Lactobacillus* only (L, n = 199), ii) Pathogens only (P, n = 56), iii) *Lactobacillus* + pathogens (L+P, n = 59) or iv) Undetected (U, n = 142).

Absence of endometrial microorganisms was significantly associated to ART success. U-patients showed significantly higher CP (64.2% vs 50.8%, Fisher's exact p=0.046) and LB rates (52.9% vs 37.2%, p=0.03) after euploid sET than patients with microorganisms. These differences were more pronounced in patients receiving donor eggs (CP 70.2% vs 50.8%, p=0.026; LB 59.5% vs 40.2%, p=0.046).

Presence of *Lactobacillus* in isolation in the endometrium showed a detrimental effect compared to U-environments. A trend towards statistical significance was detected, with L-patients showing lower LB rates compared to U-patients after euploid sET (38.8% vs 52.9%; p = 0.084).

Detection of endometrial pathogens significantly reduced ART success rates. P-patients had significantly lower CP rates (64.2% vs 41.2%, p=0.038) after euploid sET than U-patients, and significantly lower CP (70.2% vs 36%, p=0.006) and LB rates (59.5% vs 21.7%, p=0.004) if receiving donor eggs. Interestingly, *Lactobacillus* dominance in pathogen-positive patients seemed to improve outcomes after euploid sET compared to pathogen-dominated patients (CP 56.7% vs 42.2%; LB 41.7% vs 31%).

Limitations, reasons for caution: This is a retrospective study. Randomised-control trials, non-selection studies and/or other investigations including antimicrobial treatments are needed to confirm these results and the extent of any clinical benefits. Endometrial biopsies were obtained transcervically. Although utmost care was taken, risk of contamination from the lower genital tract cannot be completely excluded.

Wider implications of the findings: Our results suggest that sterile endometria provide the best conditions for embryo implantation and pregnancy. *Lactobacillus* presence in endometrium might not be essential for pregnancy, and could even be detrimental if found in isolation. Presence of *Lactobacillus* as a bioregulator might only be beneficial if other pathogens co-colonise the tissue.

Trial registration number: Not applicable

Abstract citation ID: dead093.724

P-366 Unraveling the whole transcriptome profiles of receptive phase endometrium in infertile women

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Study question: Are there differential transcriptomic profiles of the receptive phase endometria in infertile women with different infertility diagnoses?

Summary answer: Endometrium-related pathologies such as endometriosis and RIF seemed to share similar endometrial molecular profiles in mid-secretory phase,while samples from unexplained infertility were similar to controls.

What is known already: Successful embryo implantation is orchestrated by the combination of a viable blastocyst, a receptive endometrium and a perfectly synchronized molecular dialogue between them. Endometrium-associated pathologies such as endometriosis and recurrent implantation failure (RIF) are suggested to hamper endometrial receptivity, but the molecular mechanisms affected in these and other infertility diagnoses such as unexplained infertility need further investigation. Previous transcriptome studies of the human endometrium have analysed different infertility diagnoses separately, but whether and to what extent receptive-phase endometrial transcriptome profiles vary between distinct infertility diagnoses is underexplored.

Study design, size, duration: A prospective, cross-sectional study was performed at the Reproductive Unit at the University Hospital between March 2019 and April 2021. Forty-five well-characterized infertile women (age 34.7 ± 3.8 years, BMI 24.5 ± kg/m^2) were recruited before starting any infertility treatment. Four groups according to different infertility diagnoses were established: endometriosis (n = 12), RIF (n = 14), unexplained infertility (n = 10), and male factor infertility as control group (n = 9).

Participants/materials, setting, methods: Endometrial biopsies were collected using Pipelle curette during mid-secretory phase (LH + 7) of the natural cycle. Total RNA was extracted and sequenced using Illumina NovaSeq 6000 technology. Raw sequencing data was quality assessed, pre-processed and quantified, and differential expression and enrichment analyses were performed using R software.

Main results and the role of chance: Differential expression analysis revealed 743, 587 and 140 differentially expressed genes (DEGs) in endometriosis, RIF and unexplained infertility, respectively, when compared to control women of the male factor infertility group. Among these, 31 genes exhibited consistent differential expression between all infertility groups. Regarding

functional enrichment analyses, the detected DEGs were mainly related to immune response and inflammatory processes, supporting the involvement of these pathways in the impairment of endometrial receptivity. Interestingly, the comparison between different study groups showed similar transcriptome profiles in endometrium-associated pathologies such as endometriosis and RIF, while women with unexplained infertility had similar molecular profiles with the control group of male factor infertility. These study results indicate common molecular pathways in endometrial receptivity between infertile women with endometriosis and RIF, and between unexplained infertility and control male factor infertility patients.

Limitations, reasons for caution: Further research with bigger sample size is required to confirm these findings.

Wider implications of the findings: We identified transcriptome profiles associated with infertility diagnoses. Further, women with unexplained infertility tended to have similar molecular profiles with control women, meaning that other factors than endometrial receptivity issues could lead to their infertility. Our study helps to understand the molecular mechanisms underlying female infertility in different infertility diagnoses.

Trial registration number: not applicable

Abstract citation ID: dead093.725
P-367 Women with endometriosis have impaired fertility, but give birth to at least one child as often as reference group - A population-based cohort analysis

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Study question: Do women with endometriosis have decreased fertility and decreased number of children compared with women without endometriosis in a population-based study setting?

Summary answer: Women with endometriosis have decreased fertility compared with women without endometriosis but give birth to at least one child as often as women without endometriosis.

What is known already: Endometriosis is known to be associated with infertility. It is estimated that 25-50% of patients undergoing fertility treatments have diagnosis of endometriosis. Up to date, population-based studies investigating the association of endometriosis and fertility are lacking.

Study design, size, duration: In a large follow-up national birth cohort (n = 5889), a postal questionnaire was sent to women at ages 31 and 46 years (response rates 76% and 65%, respectively). At age 46, the postal questionnaire included a question on previously diagnosed endometriosis (n = 261). Furthermore, the cohort was linked to Finnish national register for health care to get the register-based diagnosis for endometriosis (n = 257) and to the national birth register for all births from 1987 to 2014.

Participants/materials, setting, methods: The final study population included 319 women with endometriosis and 2714 women without endometriosis. Time to pregnancy and fertility treatments were inquired via questionnaire at ages 31 and 46 years. Number of deliveries and mother's age at the birth of the first child was derived from national birth register between 1987-2014. Pearson's Chi-square tests, Mann-WhitneyU test and Kaplan-Meier method (with Mantel-Cox test) for survival analysis were carried out using IBM SPSS Statistics 27.

Main results and the role of chance: At age 31 years, women with endometriosis had decreased fecundability as they reported more often more often over 12 months time to pregnancy (11.2% vs. 22.9%, $p < 0.001$) when compared with women without endometriosis. At ages 31 years and 46 years women with endometriosis reported higher need for fertility treatments (OR 2.80 [95%CI 1.35 – 5.81], $p = 0.005$ and 46yrs OR 2.82 [95% CI 1.80-4.43], $p < 0.001$). Survival analysis showed that according to the birth register by age 48 years, women with endometriosis had given birth to their first child at an older age (median 29.79yrs [95%CI 28.74 – 30.85] vs. 27.51yrs [95%CI 27.24 – 27.78], $p < 0.001$) and had fewer children (mean 2.29 vs 2.63, $p = 0.001$) when compared with women without endometriosis. However, women with endometriosis had at least one child as often as women without endometriosis (85.3% vs. 88.6%, $p = 0.074$) by age 48 years.

Limitations, reasons for caution: Fecundability was asked in the postal questionnaire only at age 31 years but not at 46 years. Infertility was evaluated based on self-report.

Wider implications of the findings: This unique, population-based data showed that fertility among women with endometriosis is compromised, as these women had decreased fecundability, increased need for fertility treatments and less children compared with the reference group. The awareness of the effects of endometriosis on fertility should be increased and met with adequate care.

Trial registration number: Not applicable

Abstract citation ID: dead093.726
P-369 An innovative approach for FET that does not rely on the luteinizing hormone surge or human chorionic gonadotropin: a natural cycle with luteal support

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Study question: Can progestin supplementation in natural cycle, without reliance on LH surge or hCG ovulation triggering, effectively prepare the endometrium for a frozen embryo transfer (FET)?

Summary answer: High pregnancy rates can be achieved in natural cycle using luteal support as the main method for endometrial synchronization, without relying on the LH surge

What is known already: In recent years, frozen-thawed embryo transfer has become widely adopted and plays a significant role in assisted reproductive technology. To date, the evidence suggests that natural cycles result in higher pregnancy rates and fewer obstetric complications compared to hormonal replacement therapy, however, the most effective method for preparing the endometrium has not yet been determined.

For transfer in the natural cycle specifically, conflicting results have been reported in terms of the outcome following spontaneous or triggered ovulation. No research is available that supports a natural cycle FET without monitoring the endogenous LH surge or using hCG trigger.

Study design, size, duration: This is a prospective analysis of 508 natural FET cycles between March 2019 and December 2022, including patients receiving embryos with own oocytes (339) and donated oocytes (169).

Participants/materials, setting, methods: All patients underwent ultrasound monitoring and serum hormone levels determination of estrogen and progesterone in 3 moments of the cycle (the day of the start of luteal support, 1 week before B-HCG and the day of B-HCG). Upon ultrasound confirmation of the endometrium's optimal thickness and appearance and the presence of the pre-ovulatory follicle, patients began progesterone supplementation and the day for FET was scheduled.

Main results and the role of chance: A clinical pregnancy rate of 65.48% was reported with a miscarriage rate of 9.09%.

A higher rate of miscarriage (22.22%) was found to be associated with an endometrial thickness of less than 7mm at the time of progesterone initiation. The size of the dominant follicle did not affect pregnancy outcome, but a higher pregnancy rate was observed when the follicle measured 15 mm or larger (CPR of 66.83% in cycles with a follicle $\geq$15mm versus CPR of 60.22% in cycles with a follicle <15mm). Estrogen levels decline in the second measurement but increase again on the day of a positive beta test.

This protocol requires less monitoring compared to true and modified natural cycles and is convenient to coordinate with oocyte recipients. A thin endometrial lining, measured below 7mm, increases the risk of a clinical

miscarriage. A lack of increase in estrogen levels on the day of B-hCG is an unfavorable sign for a positive outcome.

Limitations, reasons for caution: Our analysis of data has a prospective design, although it may be limited by unmeasured factors such as the number, stage, and chromosomal testing of transferred embryos.

Wider implications of the findings: Supplementary luteal support does not affect the natural progression of the endometrium towards receptiveness and results in a key intervention for controlling the start, length and functionality of the window of implantation. A natural cycle, with progesterone supplementation but without monitoring the LH surge, results in high pregnancy rates.

Trial registration number: Not applicable

Abstract citation ID: dead093.727

P-370 Impaired endometrial decidualization with a hyperinflammation environment is involved in poor reproductive outcomes in adenomyosis patients

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Study question: Do patients with adenomyosis have an impaired inflammatory state of the endometrium that could affect implantation and pregnancy?

Summary answer: Adenomyosis patients show increased proinflammatory cytokines expression and deregulated decidualization markers expression in eutopic endometrium which could lead to altered endometrial receptivity and impaired implantation.

What is known already: Adenomyosis is an estrogen-dependent chronic inflammatory condition, characterized by the presence of endometrial glands and stroma within the myometrium. Adenomyosis patients present altered decidualization and defective embryo-endometrium communication, resulting in implantation failure, miscarriage, and other fertility-related disorders. Although the underlying mechanisms of this infertility remain unknown, it is known that a favorable immune and inflammatory uterine environment is necessary for developmental pregnancy. It has been proposed that a uterine hyperinflammatory state could be involved in adenomyosis-related infertility. For this reason, we aim to evaluate the inflammatory and endometrial receptivity status during implantation in eutopic endometrium from adenomyosis patients.

Study design, size, duration: Sixteen endometrial samples were collected from infertile patients with and without adenomyosis undergoing hormonal replacement therapy before in vitro fertilization (IVF) at IVIRMA Valencia between January to December 2022.

Participants/materials, setting, methods: Eutopic endometrial samples were obtained at secretory phase (LH+7) from patients diagnosed with adenomyosis (n = 8) by ultrasound or hysteroscopy and patients without gynecological diseases (control, n = 8). Total RNA extraction was performed to later determine the gene expression of the decidualization-related genes Prolactin (*PRL*), *SPP1* and *PAEP* by qRT-PCR. In addition, proteins were extracted from eutopic endometrium using a lysis buffer and the relative expression levels of human cytokines were measured by Human Cytokine Array (Raybiotech).

Main results and the role of chance: Gene expression evaluation in endometrium from adenomyosis patients compared to control showed a significant downregulation of the key marker of decidualization *PRL* (fold change [fc] = 0.56, p = 0.0047) and a significant upregulation of *SPP1* (fc = 1.75, p = 0.009) and *PAEP* (fc = 1.56, p = 0.0192), both involved in endometrial receptivity and in immune regulation. Regarding cytokines expression, array results showed upregulation of different interleukins (IL) involved in the mediation of the immune and inflammatory response previously described in adenomyosis. Specifically, IL1ß (12 ± 19.63 vs. 1.37 ± 2.77, p = 0.009) that regulates several inflammatory responses, including cell proliferation, differentiation, and apoptosis; and IL6 (584.7 ± 121.8 vs. 488.4 ± 57.44, p = 0.06) that acts on the inflammation and maturation of B cells, contributing to the development of autoimmune diseases. Similarly, cytokines related to the promotion of inflammation and cellular proliferation in endometriosis, IL17a (173.6 ± 44.75 vs. 102.10 ± 71.85, p = 0.04) and TNFβ (430.9 ± 139.6 vs. 315.3 ± 37.09, p = 0.04), were also upregulated in adenomyosis compared to control. There were also other cytokines upregulated with diverse functions like an anti-inflammation response, growth factors or immune modulation: IL5 (308.1 ± 88.77 vs. 202.6 ± 45.72, p = 0.01), IL2 (341.4 ± 60.38 vs. 270.8 ± 35.13, p = 0.014), TGFα (215.3 ± 27.04 vs. 117.8 ± 36.67, p = 0.002), IL31 (77.74 ± 40.57 vs. 14 ± 15.96, p = 0.0012), IL7 (739.1 ± 147.1 vs. 511.8 ± 103.7, p = 0.003) and IL15 (301.9 ± 95.73 vs. 209.8 ± 50.62, p = 0.03).

Limitations, reasons for caution: Our findings are limited by the relatively small sample size and inherent biological variability of human samples.

Wider implications of the findings: Adenomyosis patients showed impaired decidualization and a hyperinflammatory endometrial environment that could be affecting the endometrial receptivity and consequently, the embryo implantation. These findings open insight to further investigations to study the mechanism by which exaggerated inflammation affects fertility in the context of adenomyosis to define new management approaches.

Trial registration number: Not applicable

Abstract citation ID: dead093.728

P-371 Subendometrial administration of selectively enriched angiogenic precursor cells and growth factors derived from peripheral blood and bone marrow optimizes endometrialthickness and pregnancyoutcomes in thin-endometrium patients

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Study question: Can hysteroscopic instillation of progenitor cells combined with platelet derived growth factor concentrate into subendometrial region help patient with thin endometrium conceive?

Summary answer: Hysteroscopic subendometrial administration of stem cells and growth factors significantly improved endometrial thickness (EMT) and pregnancy outcomes in women with refractory thin endometrium

What is known already: Chronically thin endometrium is a challenge in ART, results in repeated expensive IVF cycles, cycle cancellations, unplanned cryopreservation of embryos and, surrogacy. Many studies have successfully used stem cells or platelet derivatives to rejuvenate reproductive tissues. When these growth-promoting cells and growth factors from platelets are delivered into basal layer which is origin of endometrial cells quality of the endometrium can be improved. While bone marrow is excellent source for obtaining stem cells main challenge is invasiveness of collection. This forms the basis for collecting mobilized endothelial progenitor cells from bone marrow into circulation for easy collection.

Study design, size, duration: This study is to evaluate effect angiogenic precursor cells and growth factors derived from peripheral blood and bone marrow in improving endometrial quality and pregnancy outcomes. In the present pilot study, 50 patients with persistent refractory thin endometrium were included and underwent frozen embryo transfer from September 2019-January 2023 at A4 fertility center, Chennai. Out of 50 patients, 43 were treated with peripheral blood and 7 were treated with bonemarrow derived cells and growth factors.

Participants/materials, setting, methods: 50 patients with EMT<6mm and >2 canceled cycles included. Two doses of subcutaneous G-CSF was given on Menstrual Cycle Days 3&4, followed by 60ml venous blood or bone marrow aspirated on MCD-5. Seragen's selective enrichment protocol was

used to prepare circulating endothelial progenitor cells and growth factors concentrate. With a 2.9mm hysteroscope and single lumen ovum pickup needle harvested cells and growth factors were injected on all four walls of cavity.

Main results and the role of chance: EMT increased significantly following bone marrow and peripheral blood cells 5 days after sub-endometrial injection (5.8 ± 0.71 vs. 7.12 ± 0.8; P = 0.0001), with average increase of 1.30mm and 1.80mm respectively and considered optimal improvement when EMT≥ 7mm. 33 (66%) had an optimal response and there was a significant improvement in the endometrial thickness(mm).Cycle cancellations were significantly lesser in both peripheral blood and bone marrow group and LBR was significantly improved ($p < 0.05$). There was no significant difference in CPR, IR and LBR between peripheral blood and bone marrow groups ($p > 0.05$). Overall, clinical pregnancy and LBR reached up to 40% and 30%, respectively. No adverse reactions were reported. There was statistically significant probability of achieving pregnancy ($p < 0.01$) when treated with both peripheral blood and bone marrow derived cells. Likewise, there was also a statistically significant probability of getting pregnant ($p < 0.05$) when embryo transfer was planned in the subsequent cycle. While bone marrow group showed 100% HCG positive in all 5 out of 7 patients underwent embryo transfer and 57% (4/7) of clinical pregnancy, the sample size was small compared to peripheral blood group.

Limitations, reasons for caution: Further research is needed to provide opportunity for women with refractory thin endometrium to conceive without surrogacy. Clinician expertise in patient recruitment, dosage personalization support, and administration is paramount. Our study provides promising information for future randomized, controlled trials with large sample size in this field.

Wider implications of the findings: We have validated feasibility and efficacy of mobilized peripheral blood cells for improving endometrial pathologies when bone marrow aspiration is a challenge. Our findings show targeted delivery of personalizeddosage from minimally invasive autologous source could be new rayof hope in females failto improve despite all possible treatment options recommended surrogacy.

Trial registration number: Not applicable

Abstract citation ID: dead093.729
P-372 RNA sequencing studies evaluating oocyte quality in women with endometriosis: a meta-analysis

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Study question: Can a network-based integrative analysis (NBIA) meta-analysis approach be used to identify clinically relevant cumulus-oocyte complex biological pathway and gene targets in women with endometriosis?

Summary answer: Bi-level meta-analysis (BLMA) of multiple RNAseq studies shows enrichment of both inflammatory and mitochondrial pathway-related differential gene expression within oocyte, granulosa and cumulus cells.

What is known already: Endometriosis-related infertility is multifactorial, and studies suggest that impaired oocyte quality plays a significant role. The close relationship between granulosa, cumulus cells and developing oocytes makes them ideal candidates for studying non-invasive biomarkers for oocyte quality. As high-throughput sequencing becomes more accessible, the volume of RNAseq molecular data in public databases such as Gene Expression Omnibus (GEO) has increased. Combined analysis of multiple datasets theoretically increases study power due to larger number of samples. Comparison of differentially expressed genes (DEGs) and biological pathways across multiple independent studies therefore requires a 'meta-analysis' framework that can effectively integrate data from different sources.

Study design, size, duration: We identified relevant RNAseq datasets through NCBI public functional genomics data repository Gene Expression Omnibus (GEO). Datasets were split into "test" and "control" groups referring to models of oocyte incompetence and competence.

Accession numbers GSEXXX or PRJNXXX represent unique identifiers for RNAseq data files contained within a given NCBI GEO series or BioProject record, respectively. Five human RNAseq datasets (PRJNA514416, GSE168214, PRJNA216966, PRJNA727838 and GSE81579) were identified and DEG lists generated for each individual dataset.

Participants/materials, setting, methods: Normalisation and differential expression between RNAseq datasets was carried out in R using the Bioconductor DESeq2 package. DEGs were identified as those whose P-value was adjusted using a false discovery rate less than 0.5, adjusted using Benjamini-Hochberg procedure. Log2 fold-change ratio (logFC) of each DEG was used to identify significantly up and downregulated genes.

The NBIA pipeline (Nguyen et al, 2020) examines over-representation of significantly impacted genes in a pathway as per KEGG pathway analysis.

Main results and the role of chance: Upregulated DEGs were identified in mitochondrial membrane ATP synthase pathways, (ATP5G1, ATP5G2, ATP5J2 and ATP5H). KEGG Pathway terms "Parkinson's", "Huntington's", and "Alzheimer disease" were also enriched, due to many genes partaking in oxidative phosphorylation in the context of these pathologies. Functional analysis also revealed significantly down-regulated genes related to inflammatory pathways (CXCL1, 2, 3, 5,6 and 8), which have a role in innate immunity. Although surprising, in our analysis, downregulated functions based on KEGG functional pathway analysis, observed that innate immune response, cellular defense response, chemokine-mediated signaling were significantly downregulated.

BLMA over-representation analysis (ORA) and impact analysis (IA) analysis showed that the Amoebiasis pathway was enriched expressed across all datasets. Genes involved in this pathway include interleukin (IL), CXCL1, CXCL2, CXCL3, SERPINB, TGFB1 and TGFB2, and its biological process is related to natural killer (NK) cell mediated cytotoxicity. Overall, the enrichment of immune and inflammation-related pathways revealed by BLMA meta-analysis underscores the relevance of inflammation to follicular function. Indeed, ovulation is inextricably linked with inflammation.

Other significantly enriched pathways highlighted by this meta-analysis are related to infection (path:hsa05134 Legionellosis; path:hsa05140 Leishmaniasis) immune response (path:hsa04145 Phagosome; path:hsa04610 Complement and coagulation cascades) and inflammation (path:hsa05323 Rheumatoid arthritis).

Limitations, reasons for caution: Methods used availed of publicly available GEO datasets and bioinformatics software (Bioconductor, R and DAVID). Although useful tools, these approaches are open to inter-user variation. Cell types compared across studies were oocytes, granulosa and cumulus cells. Controlled ovarian stimulation may also be open to influence based on stimulation protocol used.

Wider implications of the findings: Enrichment of inflammatory and mitochondrial pathways in our GO analysis is compelling. Given the role of mitochondria in oocyte developmental competence, this reinforces the role of impaired oocyte competence in women with endometriosis. Our study identifies several genes that require further elucidation as potential predictors of oocyte quality in endometriosis.

Trial registration number: N/A

Abstract citation ID: dead093.730
P-373 Low-Dose Vaginal Misoprostol Application Before Office Hysteroscopy

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Study question: In gynecological interventional procedures what should be the minimum misoprostol dose for achieving cervical maturation?

Summary answer: 50mcgr vaginal misoprostol before office hysteroscopy; decreases the VAS score, bleeding and the total procedure time.

What is known already: Misoprostol has been on the World Health Organization (WHO) essential drugs list since 2005 due to its uterotonic effect.FIGO (The International Federation of Gynecology and Obstetrics) published a posology table in 2017 that does not cause side effects in off-label use of misoprostol in the obstetric field.There are many publications that misoprostol facilitates interventional gynecologic procedures by allowing cervical maturation . However, no guidelines have yet been published on the dose and route of administration

Study design, size, duration: This Prospective Randomized Clinical Research was approved by Ankara City Hospital Clinical Research Ethics and Academic committee . Then, 100 women who underwent office hysteroscopy in Ankara City Hospital Gynecology and Obstetrics Clinic gynecology-infertility outpatient clinic between 01/12/2021-01/05/2022 were included in the study after their informed consent was obtained.

Participants/materials, setting, methods: 50 women who self-administered 50 mcg vaginal misoprostol the night before the office hysteroscopy procedure and 50 women who did not administer the drug were compared. The primary outcome of the study was the duration of the procedure, with the pain score during the procedure used being the (VAS) score system. The secondary outcome was procedure and drug-related side effects Statistical evaluation of the data was performed using the SPSS for Windows version 20.0

Main results and the role of chance: In the medicated group the mean of VAS scores during and after the procedure, and the transition and the total time of the procedure were significantly lower than non-medicated group.

Limitations, reasons for caution: In Turkey, there is only one misoprostol product (CYTOTEC Ali Raif İlaç Sanayi A.Ş) with the 200 mcg posology Off label use of misoporostol in obstetrics is legal since 2021 but not for gynecological practice. We do not have country guidelines on misoprostol posology in obstetrics and gynecology usage also

Wider implications of the findings: In gynecological interventional procedures especially before office hysteroscopy, it is important to apply a minimum dose of misoprostol for cervical maturation due to its bleeding-increasing effect in patients whith endometrial polyps or hyperplasia .Because bleeding is the most important factor affecting image quality and procedure efficacy during hysteroscopy.

Trial registration number: E2-21-1068 dated 24.11.2021

Abstract citation ID: dead093.731
P-374 Impact of different luteal phase support protocols in fresh embryo transfers on perinatal outcomes of singleton in vitro fertilization pregnancies: a three-decade experience.

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Study question: How can stimulation of corpus luteum in the luteal phase with human chorionic gonadotropin (hCG) improve obstetric and perinatal outcomes?

Summary answer: There is currently no evidence that the use of hCG as an alternative treatment for luteal-phase support (LPS) improves obstetric and perinatal outcomes.

What is known already: There are different approaches for providing LPS, although none have shown differences in pregnancy rates. However, emerging evidence has also revealed the important role of corpus luteum in obstetric and perinatal outcomes after *in vitro* fertilization (IVF) (preeclampsia or intrauterine growth restriction, among others) in the production of progesterone (can be administered exogenously) as well as the secretion of multiple vasoactive active substances.

Study design, size, duration: We performed a retrospective, single centre study between 1991-2021. A total of 2592 singleton pregnancies were analysed after fresh embryo-transfer according to LPS.

Participants/materials, setting, methods: IVF singleton pregnancies were stratified into two cohorts according to the LPS protocol: 1) trigger with 5000 IU urinary hCG (U-hCG) and LPS protocol with progesterone 200 mg x3/day/vaginally beginning the day after oocyte retrieval and one u-hCG bolus of 2500 IU, the day and 3 days after oocyte retrieval; and 2) trigger with 250 µgr of recombinant hCG and LPS protocol with only progesterone (200 mg x3/day/ vaginally).

Main results and the role of chance: We compared the reproductive outcomes between cycles in cohort 1) n = 527 and cohort 2) n = 2065. The two cohorts were stratified and analysed in three groups according to maternal age to reduce possible bias: 35 years old or less, between 36 and 39 years old, and 40 or more years old. In cohort 1, all the cycles followed a long agonist GnRH protocol, while in cohort 2, 78.11% received an agonist GnRH protocol and 21.89 % a GnRH antagonist protocol. Recombinant gonadotropins for ovarian stimulation was administered in all cycles. We observed a greater number of eutocic deliveries, a lower number of instrumentalized births and a stable caesarean rate along the study period. There were no statistical differences between the two treatment groups regarding hypertensive pregnancy disorders, intrauterine growth restriction, small for gestational age or large for gestational age. Group 2 showed an apparent greater tendency, albeit not significant, to preeclampsia and a significant increase in very preterm birth. Emerging evidence has also revealed the important role of corpus luteum in obstetric and perinatal outcomes following IVF.

Limitations, reasons for caution: The large number of pregnancies evaluated is a clear strength of this study. This is the first series comparing different perinatal outcomes according to LPS. In contrast, the retrospective design and long time period involving changes in laboratory and patient profiles is a clear limitation.

Wider implications of the findings: The optimization of IVF treatments is especially important and is related to multiple factors, including the individualization of treatments to ensure the safety of IVF treatment as well as that of the pregnancies derived thereof. More studies on the different LPS protocols available are needed.

Trial registration number: not applicable

Abstract citation ID: dead093.732
P-375 Effect of D-Chiro-Inositol in a mouse model of endometriosis

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Study question: Can D-Chiro Inositol administration mitigate the phenotype of endometriosis in a mouse model?

Summary answer: Based on an endometriosis mouse model, we demonstrated that administration of D-Chiro Inositol can reduce development of endometriotic lesions.

What is known already: Endometriosis, a disease affecting 5-10% of women of reproductive age, is characterized by the spread of endometrial-like tissue outside the uterine cavity that produces ectopic endometriotic lesions causing pain and infertility. The sensitivity of endometriosis to estrogens is a characteristic that can be used for therapeutic purposes. D-Chiro Inositol (DCI), one of the nine isomers of Inositol, is known to decrease the CYP19A1 aromatase gene expression in granulosa cells. Based on these premises, it was suggested that treatment with DCI may have clinical application in conditions where decreased estrogen levels is required.

Study design, size, duration: To address the study question, a mouse model of endometriosis was generated. Out of 20 CD1 mice, 4 mice were randomly selected as donors of uterine fragments and the remaining 16 were recipient mice. The first day after transplantation, mice were randomly assigned to four experimental group which received for 28 days 2ml of water containing: none (CTRL); DCI 0.4mg (DCI 0.4); DCI 0.2mg and Dienogest 0.33ng (DCI 0.2+DG 0.33); DG 0.67ng (DG 0.67).

Participants/materials, setting, methods: Uterine horns were removed from donor mice at the diestrous stage of the reproductive cycle. The tissue cut into fragments was inoculated in recipient mice by intraperitoneal injection. Four weeks after induction, all mice were sacrificed. Their endometriotic lesions were excised, measured by number and size, and examined for the presence of blood vessels vascularization under stereomicroscope. Then, lesions were processed for histology examination by hematoxilin-eosin (H&E) and Azan Mallory staining.

Main results and the role of chance: Endometriotic lesions developed in recipient mice met all criteria for endometriosis, including the presence of endometrial epithelial and stromal cells, and encapsulation in neighboring tissues or organs. The lesions number was reduced in all the treatment groups when compared to control ($p < 0.05$, t-test), and no differences were observed among DCI 0.4, DCI 0.2+DG 0.33 and DG 0.67. Concomitantly the rate of vascularized lesions was lower in the treated groups, with more pronounced effect in the DCI 0.4 group where no vascularized lesions were observed ($p < 0.05$, t-test). The histological analysis revealed a marked reduction of endometriotic foci in all groups. These results provide evidence that DCI can reduce development and vascularization of endometriotic lesions in a mouse model, an effect that is not observed when it is employed at lower dose in association with DG. Although molecular mechanisms underlying DCI effects requires further investigation, present findings support the hypothesis that DCI could be more effective than DG in mitigating endometriosis phenotype.

Limitations, reasons for caution: Results from animal studies should be extrapolated to humans with caution.

Wider implications of the findings: Present findings may open new avenue in testing whether DCI may have clinical application in endometriosis therapy.

Trial registration number: Not Applicable

Abstract citation ID: dead093.733
P-376 Restoration of mRNA expression level of endometrial MEIS1, as a co-factor of HOXA10, after laparoscopic salpingectomy of women with hydrosalpinx

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Study question: Do salpingectomy could restore HOXA10 co-factor MEIS1 expression in endometrial tissues of hydrosalpinx patients?

Summary answer: Laparoscopic salpingectomy could restore endometrial HOXA10-cofactor- MEIS1 expression level, in addition to HOXA10, in hydrosalpinx women.

What is known already: Hydrosalpinx is defined as a distally blocked and dilated fallopian tube which has been filled with serous fluid. Evidence suggests that hydrosalpinx is associated with adverse effect on endometrial receptivity by abnormal expression of key molecules such as HOXA10 in the pre-implantation endometrium. Previous data showed salpingectomy results in statistically significant increase in endometrial HOXA10 expression.

MEIS1 is a three-amino-acid loop extension (TALE) family homeobox gene which has been proven to be a novel coactivator of HOXA10 during human and mouse endometrial decidualization. MEIS1-HOXA10 complex binds to the promoters of the target genes which are beneficial for human stromal cell decidualization.

Study design, size, duration: This prospective study was conducted from January 2021 to January 2022 to determine whether salpingectomy would reverse HOXA10-cofactor- MEIS1 expression levels after salpingectomy. All women signed the informed consent and did not receive any hormonal medication during the last three months. In this study 10 infertile women with moderate to severe hydrosalpinx proven by hysterosalpingography or laparoscopy undergoing salpingectomy were recruited. Ten healthy fertile age-matched women considered as control group.

Participants/materials, setting, methods: Ten infertile women, aged 20-40, with sonovisible hydrosalpinx (diameter >10mm), AMH>1.2 ng/dL, BMI (18–28 kg/m^2) and regular menstrual cycle indicated by mid-luteal progesterone levels of > 10 ng/mL were included. Ten healthy fertile age-matched women with a history of successful pregnancy considered as control group. Mid–luteal-phase endometrial sampling (by pipelle) was performed at the time of surgery and second sampling was obtained forth-post treatment cycle. RNA extraction and cDNA synthesis were done.

Main results and the role of chance: Quantitative mRNA expression of MEIS1 was determined by real-time PCR technique. Gene expression data were analyzed based on $2^{-\Delta\Delta CT}$ to estimate the relative fold change value. The non-parametric Wilcoxon signed rank test was used for data analysis. P value less than 0.05 was considered statistically significant.There was a significant reduction in the expression levels of MEIS1 gene in the endometrial samples of hydrosalpinx group before surgical removal. The obtained data showed that salpingectomy resulted in a statistically significant increase (about 6-fold) in endometrial MEIS1 expression ($P = 0.02$). Moreover, there was not significant difference in MEIS1 mRNA expression of hydrosalpinx group after salpingectomy and the control group ($P > 0.05$).

Limitations, reasons for caution: Larger sample size for the confirmation of these data is needed. Epigenetic evaluation of hydrosalpinx endometrial tissues especially in HOXA10 promoter is recommended.

Wider implications of the findings: This study suggests that the restoration of MEIS1 gene expression observed in hydrosalpinx women after salpingectomy can be considered as a molecular mechanism by which salpingectomy results in improvement of pregnancy rate in IVF cycle.

Trial registration number: not applicable

POSTER VIEWING
ETHICS AND LAW

Abstract citation ID: dead093.734
P-377 Artificial Intelligence (AI) for Pre-implantation Genetic Test for Aneuploidy (PGT-A): important epistemic and ethical considerations

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Study question: What are the epistemic and ethical considerations if it's used AI for PGT-A?

Summary answer: The AI used to date presents significant epistemic and ethical problems and there are no studies evaluating its clinical efficacy. This topic requires more attention.

What is known already: The introduction of time-lapse systems offers the possibility of generating large amounts of data to evaluate the state of health of embryos. With the introduction of AI and machine learning this data can be analyzed by algorithms that improve automatically as they are exposed to more data. Almost all AI is based on algorithms developed using neural networks. Many algorithms are difficult to understand or even uninterpretable

because they are covered by company secrets. Not having access to the algorithms causes problems of an ethical nature and caution is required in its use for the identification of healthy embryos.

Study design, size, duration: We performed a study of the articles that mention a possible use of AI as a tool to be used within the PGT-A. The epistemic and ethical implications of current approaches have been considered. We also considered the oral communications presented during the last ESHRE annual meeting and the sensationalist communications associated with them.

Participants/materials, setting, methods: We used PubMed for article search which includes more than 35 million citations for biomedical literature from MEDLINE, life science journals and online books. To carry out a targeted search we used the following keywords: Artificial Intelligence* OR AI OR Neural Network* OR Machine Learning OR Support Vector Machine OR Automated Classification AND IVF OR IVF OR Embryo OR Pre-implantation Genetic OR PGT*.

Main results and the role of chance: The results obtained were difficult to interpret. We found articles where they looked at 2 types of results: accuracy for predicting euploidy and agreement with the molecular assay assessment. It has been demonstrated that some algorithms are not always able to totally differentiate a euploid from an aneuploid embryo, especially when speaking of segmental aneuploidies. This data lends many concerns. Almost universally, AI models were opaque in that at least part of the process was not accessible. If not fully accessible, these models are problematic for epistemic and ethical reasons. Epistemic concerns include information asymmetries between algorithm developers and all the operators who routinely perform these analyses. There is another risk of biased predictions caused by known and/or unknown confounders during the algorithm auto-improvement process. Furthermore, we must consider the high difficulty in checking for any errors in real time due to the total non-accessibility.

The ethical trap includes: the risk of misrepresenting important values for the health of the embryo and the patient; the concern of incorrect deselection or worse a risk to the health and well-being of future children. There are also possible social implications; and a hole of responsibility, in case of adverse events.

Limitations, reasons for caution: Our search was limited to a single major medical research database and to what was presented during the last ESHRE annual meeting. Although there are other databases where it might be possible to find different articles. The application of AI in PGT is a very recent topic.

Wider implications of the findings: It is premature to implement AI for the identification of aneuploidies in embryos. AI for aneuploidy embryo deselection has enormous potential but needs to be done with care and transparency, as epistemic and ethical issues are significant. Currently the only possible use is to identify priority embryos for the transfer.

Trial registration number: not applicable

Abstract citation ID: dead093.735
P-378 Does surrogacy (unexpectedly) provide protection of the right to life of the unborn child?

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Study question: How surrogacy contracts provisions provide greater protection to the unborn children compared to the case of natural conception?

Summary answer: The right to life of the unborn child seems to have a broader scope of protection than it does in the case of natural conception.

What is known already: The surrogacy contract usually contains a provision according to which a surrogate will not terminate the pregnancy unless her own life is at risk. On the other hand, intended parents have the right to request the termination of pregnancy only when there are eugenic indications. It is evident that the conditions under which pregnancy can be terminated are more rigid than in the case of natural conception.

Study design, size, duration: The research was conducted based on different types of surrogacy contracts applied in different legal systems. Countries where surrogacy is allowed, but also where it is widespread, such as certain US states, are covered. In addition, an overview of the legal texts of individual countries, which refer to the issue of termination of pregnancy in the case of surrogacy, is given.

Participants/materials, setting, methods: During the research, the comparative legal method was used, as well as the analysis of various contracts on surrogacy, as well as the judicial practice of national and supranational courts.

Main results and the role of chance: The right to life of the unborn child in the case of surrogacy enjoys protection from the moment the embryo is implanted in the surrogate mother, while in the case of natural conception, in most legal systems, this protection of the right to life is shifted to a certain gestational age, up to which termination of pregnancy is completely free. In this way, the rights of the unborn child are placed above the rights of the surrogate mother and that was exactly what was unacceptable for the UN Convention on the Rights of the Child and why the protection of the right to life of the unborn child is missing in the text. The right to life of an unborn child almost completely depends on the desire of the intended parents to have a child that will be completely healthy. However, if during the pregnancy of the surrogate mother, no danger to the life and health of the child is established, it seems that the intended parents do not have the right to demand termination of the pregnancy. All of the above implies a lot of ethical and legal dilemmas, which require careful scientific analysis.

Limitations, reasons for caution: It is disputable what would happen if serious indications for termination of pregnancy were determined and if intended parents demanded termination of the pregnancy, but the surrogate mother refused. More precisely, whether the surrogate mother is obliged to terminate the pregnancy when she is required to do so?

Wider implications of the findings: Forasmuch that in the case of surrogacy, several persons can request termination of pregnancy, this right becomes limited, and it no longer belongs only to the woman. This could affect the change of discourse in the case of natural conception and limit the right of the pregnant woman.

Trial registration number: not applicable

Abstract citation ID: dead093.736
P-379 Managing genetic information generated by non-invasive prenatal testing: Views of healthcare professionals and test users in Australia

Abstract withdrawn by the authors

Abstract citation ID: dead093.737
P-380 Reproductive autonomy in Spain - reproduction as a negative right and the obligations of the State

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Study question: Even when considering the right to reproduction as a negative right are there actions to be taken in order achieve a better reproductive autonomy?

Summary answer: Specific actions become a necessary positivisation of the right to reproduction. Ensuring socio-economic stability and promoting specific education are key parameters for promoting fertility.

What is known already: The reproductive autonomy of individuals consists of being able to choose the number of children desired and the appropriate timing between deliveries. However, there are many factors that prevent reproductive autonomy from being fully developed in Spain, being the country with the second lowest fertility indicator in Europe. There are texts that argue that reproductive autonomy should be approached from a Human Rights perspective and urge governments to adopt positive measures in accordance. However, the European Court of Human Rights' rulings on this issue are far from this perspective. Nevertheless, states have obligations to enhance the reproductive autonomy of individuals.

Study design, size, duration: In order to stablish this position, we studied the Spanish demographic and fertility indicator data and examined the existing Spanish laws regarding assisted reproductive techniques (such as Law 14/2006), the European Court of Human Rights' ruling on this matter, United

Nations consensus related to this field, the Guttmacher-Lancet commission and recommendations of scientific societies such as ESHRE.

Participants/materials, setting, methods: Bibliography was achieved using the European Court Human Rights' ruling HUDOC database, the Spanish State Official Newsletter and United Nations Library, between others. Socio-economic and demographic indexes were obtained with Eurostat and the Spanish National Statistics Office. Selected legal aspects were included in the revision and manuscript.

Main results and the role of chance: The Spanish socio-economic panorama is unfavorable for the emancipation of young people and the consequent formation of families with offspring, which aggravates the existing demographic crisis and generational turnover. Importantly, people are not being fully autonomous in their reproductive decisions, mainly due to a lack of information and foresight regarding real fertility and infertility expectations.

States must ensure comprehensive sexual and reproductive health promotion, which includes reproductive autonomy. The wide range of rights that constitute the so-called reproductive rights is encompassed within the framework of human rights. However, the right to reproduction is not always considered as a positive right, although the legislator must ensure that people can fully exercise their autonomy. The fact that the full positivisation of the right to reproduction is not considered does not exempt the State from obligations to ensure the reproductive autonomy of its citizens. Therefore, the positivisation of the right to reproduction must ensure socio-economic stability and reproductive health education, thus preventing future infertility problems.

Public and private clinics and health centers must be provided with the necessary means to diagnose possible reproductive pathologies. Education and economic stability are solutions to most of the problems related to population growth and infertility.

Limitations, reasons for caution: In this study we have interpreted international Law, soft-law and reccommendations according to the actual Spanish socio-economic and demographic context, which may not be adequate to extrapolate to other countries or populations.

Wider implications of the findings: To consider the right to reproduction as a positive right may mean converting the State into a provider of resources for this purpose. However, positivisation of the right to reproduction materializes in education and socio-economic stability, actions worth considering, such as creating public campaigns aimed at fertility education and awareness.

Trial registration number: Not applicable

Abstract citation ID: dead093.738
P-381 "An Analysis of the Legal Gaps and Jurisprudential Paradigms Surrounding Supernumerary Embryos in Italy: A Comparative Law Approach to Propose Practical Solutions"

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Study question: What is the current state of resolving the issue of surplus embryos in Italy, in the absence of clear legislation?

Summary answer: This paper proposes organic reform of Italian law and European harmonization for informed and conscious patient decision-making on excess embryos.

What is known already: According to Italian law, embryos obtained through in-vitro fertilization for assisted reproduction that have not reached the transfer phase (including unsuitable ones) cannot be destroyed, resulting in significant resource allocation for indefinite preservation in biobanks. This stems from the legal and ethical recognition of the human embryo as a subject of rights that in turn prohibits destruction, donation to other couples, or use for scientific research.

Study design, size, duration: My study compares European legal systems and analyzes the evolution of Italian law on medically assisted fertilization (L. 40/2004) since its implementation, with a focus on legal and ethical issues that have been subject of ongoing debate.

It also examines the Italian Ministerial Guidelines, the only text where the legislator has dealt with the issue of surplus embryos.

Participants/materials, setting, methods: Not applicable.

Main results and the role of chance: The present study examines the various approaches taken by countries to address the issue of surplus embryos. Regulations in countries such as Spain, France were taken into consideration. The British legal system is also examined for its regulations on the donation of embryos and reproductive cells. The Spanish regulation of embryo-sharing (L. 14/2006) is the most organic approach. It grants three options to couples with respect to their remaining embryos: preservation for personal use, donation to other couples, or donation for research. In the United States, the increase in cryopreserved and abandoned blastocysts has led to the spread of conditional embryo adoption and Snowflakes adoption. The Italian provision, however, fails to adequately balance the constitutionally protected interests of couples' self-determination and scientific research for the protection of individual and collective health.

Role of chance not applicable here.

Limitations, reasons for caution: Not applicable.

Wider implications of the findings: Italian legislation neglecting to intervene has caused numerous issues, such as increased costs for fertility centers and poor practices. Clinics advertising embryo adoption, despite regulatory uncertainty, have created unfair competition leading to psychological distress for couples with cells in limbo and fears of financial obligations.

Trial registration number: NOT APPLICABLE

Abstract citation ID: dead093.739
P-382 A moral appraisal of utilization of the sex selection method to avoid sexual discrimination

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Study question: Is it morally justified to utilize the sex selection method in countries where sex discriminations against one sex are prevalent?

Summary answer: Sex selection is not morally justifiable except in certain specific cases. Avoidance of sexual discrimination may not be included in the said exceptional cases.

What is known already: No enactment exists in Iran that authorizes or forbids sex selection. Given this legal gap, various clinics have adopted diverse practices. For instance, a number of them have embraced sex selection methods just for therapeutic purposes, while other ones utilize them for non-therapeutic aims. In countries like Iran where cultural structures and laws provide better life conditions for a particular sex, i.e. the male one, a group of parents tend to resort to the sex selection method so as to bring about a thriving and more prosperous circumstance for their future child.

Study design, size, duration: Cases of couples who have referred to infertility treatments centers over the last six years have been studied. About two third of them have applied for a male sex embryo. Upon interview, it is found out that one third of them, despite not believing in the supremacy of the male sex, have inevitably applied for this sex so as to secure a better future for their child.

Participants/materials, setting, methods: Experts of various fields, i.e. philosophy, law, sociology and medicine, have contributed to this research. The research materials are drawn from existing cases in an ARTs clinic in Tehran, plus the related humanities literature (in particular, those of law, philosophy and sociology). The research method, hence, is an empirical-cum-analytical one.

Main results and the role of chance: Although it is a moral duty to resist discrimination and to bring about a just structure for the growth and development of all individuals in the society, it does not seem that this can be achieved by preventing the birth of a particular sex. This way of tackling the problem is equal to the dissolution of the problem, rather than a resolution for such a profound human conundrum. First, the sex selection method is not accessible to all parents, say (at least) due to the financial costs, a fact that in its turn is a grave discrimination against the un-wealthy parents. Secondly, the

fact that the sex selection applicants are dominantly going for the male sex intensifies the male oriented prevalent culture and, hence, deepens sex discrimination practices. Thirdly, provision of sex selection facilities in an unfettered way will harm the population balance, in terms of sexes, and create more problems for the whole society. On this basis, making a recourse to sex selection methods in order to counter sex-based discriminations does not seem to be a morally right measure. This is deeply dubious from a moral vantage point.

Limitations, reasons for caution: Applicants' cases were anonymized so that their privacy could be protected. Researchers are also bound not to mention the name of the infertility clinic due to the particular situation in the society.

Wider implications of the findings: It seems that attempts, such as civil struggles and public awareness broadening endeavours, which are made to change prevalent discriminative attitudes, although time consuming, would be much more appropriate, in moral terms, and more effective in tackling discriminations against women.

Trial registration number: not applicable

Abstract citation ID: dead093.740
P-383 The risks and opportunities of building a family at an advanced parental age: A systematic review

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Study question: What empirical evidence exists on the well-being and psycho-social health of couples who become parents at an advanced age and their children?

Summary answer: Empirical evidence mostly focus on the well-being and psycho-social health of offspring (psychotic disorders, (neuro-)developmental disorders) and only little knowledge exists about their parents' well-being.

What is known already: In many societies, people tend to have children at an increasingly advanced age and there is increasing attention in the medical literature towards the consequences of this trend. This systematic review is the first one to critically synthesize the existing empirical literature on the psycho-social health and well-being of parents who had their children from the age 40 onwards and their offspring.

Study design, size, duration: We conducted a Systematic Review and registered its protocol in Prospero (CRD42022304564). The search strategy was designed based on a Population-Context-Outcome (PCO) structure, an adaptation of the traditional Population-Intervention-Comparison-Outcome (PICO) scheme to fit the specific aims of our study. The search was conducted in six electronic databases (namely Pubmed incl. MEDLINE, Embase, Scopus, PsycInfo, CINAHL and SocINDEX) and was limited to include empirical studies published between 01.01.2000 and 31.12.2021.

Participants/materials, setting, methods: Studies included are empirical studies – qualitative or quantitative – where subjects were children born to 40+ parents and their parents. Studies either examined the well-being and psycho-social health of parents and/or their children or focused on the social and ethical discussions surrounding risk and benefits of advanced parental age for parents and/or the offspring.

Main results and the role of chance: 5'403 articles were identified, leaving 2'543 after the removal of duplicates and 98 after the screening of titles and abstracts to be included for a full-text screening. Thereafter another 30 additional articles were excluded because they did not fulfil the inclusion criteria. Simultaneously, citation searching brought up 10 additional articles to include, of which 4 were in line with the defined inclusion criteria, leading to 69 total articles included in the final sample of the present systematic review.

The key results concern four aspects relevant for the research question: (1) studies show discrepancies in defining who is a parent of advanced parental age (APA); (2) there is an imbalance in the empirical evidence produced for different participant groups (e.g. mothers, fathers, offspring); (3) aspects of wellbeing (e.g. psychotic disorders, (neuro-)developmental disorders, general well-being) discussed for the specific participant group studied; and (4) a narrative synthesis of the advantages and disadvantages related to psycho-social health and wellbeing for the specific participant group.

Limitations, reasons for caution: Only empirical studies in English, published between 01.01.2000 and 31.12.2021 were included in this review. Also, the review focuses only on empirical evidence produced in studies where parents were 40 years or older at the time of birth.

Wider implications of the findings: There are many aspects of the well-being of children born by older parents which remain unknown, thus requiring more research (esp. on psychosocial wellbeing of children) to understand the (non-medico-somatic) risks and benefits of becoming parents at an older age and designing evidence-informed policies.

Trial registration number: Not applicable

Abstract citation ID: dead093.741
P-384 Advanced paternal age: a mapping of the ethical issues

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Study question: What are the ethical issues raised by advanced paternal age?

Summary answer: Advanced paternal age is an ethically challenging phenomenon at the intersection of perinatal ethics, public health ethics, research ethics and policymaking.

What is known already: Although there is no clear indication on when begins advanced paternal age (APA), there is a tendency to consider 40 years old and over as a turning point in male reproduction. APA is now associated with several risks for male fertility, miscarriages, future child health and psychological development. Clinicians, scientists, social science researchers and ethicists have pointed several ethical issues raised by APA. For the moment, the scope of this discussion has not been fully assessed in a systematic way.

Study design, size, duration: To support a comprehensive evaluation of APA, we propose a mapping of the ethical issues of APA.

Participants/materials, setting, methods: First, we conducted a systematic literature review to identify ethical issues pointed by researchers and clinicians. Second, this first list of issues was discussed by a panel of experts (N = 23) through a web survey. The results from both data collections were triangulated by qualitative analysis into an integrated list of issues.

Main results and the role of chance: Six groups of issues emerged from data analysis. The first group relates to issues associated with the family project. In other words, how to balance the risks of APA with the benefits of the family project. The second group looks at the issues for healthcare professionals, i.e., how to support the family project while respecting the professional duty to protect their patients' wellbeing. The third group refers to the gender justice issues and how APA generates inequities between gendered reproductive roles. The fourth group covers the public health dimension of APA and the justification to create intervention to respond to a collective and potentially intergenerational phenomenon. The fifth group is concerned about ways to develop sound and fair policies to respond to the challenges of APA. The sixth group encompasses research ethics issues on how to respectfully develop the field of research on APA.

Limitations, reasons for caution: APA is a burgeoning field of research, and it faces two principal limitations: (1) there is no clear understanding of the molecular mechanism of APA effect, and (2) data on the psychosocial dimension of APA is scarce. For these reasons, the ethical reflection is still speculative.

Wider implications of the findings: These findings offer a roadmap for future research on the ethics of APA. They provide a comprehensive understanding of issues that can be discussed during preconception counselling. At last, they suggest indications for the development of public health interventions or policies dedicated to address the most challenging aspects of APA.

Trial registration number: not applicable

Abstract citation ID: dead093.742

P-386 The impact of cross-border surrogacy practices on the welfare of children

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Study question: What are the clinical characteristics and medical outcomes of cross-border surrogacy (CBS) arrangements?

Summary answer: CBS practices commonly involve anonymously donated oocytes and multiple embryo transfers which can adversely impact the psychological and physical welfare of the children born.

What is known already: Intended parents may seek CBS when surrogacy in their home country is prohibited or when access is restricted to heterosexual couples, or when they cannot find a surrogate in their home country. Standards of clinical care can differ between the parents' home country and the CBS destination. Surrogacy is generally unregulated in CBS destinations, making it difficult to monitor clinical practices and the outcomes for the children born.

In Australia, where this study is set, anonymous gamete donation and multiple embryo transfers for patients engaged in surrogacy is prohibited. The rate of twin surrogacy deliveries is 2.2%.

Study design, size, duration: This cross-sectional study collected data through an online, anonymous survey open between April and November 2021.

Participants/materials, setting, methods: A survey with predominantly fixed-choice questions was developed and informed by the authors' prior research, the literature, and one author's experience of surrogacy and surrogacy advocacy. Questions were refined through an iterative process involving consultation with parents through surrogacy. Australian parents through surrogacy were eligible to participate and the study was advertised to personal contacts, members of a surrogacy non-profit organisation and members of a surrogacy related Facebook group. Data were analysed descriptively.

Main results and the role of chance: One hundred and eight Australian parents through CBS completed the survey. Surrogacy was undertaken in twelve destinations, with approximately half of respondents completing surrogacy in the United States of America (34%, n = 37) or Canada (17%, n = 18).

Almost all respondents reported the pregnancy was a result of an embryo transfer (92%, n = 98) and not artificial insemination (8%, n = 10). Of those reporting embryo transfer, 41% reported the transfer of multiple embryos (n = 40) and 79% reported the use of donor oocytes (n = 77). Of the respondents that used donor oocytes, almost half were from an anonymous donor (47%, n = 36) and all but one noted their intent to disclose the use of donor oocytes to their child (97%, n = 76).

Pregnancy or birth complications were reported by 29% of respondents (n = 31). There were 12 twin births (11%), 22 preterm births (20%) and 24 births requiring neonatal intensive care (22%). The median time spent in the neonatal intensive care unit was 6 days (range 1-60). All but one twin birth arose from pregnancies resulting from a multiple embryo transfer and the majority were preterm (75%, n = 9) and required neonatal intensive care (59%, n = 7).

Limitations, reasons for caution: It is not known if those who completed the survey are representative of all parents through CBS. However, the respondents' sociodemographic characteristics and motivations for surrogacy were similar to those in previously conducted studies, both within Australia and internationally.

Wider implications of the findings: The welfare of children born through surrogacy can be protected by addressing the barriers to undertaking surrogacy domestically and thereby reducing the number of people crossing borders, and by promoting identity release or known donation and single embryo transfer as best practice in surrogacy internationally.

Trial registration number: Not applicable

Abstract citation ID: dead093.743

P-389 "Time is the nurse and breeder of all good": storage periods for embryos and gametes across Europe

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Study question: What are the justifications, advantages and disadvantages of contrasting storage regimes for embryos and gametes across Europe?

Summary answer: Permitted storage periods vary significantly between countries, often lack justification for the limitations they impose, and may compromise individual rights.

What is known already: Storage periods for gametes and embryos vary significantly across Europe, as does the margin of discretion to grant extensions and adjustments to such periods. This fragmented regulatory landscape adds significant complexity to how cryobanks, clinics and patients are able to plan future treatment options. In addition, it creates an effect which may negatively impact on stakeholders' ability to freely choose *where* in Europe services are obtained, as well as *when*: when treatment is delayed because of personal health issues, the clock may not stop ticking on the maximum storage period.

Study design, size, duration: We have analysed and reviewed the regulation of storage periods in a number of European countries, and have put a particular focus on states which have strongly diverging periods. This assessment examined the policy underpinning the relevant rules, and the consequences of the regime for stakeholders. Our review considered both the legal and ethical aspects of each system and its consequences.

Participants/materials, setting, methods: Information about storage periods was obtained from legislation, policy documents and publications from national regulators. We also looked at case-law regarding storage issues in certain states.

Main results and the role of chance: Storage periods for gametes and embryos vary significantly across Europe, as does the margin of discretion to grant extensions and adjustments to such periods. This fragmented regulatory landscape adds significant complexity to how cryobanks, clinics and patients are able to plan future treatment options. In addition, it creates an effect which may negatively impact on stakeholders' ability to freely choose *where* in Europe services are obtained, as well as *when*: when treatment is delayed because of personal health issues, such as concurrent cancer treatment, the clock may not stop ticking on the maximum storage period.

A regulatory field which so clearly impacts EU Freedom of movement rights, as well as human rights contained both within EU law and the Council of Europe's Convention on Human Rights, should be capable of justification based on robust scientific and clinical evidence. This appears to be inconsistent with the variation we have identified in storage periods across the jurisdictions we have considered.

Limitations, reasons for caution: The assessment of domestic legal approaches requires an appropriate understanding of the language, as well as of the legal system. Whilst an informative assessment is possible based on policy overviews, a robust assessment of jurisdictions where the official language is not English or German is outwith the paper's scope.

Wider implications of the findings: The paper's conclusion is that the fragmentation of legal requirements in relation to the storage of embryos and gametes is neither ethically sound nor appropriately legally justified. It impacts negatively on individuals' rights and on commercial interests. More harmonisation is desirable in this field.

Trial registration number: Not applicable

POSTER VIEWING
FERTILITY PRESERVATION

Abstract citation ID: dead093.744
P-392 Controlled ovarian hyperstimulation applied before unilateral oophorectomy improves fertility preservation in cancer patients

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Study question: Can ovarian tissue cryopreservation (OTC) be performed after controlled ovarian hyperstimulation (COH)?

Summary answer: Unilateral oophorectomy after transvaginal oocyte retrieval is feasible on stimulated ovaries during one surgical step.

What is known already: In the fertility preservation (FP) field, the timeframe between patient referral and start of curative treatment is limited. Combining oocyte pick-up with ovarian tissue (OT) extraction has been reported to improve FP but COH applied before OT extraction is not currently recommended.

Study design, size, duration: This retrospective cohort-controlled study involved 58 patients who underwent oocyte cryopreservation immediately followed by OTC between September 2009 and November 2021. The exclusion criteria were a delay between oocyte retrieval and OTC of greater than 24 hours (n = 5) and *in vitro* maturation (IVM) of oocytes obtained *ex-vivo* in the ovarian cortex (n = 2). This FP strategy was performed either after COH (stimulated group, n = 18) or after IVM (unstimulated group, n = 33).

Participants/materials, setting, methods: Oocyte retrieval followed by OT extraction on the same day was performed either without previous stimulation or after COH. Adverse effects of surgery and ovarian stimulation, mature oocyte yield and pathology findings of fresh OT were retrospectively analysed. Thawed OTs were analysed prospectively, for vascularisation and apoptosis using immunohistochemistry, when patient consent was obtained.

Main results and the role of chance: No surgical complication occurred after OTC surgery in either group. In particular, no severe bleeding was associated with COH. The number of mature oocytes obtained increased after COH (8.5 [5.3–12.0] MII oocytes) compared to the unstimulated group (2.0 [1.0–5.3] MII oocytes, $p < 0.001$). Neither ovarian follicle density nor cell integrity was affected by COH. Fresh OT analysis showed congestion in half of the stimulated OT which was higher than in the unstimulated OT (3.1%, $p < 0.001$). COH also increased haemorrhagic suffusion (COH+OTC: 66.7%; IVM+OTC: 18.8%, p=0.002) and oedema (COH+OTC: 55.6%; IVM+OTC: 9.4%, $p < 0.001$). After thawing, the pathological findings were similar between both groups. No statistical difference in the number of blood vessels was observed between the groups. The oocyte apoptotic rate in thawed OT was not statistically different between the groups (ratio of positive cleaved caspase-3 staining oocytes/total number of oocytes equal to median 0.50 [0.33-0.85] and 0.45 [0.23-0.58] in unstimulated and stimulated groups respectively, $p = 0.720$).

Limitations, reasons for caution: The study reports FP from a small number of women following OTC. Follicle density and other pathology findings are an estimate only.

Wider implications of the findings: This approach could be proposed to post pubertal patients when the number of mature oocytes expected is low or when the risk of residual pathology is high. The reduction of surgical steps for cancer patients also has positive implications for introducing this approach into clinical practice.

Trial registration number: not applicable

Abstract citation ID: dead093.745
P-393 The mitochondrial targeted antioxidant Mitoquinol increases survival of isolated human preantral follicles in vitro

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Study question: Does Mitoquinol have a protective effect on survival and mitochondrial oxidative stress in cultured human preantral follicles?

Summary answer: Mitoquinol significantly increased survival of human preantral follicles cultured for 8 days and significantly decreased expression of genes related to the mitochondrial oxidative stress response.

What is known already: Culturing human ovarian follicles is a promising new source of mature oocytes for fertility preservation, as well as a key model system to investigate fundamental biology. Nevertheless, only a handful of studies have yielded mature human oocytes from preantral stage follicles and optimizing culture systems is challenging due to the scarcity of human material. Mitoquinol is an antioxidant that contains a form of Coenzyme Q10 called ubiquinone that has been modified to selectively target and protect mitochondria from oxidative damage. In human oocytes, the presence of Mitoquinol in the IVM media increased nuclear maturation and protected against chromosomal misalignment.

Study design, size, duration: Human preantral follicles (n = 213; mean diameter: 71 µm; range: 26-189 µm) were isolated from ovarian medulla tissue. Preantral follicles were mechanically and enzymatically isolated, encapsulated in 0.5% alginate and cultured for 8 days in three experimental groups: Control (n = 68); 50nM Mitoquinol (n = 71), 250nM Mitoquinol (n = 74). The primary endpoints were follicular growth and survival. Secondary endpoints included follicular gene expression and hormone analysis of the spent culture media.

Participants/materials, setting, methods: Ovarian medulla tissue was donated by 5 patients (aged 30-37 years) undergoing unilateral oophorectomy for ovarian tissue cryopreservation. Follicular growth and survival were assessed every second day during culture by microscopy. For follicles bigger than 95 µm, AMH and Estradiol concentrations were measured by ELISA in the spent media on day 8. Finally surviving follicles were snap-frozen and gene expression was analysed by qPCR; mitochondrial oxidative stress genes: *HSP-60*, *TFAM* and apoptosis genes: *BAX/BCL-2*.

Main results and the role of chance: After 8 days in culture, the follicular survival rate in the 50nM Mitoquinol group (68%; n = 51/74) was significantly higher than the control group (49%; n = 32/68; p = 0.0344) and the 250nM Mitoquinol (54%; n = 39/71; p = 0.0443). The protective effect of 50nM Mitoquinol was more pronounced in the subset of small follicles (Day0 < 70 µm, n = 135) in which survival rate of the 50nM Mitoquinol group (65%; n = 31/47) was significantly higher than the control (41%; n = 18/43; p = 0.005) and the 250nM group (44%; n = 20/4; p = 0.019). The average growth of surviving follicles was similar in the three experimental groups (Control: 40.7 ± 17.4 µm; Mitoquinol 50nM: 36.9 ± 19 µm; Mitoquinol 250nM: 36.4 ± 16.5 µm). A significant positive association between the diameter of the follicle and the concentration of AMH and Estradiol was observed (AMH: p = 0.0215, R = 0.43 and Estradiol: p = 0.0413, R = 0.38) but no differences were found between the experimental groups. The relative expression of genes involved in mitochondrial oxidative stress response were significantly lower in the 50nM Mitoquinol group compared to the 250mM

Mitoquinol group (*HSP-60* p = 0.018; *TFAM* p = 0.013) and similar to the control group (*HSP-60* p = 0.056; *TFAM* p = 0.071). Moreover, the gene expression of the ratio *BAX/BCL2* was similar in the three groups.

Limitations, reasons for caution: A larger sample size is required to confirm the statistical analysis and to extrapolate the results to primordial, primary, and secondary stage follicles. Furthermore, long-term culture studies are required to determine the impact of Mitoquinol on antral follicle development and oocyte maturation

Wider implications of the findings: Our findings show that Mitoquinol can be used as an antioxidant in the culture of human preantral follicles since it increased follicle survival and reduced gene expression of genes related to mitochondrial oxidative stress response.

Trial registration number: Not appliclable

Abstract citation ID: dead093.746

P-395 Protective effect of metformin on the ovarian reserve of prepubertal mice under cyclophosphamide chemotherapy

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Study question: Can metformin reduce the side effects of cyclophosphamide on the ovaries of prepubertal mice treated with cyclophosphamide?

Summary answer: Yes, metformin, as an inhibitor of mTOR and, by affecting the HIPPO pathway, can exert its protective effect in the chemotherapy mice model.

What is known already: PI3K/AKT and HIPPO pathways are influential pathways in the process of activation of primordial follicles and proliferation of cell growth, which are affected by various agents and drugs such as cyclophosphamide. One of the side effects of cyclophosphamide is the reduction of the ovarian reserve through abnormal changes in the PI3K/AKT and HIPPO pathways. Metformin is a drug that is used to treat type 2 diabetes and at the same time regulates PI3K/AKT and HIPPO pathways and prohibits the proliferation of cancer cells.

Study design, size, duration: Controlled experimental study.

After metformin and cyclophosphamide dose adjustment, 12 female NMRI 14-day-old mice were randomly divided into four groups: the control group (CONT), metformin group (MET), cyclophosphamide group (CYC) and metformin with cyclophosphamide group (MET-CYC).

Participants/materials, setting, methods: Mice were treated by intraperitoneal injection of a dose of 150mg/kg of metformin for 11 consecutive days, and/or a dose of 65mg/kg of cyclophosphamide every three days (The third, sixth and ninth days). The mice were sacrificed 24 hours after the last metformin injection or 3 days after the last cyclophosphamide injection. The ovaries were collected for stereological evaluation, and expression of *Pten*, *Mtor*, *Yap-1*, *p53*, *Bcl-2*, and *Bax* genes by qRT-PCR method.

Main results and the role of chance: The stereological evaluation showed that the ovarian volume and primordial follicle number in the CYC group were decreased compared to the other groups ($P < 0.05$), and also in the MET-CYC group reduced compared to the CONT and MET groups, but increased significantly compared to the CYC group ($P < 0.05$). A significantly increased number of growing follicles was observed in the CYC group compared to the other groups ($P < 0.05$). The molecular analysis showed that the *Pten* expression in the CYC group was lower than CONT and MET groups ($P < 0.05$). *Mtor* expression in the CYC group was significantly increased compared to other groups ($P < 0.05$). The expression of *Yap-1* was significantly decreased in the MET group compared to other groups ($P < 0.05$), and was lower in the MET-CYC than in the CYC group ($P < 0.05$). The of *P53* expression in the MET-CYC group was higher than CONT and MET groups ($P < 0.05$), and was increased in the CYC compared to the CONT group ($P < 0.05$). *Bcl-2* expression was higher in CONT and MET groups than in MET-CYC and CYC groups ($P < 0.05$). The expression of *Bax* in the CYC group was significantly increased compared to CONT and MET groups ($P < 0.05$).

Limitations, reasons for caution: Due to ethical issues in less use of mice, dose of metformin was obtained according to the articles used in adult mice and also by using the human to animal dose conversion formula.

Wider implications of the findings: It can be concluded that metformin during cyclophosphamide treatment can maintains ovarian volume, reserve, and activity in prepubertal mice by regulating PI3K/AKT and HIPPO pathways. Considering the relatively acceptable cost and long-term safety of metformin, it is a promising factor to be used to preserve fertility during chemotherapy.

Trial registration number: not applicable

Abstract citation ID: dead093.747

P-396 Modified Flare/GnRH antagonist protocol versus GnRH antagonist protocol in oocyte cryopreservation cycles

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Study question: Is there an advantage for modified flare/ GnRH antagonist protocol in comparison to antagonist protocol in oocyte cryopreservation cycles?

Summary answer: Modified flare/ GnRH antagonist protocol is at least as good as antagonist protocol in oocyte cryopreservation cycles, potentially improving maturity rate in subgroup of patients.

What is known already: Ultrashort flare GnRH agonist (GnRHa)/GnRH - antagonist protocol offer benefits of stimulatory effect of repeated microdose flare on endogenous FSH and the advantages of GnRH antagonist suppression, including the option to use GnRHa ovulation trigger to avoid ovarian hyperstimulation syndrome. GnRHa ovulation trigger has been associated with suboptimal response that refers to lesser than expected oocyte yield. Our research group previously demonstrated the value of GnRHa follicular challenge test (FACT) in prediction of suboptimal response assessed by post ovulation trigger LH levels and as a dynamic ovarian reserve test (not published yet).

Study design, size, duration: A retrospective, age- matched, cohort study that included all non-medical oocyte cryopreservation cycles from October 2020 to December 2021. The study group included 110 women that received modified flare/ GnRH antagonist protocol and were matched with 110 women that received antagonist protocol.

Participants/materials, setting, methods: Women in the modified flare/ GnRH antagonist protocol were administered GnRHa (Decapeptyl 0.2 mg) on day 2 to menstrual cycle followed by flexible antagonist protocol. They were compared to age matched women that received flexible antagonist protocol. In both study groups GnRHa final ovulation trigger was administered.

Main results and the role of chance: Among 110 women that underwent non-medical fertility preservation with modified Flare/GnRH antagonist protocol, in comparison to 110 women that received antagonist protocol, a reduced dosage of gonadotropins was consumed with more oocytes retrieved (14.63 vs. 13.86) and more mature oocytes cryopreserved (11.32 vs. 10.14), but differences were not significantly different. In a subgroup with more than 6 oocytes retrieved significantly more mature oocytes were cryopreserved in women that received modified flare/antagonist protocol (14.03 ± 0.90 vs. 11.63 ± 0.71, $p = 0.03$) in comparison to women that received antagonist protocol.

Limitations, reasons for caution: Limitations of our study are its retrospective nature and lack of information on the oocyte's fertilization competence and pregnancy outcomes.

Wider implications of the findings: Future studies will further explore the advantages of this protocol in terms of higher oocytes maturity rate, intra

cycle prediction of ovarian response and suboptimal response to GnRHa triggering.

Trial registration number: not applicable

Abstract citation ID: dead093.748

P-397 The effect of letrozole overlapped with gonadotropin on IVF outcomes in women with DOR or aged over 40 years old

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Study question: Evaluating the efficacy of modified letrozole protocol (mLP) for diminished ovarian reserve (DOR) or advanced age women.

Summary answer: The modified letrozole protocol provides another option for women with DOR or advanced age, especially those who experienced ovarian stimulation previously.

What is known already: For years, numerous methods have been conducted to obtain better outcomes in women with advanced age or DOR. Increasing doses of gonadotropin (Gn), exogenous luteinizing hormone (LH) or growth factor (GH) supplementation, and various kinds of controlled ovarian stimulation (COS) protocols were reported. However, there is still no consensus due to the contradictory reports of comparable reproductive outcomes. During ovarian stimulation, estrogen and androgen are important in the recruitment of primordial follicles and promotion of follicular growth at the preantral and antral stages across different species.

Study design, size, duration: This is a paired-match study including 243 women with DOR and 249 women aged over 40 years old who received *in vitro* fertilization (IVF) treatment.

Participants/materials, setting, methods: 123 women received stimulation with mLP. GnRH agonist (GnRH-a) long, GnRH antagonist (GnRH-anta), and mild stimulation protocol were used as controls with 123 women in each group. Clinical pregnancy rate (CPR) and cumulative clinical pregnancy rate (CCPR) were main outcomes.

Main results and the role of chance: CPR in the mLP group (38.46%) was significantly higher than mild stimulation (17.11%), but not significantly different from GnRH-a long (26.13%) and GnRH-anta (29.17%) group. CCPR showed an increasing trend in the mLP group (33.33%) although without significance when compared with controls. The CCRP of GnRH-a long, GnRH-anta, mild stimulation group were 21.68%, 29.03%, and 13.01%, respectively. In women with repeated cycles, mLP achieved higher available embryo rate and top-quality embryo rate. Further study showed positive correlation between testosterone and the number of oocytes retrieved in mLP group ($r = 0.395$, $P < 0.01$).

Limitations, reasons for caution: It is a retrospective study which resulted in minor bias among mLP and the other control protocols. Although we have already paired for age and AMH, the presence of biases cannot be totally excluded. Further prospective studies need to be done to validate the effect of mLP.

Wider implications of the findings: The modified letrozole protocol may be effective for women with DOR or advanced age, especially those who have experienced previous cycle failure. An increasing serum testosterone level may play a role and reflect follicular growth during ovarian stimulation.

Trial registration number: not applicable

Abstract citation ID: dead093.749

P-398 Elective Oocyte Cryopreservation – a viable alternative as a means of fertility preservation

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Study question: What are the trends in women accessing elective oocyte cryopreservation (EOC) across a multi-site clinic in Sydney, Australia, thaw rates, utilisation and live birth rates

Summary answer: Over 9 years, there was a significant increase in women having EOC. 2.7% returned to use. Oocyte thaw survival rates were 89.5% with LBR 35.5%.

What is known already: Data from Australia/New Zealand and USA demonstrated a 3 to 8-fold increase in EOC cycles, with a reduction in mean age of women accessing treatment. <5% of women returned to use their frozen gametes and live birth rates were better in women aged < 35 years. HFEA data demonstrated the use of frozen oocytes did not appear to increase risk of adverse perinatal outcomes. Research has shown that almost half of the women who returned to use their frozen oocytes used donor sperm with an overall LBR of 20.9%. 91% of women had no regrets over their decision for EOC.

Study design, size, duration: This was a retrospective cohort study performed at a multi-site large private fertility clinic in Sydney, Australia. This clinic was the first in Sydney to offer EOC to women. All women accessing EOC from 2014 – September 2022 were included. Ethics approval was obtained.

Participants/materials, setting, methods: Data was collected from women undergoing EOC; including demographic information, controlled ovarian stimulation parameters, return to use, oocyte thaw survival rates, source of sperm used for fertilization, embryo biopsy rates, aneuploidy rates, as well as pregnancy rates.

Main results and the role of chance: There was a 17.5-fold increase in cycles of EOC over the 8-year study period; with a reduction in average age from 38.13 to 35.85 years. Overall the mean age was 36.6 of the 2278 women who underwent EOC, of which 30% were aged >38 years. The mean oocyte yield was 8.85.

Of the 62 (2.7%) women who returned to use their frozen oocytes, 54.8% were < 38 years of age. Oocyte thaw survival rates were 89.5%. The majority of women who returned to use their frozen oocytes, used donor sperm for conception. Fertilization rates with intra-cytoplasmic sperm injection (ICSI) was 65.3%; with 8.3% of oocytes degenerating post ICSI. 41.6% of embryos developed into blastocysts with 27.8% utilization rate (good quality blastocyst formation rate).

Overall LBR was 35.5% for all women. Clinical pregnancy rates (CPR) from warmed oocytes were 46.2% from women aged <38 years old and 22.2% from women >38 years old. Of women who returned for a frozen embryo transfer, LBR was 25%; all occurring in women who froze their oocytes prior to age 38.

Limitations, reasons for caution: In parallel to international studies, very few women returned to use their oocytes; results need to be interpreted with care when counselling women considering EOC. Lifestyle factors were unable to be accounted for. Most women have opted to continue storing and not dispose/donate their oocytes at the time of publication.

Wider implications of the findings: EOC proves to be a viable and useful option for fertility preservation. Due to the small proportion of women who return to use their oocytes; and paucity of data; it would be prudent to improve standardisation of data collection and outcome measure reporting.

Trial registration number: Not Applicable

Abstract citation ID: dead093.750

P-399 Oocyte vitrification does not impair clinical outcomes in women of advanced maternal age. An analysis of 1268 cycles using fresh and vitrified oocytes

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Study question: Does oocyte vitrification impair clinical outcomes in women of advanced maternal age (AMA, >35 years old)?

Summary answer: Up to the age of 39 years, clinical outcomes were comparable amongst cycles using fresh and vitrified oocytes.

What is known already: Primarily recommended for young women, oocyte vitrification is the most efficient method for elective fertility preservation. Nevertheless, various social and economic circumstances often compel women to undergo fertility preservation when already at an AMA. Recent studies in mouse suggest that AMA increases the vulnerability of oocytes to molecular and subcellular damage during vitrification, ultimately compromising embryo viability and implantation potential. However, the effects of oocyte vitrification on human preimplantation development and clinical outcomes in women of AMA currently remain unknown.

Study design, size, duration: This is a retrospective cohort single-center study. We compared outcomes of 1268 patients undergoing their first fresh ICSI cycle using autologous fresh (n = 1087) or vitrified oocytes (n = 181), performed between January 2019 and October 2022. All vitrified oocytes were obtained following elective fertility preservation for age-related fertility decline. Outcomes were stratified according to maternal age at oocyte pick-up: control group (≤35) and AMA groups (36-37, 38-39 and ≥40 years).

Participants/materials, setting, methods: We compared outcomes between fresh and vitrified oocytes across matching maternal age groups. These included fertilization rates, proportion of viable embryos on day 3, clinical pregnancy and live birth rates. Univariate and multivariate analyses (2-contrast linear and logistic regression) were used to establish correlations. Analyses were adjusting for sperm origin (partner or donor), day of transfer (3 or 5), number of embryos transferred and endometrial age. All p values <0.05 were considered significant.

Main results and the role of chance: Sperm donation and homologous cycles were equally distributed among the study groups (45% sperm donor and 55% male partner across the entire cohort). In our cohort, blastocyst transfer was performed less frequently in older women, 23.8% for women ≥40 years compared to 45.6% for women ≤35 years ($p < 0.0001$). When comparing oocyte status across matching maternal age groups, fertilization rates were ~10% lower when vitrified oocytes were used ($p < 0.05$). Our adjusted analysis confirmed this negative impact of oocyte vitrification on fertilization rates. Oocyte vitrification also affected ongoing cleavage, as the proportion of viable embryos on day 3 was significantly lower across all groups when vitrified oocytes were used ($p < 0.05$). Nevertheless, this effect did not translate to clinical pregnancy rates, which were comparable across all maternal age groups regardless of oocyte status. As expected, live birth rate decreased steadily with advancing maternal age (from 38.5% in women ≤35 to 15.0% in women ≥40). While our adjusted analysis showed a negative effect of oocyte vitrification on live birth rates for patients ≥40 (OR: 0.60, 95%CI: 0.18-2.06), we observed no such effect in AMA patients ≤39 years.

Limitations, reasons for caution: The main limitation of this study is its retrospective design. The low number of women ≥40 undergoing fertility presentation (n = 39) warrants careful interpretation. Our results cannot be generalized to all patient populations and indications for fertility preservation.

Wider implications of the findings: Clinical outcomes were comparable between fresh and vitrified oocytes up to the age of 39 years. While oocyte vitrification before 35 years old remains the most established and effective method for preventing age-related fertility decline, our findings offer a valuable clinical resource for counselling AMA patients considering elective fertility preservation.

Trial registration number: Not applicable

Abstract citation ID: dead093.751

P-400 Fertility preservation in male patients with cancer: a systematic review and meta-analysis

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Study question: Whether the fertility preservation can benefit male patients with cancer.

Summary answer: Sperm cryopreservation is an effective fertility preservation method and may benefit patients with cancer.

What is known already: Sperm cryopreservation is the only way to efficiently preserve male fertility. It is an important procedure in assisted reproductive technologies (ART). Recently, due to remarkable advances in cancer treatment, an increasing number of studies have reported the outcomes of sperm cryopreservation in patients with cancer.

Study design, size, duration: Systematic review and meta-analysis to summarize the current evidence on sperm cryopreservation and reproductive outcomes in male patients with cancer. 69 non-randomized studies were included in the meta-analysis. These included 32,234 patients referred for sperm analysis and 23,178 whose sperm was cryopreserved at least once. CENTRAL, CNKI, Cochrane Systematic Reviews, EMBASE, MEDLINE, PUBMED, and Web of Science were searched from inception through December 31, 2021.

Participants/materials, setting, methods: All studies reporting on offering or attempting to cryopreserve sperm before or during cancer treatment in male patients considered at risk of treatment-related fertility impairment were included. The pooled failed-to-cryopreserve, sperm disposal and sperm use rate in male patients with cancer. Meanwhile, the pooled pregnancy, miscarriage and delivered rates after sperm cryopreservation in male patients with cancer.

Main results and the role of chance: 69 non-randomized studies were included with 32,234 patients referred for sperm analysis and 23,178 patients cryopreserving at least one sperm sample. The pooled failed-to-cryopreserve rate was 10% (95% confidence interval [CI], 8–12%), and the sperm disposal and sperm use rate were 23% (95% CI, 16–30%) and 9% (95% CI, 8–10%). The pregnancy, miscarriage, and delivery rates were 28% (95% CI, 22–33%), 13% (95% CI, 10–17%), and 20% (95% CI, 15–25%), respectively. Subgroup analysis showed higher pregnancy and delivery rates and a lower failed-to-cryopreserve rate in recent studies than in older ones. Clinical pregnancy rates per cycle of 34% (27–41%), 24% (14–35%), and 9% (5–15%) and delivery rates per cycle of 23% (17–30%), 18% (11–26%), and 5% (1–9%) for ICSI, IVF, and IUI, respectively.

Limitations, reasons for caution: The use of average results obtained in each included study without the patient-level data might represent the first source of bias. Furthermore, we did not analyze data on congenital abnormalities based on pregnancy outcomes because congenital abnormalities were rarely reported.

Wider implications of the findings: Our study supported previous reports that sperm cryopreservation was an effective method of fertility preservation in male patients with cancer. Meanwhile, frozen sperm use rate in our review underestimated the actual rate, making it meaningful to actively recommend fertility preservation to patients with cancer.

Trial registration number: not applicable

Abstract citation ID: dead093.752

P-401 Is cancer type associated with semen quality and infertility among men who desire fertility preservation?

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Study question: To investigate the association between cancer-types and semen-parameters before gonadotoxic-treatments, and to evaluate which patients have the highest risk of diminished spermatogenesis prior to therapy.

Summary answer: sperm quality among cancer patients before gonadotoxic treatment can differ according to the type of cancer.

What is known already: Many studies have reported impaired sperm quality at cancer diagnosis before any given gonadotoxic regiments. Except for testicular cancer and Hodgkin's Lymphoma, baseline data on semen quality in case of different types of malignancies among male cancer patients are limited or based on low sample size

Study design, size, duration: A retrospective population-based cohort analysis was performed, 237 oncological male patients who were referred for fertility preservation between 1995-2020 within 90 days of cancer diagnosis and prior to cancer treatment

Participants/materials, setting, methods: The study population included 237oncological male patients who were referred to the Sperm Bank, Fertility, and IVF unit for the purpose of fertility preservation. Sperm analysis was performed within 90 days of cancer diagnosis and prior to therapy. The pre-treatment sperm analyses were based on the World Health Organization (WHO) guidelines

Main results and the role of chance: Histological examination of malignant diseases demonstrated 20 subtypes of cancers, the major groups included 49 patients with testicular cancer (TC), 72 patients with Hodgkin lymphoma (HD), 35 patients with other lymphoma, 29 patients with other hematological cancer, 16 with sarcoma and 36 with other solid malignant tumors. Our results show significant difference in sperm concentration between the different cancer groups (P value 0.007), the lowest median concentration of spermatozoa was found in the testicular cancer and sarcoma cancer patients 10 million per ml compared to the other cancer groups (other hematologic, Hodgkin lymphoma, other lymphoma and other solid tumors). Oligospermia was observed in 59% of testicular cancer patients and 50% of the sarcoma patients. Fertility outcomes were analyzed as a secondary objective. We found that only 37 patients' partners conceived and delivered offspring. Only 16.2% of those patients used their banked sperm and conceived via IVF-ICSI. We found that 20% of testicular cancer patients and other lymphoma patients including NHL conceived spontaneously after cancer diagnosis and treatment.

Limitations, reasons for caution: This is a retrospective cohort study and the oncology patients sample size is relatively small.

Wider implications of the findings: Our study emphasizes that sperm quality among cancer-patients before gonadotoxic-treatment can differ according to the cancer type. Significant reduction in sperm quality in testicular cancer patients was demonstrated, with a significant association was found between sperm concentration and various cancer-type. Further studies are warranted to confirm our results.

Trial registration number: not applicable

Abstract citation ID: dead093.753

P-402 What is the efficacy of planned oocyte cryopreservation? A systematic review and meta-regression analysis

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Study question: What are the chances of achieving a live birth after planned oocyte cryopreservation (POC)?

Summary answer: The mean thawing rate is 11.9% (± 4.9). Among women who thawed their oocytes, the mean live birth per patient is 28.1% (± 13.0).

What is known already: Trends of delayed childbearing have become increasingly common. The age-related decline in fertility potential increased the popularity of planned oocyte cryopreservation. However, Data on the outcomes of planned oocyte cryopreservation including a return for a thaw, pregnancy, and live-birth rates are scarce, being based mostly on small case series.

Study design, size, duration: A systematic review and meta-regression followed the PRISMA and MOOSE guidelines. A systematic search was conducted in Medline, Embase, and the Cochrane Library. The search strategies incorporated index terms (Mesh) and free text words for the search concepts. The first domain contained terms on indication the second domain related to oocytes cryopreservation. The detailed protocol is documented online in the International Prospective Register of Systematic Reviews registry.

Participants/materials, setting, methods: POC was defined as cryopreservation for future age-related fertility loss exclusively. All studies that reported primary data on POC were considered eligible for screening. Observational and non-observational studies were included. The primary outcome was the live birth rate per woman. The secondary outcomes included the thawing rate and other laboratory outcomes. Meta-regression analyses regarding the association between oocyte survival after thawing and live birth and age above 40 or method of freezing were conducted.

Main results and the role of chance: A systematic search from inception to October 2022 yielded 3847 citation**s.** After the selection process, 9 studies, conducted from 1999 to 2020, were included. In total, 8059 women underwent POC, and 1463 returned to use their oocytes. The mean thawing rate was 11.9% (± 4.9). The mean age at thawing was 41.9 years (± 0.8) with a mean time from freezing to thawing of 4.0 years (± - 0.4). 68.3% (± 18.4) of patients returned with a partner while the others used donor sperm. Five studies used vitrification exclusively while the others included both slow-freezing and vitrification. The oocytes survival rate after thawing was 79.0% (± 5.4) and the mean live birth per patient was 28.1% (± 13.0). A meta-regression analysis revealed an association between age above 40 years and decreased live birth (R^2=0.08) and oocyte survival (R^2=0.16). No statistically significant association was found between the method of cryopreservation to oocyte survival rate or live-birth

Limitations, reasons for caution: Variations in expertise and technical experience between different centers may yield different results. In addition, most data reports outcomes for women who underwent POC aged 35-40, while information on women who had this procedure at younger or older ages is scarce.

Wider implications of the findings: Data presented here may be valuable for consultation and informed decision-making of women considering POC

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Abstract citation ID: dead093.754

P-403 Oocyte usage and disposition outcomes following oocyte cryopreservation

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Study question: What is the fate of cryopreserved oocytes?

Summary answer: Very few people return to use their cryopreserved oocytes in assisted reproduction and while some surplus oocytes are donated for reproduction, the majority are discarded.

What is known already: The number of oocyte cryopreservation cycles performed around the world dramatically outpaces the number of people who return to use their oocytes in assisted reproduction. This imbalance is leading to the rapid accumulation of oocytes in cryostorage. Previous studies have reported that many people intend to donate their surplus cryopreserved oocytes, and some commentators suggest that this may help relieve donor shortages. However, there have been no reports to date about what *actually* happens to surplus oocytes following oocyte cryopreservation.

Study design, size, duration: A retrospective cohort study was performed, analysing the outcomes for cryopreserved oocytes from 2012 - 2022.

Participants/materials, setting, methods: Data were collected from seven IVF clinics, located in Victoria, Australia. De-identified data were aggregated on the outcomes following oocyte cryopreservation, including duration of cryostorage, assisted reproduction involving thawed oocytes, and disposition outcomes of surplus oocytes.

Main results and the role of chance: Across the study period, the number of patients with oocytes in storage grew rapidly from 144 in 2012 to 2015 in 2022. Regarding the current status of oocytes in storage, in 2022, 73% of patients had had their oocytes in storage for <5 years; 25% for 5 - 10 years, and 2% for ≥10 years. The majority of oocyte thaw cycles (600/645) involved oocytes frozen for <5 years; of which 47% were frozen for <6 months. Overall, the live birth rate from the total number of oocyte thaw cycles initiated was 12% (78/645), however, this varied depending on the reason oocytes were frozen. In comparison to the number of patients still with oocytes in storage, very few patients relinquished surplus oocytes across the study period (2015 vs 151, respectively). Of the 128 patients whose surplus oocytes were discarded, 32% had had their oocytes stored for <5 years; 32% for 5-10 years, and 36% for >10 years. Among patients who elected to donate their surplus oocytes (n = 23), all but four patients made this decision having had their oocytes in storage for <5 years. Although a legal option in Australia, no oocytes were donated to research over the period analysed.

Limitations, reasons for caution: Data were collected from one fertility group based in one state of Australia, thus it may not be representative of wider trends seen across other clinics or states/territories. Further, specific state laws govern the management, storage, and use of surplus oocytes, which may influence the reported disposition outcomes.

Wider implications of the findings: Our findings suggest that cryopreserved oocytes are rarely used or donated, rather they are left in storage, often for long durations. To relieve some of the pressure this may create on cryostorage facilities, more attention is needed to better understand the experience of oocyte disposition and the barriers to donation.

Trial registration number: not applicable

Abstract citation ID: dead093.755

P-404 Sexual function, psychological distress, and remaining fertility in female cancer survivors

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Study question: How many women after fertility preservation and gonadotoxic treatment suffer from sexual dysfunction und psychological distress?

Summary answer: Sexual dysfunction was detected in 60.4% of 53 patients with no increased presence of psychological distress compared to the general population.

What is known already: Gonadotoxic treatment in female cancer patients can lead to reversible or permanent impaired fertility. The risk depends on the agent, number of cycles and age of the women. Different methods of fertility preservation are offered, including cryopreservation of (non-) fertilized oocytes, cryopreservation of ovarian tissue, and GnRH analogues. Improved survivorship leads to new challenging issues such as comorbidities resulting from the cancer treatment itself, secondary malignancy, and potential impairment of quality of life, sexual function, and fertility.

Study design, size, duration: This prospective single center study was conducted at the Department of Gynecological Endocrinology and Reproductive Medicine, Medical University of Innsbruck, Innsbruck, Austria between June 2021 and November 2021. The department holds the biggest cryobank of ovarian tissue in Austria, offering the procedure since 2007.

Participants/materials, setting, methods: In this prospective study, 202 female cancer survivors who underwent fertility preservation methods at time of cancer diagnosis between 2010 and 2020 were invited to a gynecological exam, laboratory assessment and two questionnaires (Female Sexual Function Index (FSFI) and Hospital anxiety and depression scale (HADS)) in 2022. FSFI and HADS scores were compared depending on Anti-Mullerian-hormone (AMH) levels, current desire to have a child, and age.

Main results and the role of chance: At time of cancer diagnosis, these patients underwent at least one of the following fertility preservation methods: cryopreservation of (non-) fertilized oocytes (n = 27 [48%]), cryopreservation of ovarian tissue (n = 38 [68%]), GnRH analogues (n = 53 [95%]). After a mean follow up time of 70 +/-50 months, sexual dysfunction was detected in 60.4% of the 53 patients. Normal results regarding HADS-D/Anxiety and HADS-D/Depression were found in 88.7% and 94.3% of patients, respectively. At time of follow up, 69.9% of patients regained regular menstrual cycles, 53% of patients <40 years showed a diminished ovarian reserve with AMH levels < 1.1ng/ml. and 15 patients (28.3 %) suffered from infertility. 14 (25%) women already fulfilled their desire to have children, whereas 16 (30%) women expressed the desire to have children at time of follow up. Out of 9 women, who conceived after gonadotoxic treatment, four used cryopreserved oocytes and one underwent ovarian tissue transplantation.

Limitations, reasons for caution: A possible limitation is the small sample size and varying follow up intervals.

Wider implications of the findings: Female cancer survivors are at highly increased risk for SD. Cancer patient should be informed about possible sexual dysfunction already at the start of cancer treatment and during follow up.

Trial registration number: 1471/2020

Abstract citation ID: dead093.756

P-405 Nuclear transfer overcomes poor embryo development of in vitro grown mouse oocytes

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Study question: Is the nuclear transfer technology able to overcome the compromised embryonic development of *in vitro* cultured secondary follicles from young B6D2 mice?

Summary answer: Nuclear transfer technology was able to restore embryonic development to levels similar of *in vivo* grown oocytes.

What is known already: *In vitro* follicle culture has been proposed as a strategy for fertility preservation in cancer patients. Studies in human follicle culture remain scarce, due to the low availability of human tissue. Mouse models have been extensively studied to improve follicle maturation and investigate the potential of *in vitro* grown (IVG) gametes. Despite significant improvements reported over the years, including increased maturation rates, advanced embryonic development and generation of fertile offspring, the quality of IVG oocytes remains inferior compared to their *in vivo* grown counterparts.

Study design, size, duration: The experimental study was conducted between October 2020 and January 2023, after approval by the Animal Ethics Committee of Ghent University Hospital (ECD no 19/60). In total, 108x 16-days-old B6D2 females were used for secondary follicle isolation and *in vitro* culture, 81x 8-12-week-old females for collection of *in vivo* grown oocytes, 4x 9-14-week-old B6D2 males for sperm freezing, 5x 10-24-week-old CD1 vasectomised males and 10x 8-12-weeks-old CD1 females as surrogate mothers for embryo transfer.

Participants/materials, setting, methods: Follicles isolated from 16-days-old B6D2 mice were cultured for 9 days, followed by maturation for 16-18hrs. Mature oocytes were assessed on diameter, spindle morphology, calcium releasing ability, mitochondrial membrane and embryonic developmental potential. *In vivo* grown oocytes from stimulated mice were used as controls and cytoplasmic donors for nuclear transfer. Spindle (ST) and Pronuclear transfer (PNT) were applied to overcome poor development of IVG embryos, by transferring the spindle/pronuclei of IVG oocytes/zygotes to enucleated controls.

Main results and the role of chance: In total, 1509 secondary follicles were cultured and 73.6% survived to Day 9. From the 719 cumulus oocyte complexes, 364 (50.6%) oocytes matured. Oocyte diameters were significantly smaller between IVG (67.4μm) and controls (73.1μm) ($p < 0.001$). Spindle staining revealed a similar number of normal spindles between the two groups (71.4% and 82.6% respectively, $p = 0.138$). Calcium release was significantly lower in IVG oocytes (1.6) vs controls (5.7) ($p < 0.001$), as well as mitochondrial membrane potential ((0.9) vs (2.2), $p < 0.001$). These data implicate a cytoplasmic inferiority in IVG oocytes. Following parthenogenetic activation, 2-cell (59.4%) and blastocyst rate (36.3%) in IVG group was significantly lower than control (89.4% and 88.2% respectively, $p < 0.001$). For this reason, we performed ST, using controls as cytoplasmic recipients for IVG spindles. The 2-cell rate significantly increased to 100% ($p < 0.001$) in ST oocytes, reaching 100% blastocyst formation. Next, to create bi-paternal embryos, we performed IVF. From the IVG group, 2-cell (40%) and blastocyst (0%) rates were significantly lower than control (94.4% and 83.3%, $p < 0.001$). Embryo development was restored following PNT (98% 2-cell and 86% blastocyst rate, $p < 0.001$). Genetic analysis of PNT embryos revealed that 5/6 were chromosomally normal, similar to controls (8/10). Embryo transfer of PNT and control embryos is ongoing.

Limitations, reasons for caution: Both ST and PNT are promising technologies to overcome poor embryonic development of IVG oocytes, but the benefit for human IVG oocytes remains to be investigated, along with the safety of the technique.

Wider implications of the findings: Our results demonstrate that ST and PNT have the potential to restore embryonic developmental competence of IVG oocytes, without increasing chromosomal abnormalities. This technology could be investigated in the future, as a mean to overcome poor quality of IVG oocytes in human.

Trial registration number: Not applicable

Abstract citation ID: dead093.757

P-406 Metal-phenolic network cell-membrane modification-mediated assembly strategy for re-establishing oocyte-granulosa cell communications and improving oocyte developmental potential

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Study question: Re-establish the cell-cell communication of isolated oocyte and granulosa cells (GCs) to mimic the follicle microenvironment and promote the success rate of in-vitro maturation (IVM).

Summary answer: The Metal-phenolic network (MPN) nanoparticles-mediated cell membrane modification system was used to achieve self-assembly of GCs on the oocyte membrane and re-establishing the Oocyte-GCs communication.

What is known already: How to preserve the fertility of female patients of childbearing age is an important problem faced by reproductive medicine. IVM of oocytes is an important means for female fertility preservation, but the problem of oocyte maturation rate needs to be solved urgently. The Oocyte-GCs interaction determines the key events in the oocyte development and maturation. Biological materials are gradually used for IVM, but there are still significant disadvantages. MPN has been widely used for the functionalization of bacteria and cell surface due to its unique nano-network structure and good biocompatibility.

Study design, size, duration: The MPN nanoparticles are respectively modified on GCs and oocytes, and the GCs can be self-assembled on the surface of the oocytes to re-establish cell communication. Then explore the biosafety and cytotoxicity of MPN nanoparticles on GCs and oocytes to rule out potential impact on the secretory function of GCs, reveal the formation of gap junction and substance exchange of Oocyte-GCs, and evaluate the effects of this technology on oocyte maturation, fertilization and embryo development.

Participants/materials, setting, methods: The oocyte-GCs assembly could be achieved through the MPN(EGCG-Zn^{2+}). Then MPN@GCs and MPN@Oocytes were characterized by fluorescent protein, Zeta, SEM and TEM; the cytotoxicity was assessed by Live/Dead staining, CCK-8 and flow cytometry assays. Gap junction was observed by TEM; the expression levels changes of signaling pathways for assembled oocyte and GCs were detected by single-cell RNA-seq, RT-qPCR and WB. The oocyte and embryo developmental potential were also analyzed.

Main results and the role of chance: We confirmed the formation of a nano-network surrounding GCs and oocytes and achieved the assembly of GCs on the surface of oocytes. Furthermore, we illustrated that MPN was non- cytotoxicity for GCs and oocytes and have no negative effect on the secretion function of GCs, indicating high biocompatibility of MPN and shows the potential application on the re-establishment of oocyte-GCs communication. Gap junctions formation and substance exchange establishment between GCs and oocytes were also proved in the study. The single-cell RNA-seq uncovered key pathways involved in oocyte-GCs interactions after the oocyte-GCs assembly. In detail, the SUMO1 ubiquitination modification of PTEN was inhibited after re-establishing cell-cell communication and decreased the localization of PTEN at the GCs membrane, which weakened its inhibition on PI3K. Higher PI3K level further up-regulated AKT level and stimulated its activation by increasing phosphorylation modification, finally improved the developmental potential and maturation rate of oocytes. The re-establishment of cell communication significantly down-regulated the P38/MAPK pathway in oocytes, including inhibited P38/MAPK expression, thereby reducing the phosphorylation of MSK1 and the activation of ATF-1, and effectively weakened the apoptosis controlled by P38/MAPK. Finally, a significantly higher fertilization and blastocyst formation rate were observed as compared to the control group.

Limitations, reasons for caution: Although this study has achieved promising results, there is still gap between basic research and applications, and more fundamental research should be conducted to make it a powerful tool for clinical application in the future.

Wider implications of the findings: This study not only provided a promising tool for establishing oocyte-GCs communication and improving oocyte maturation rate for IVM, but also showed a versatile and powerful cell-cell assembly platform for regenerative medicine including tissue engineering and organoid studies.

Trial registration number: not applicable

Abstract citation ID: dead093.758

P-407 Artificial ovary construction: short-term in vitro culture of human primordial and primary follicles in a 3D environment on a stromal cell feeder layer

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Study question: Do ovarian stromal cells (OSCs) influence viability and growth of human pre-antral follicles *in vitro*?

Summary answer: Feeder layer of OSCs advanced growth and transition of primordial follicles to primary/secondary stage while keeping a high proportion of viable follicles.

What is known already: In the ovary, follicles require the support of ovarian cells through the secretion of essential factors for their survival and development. This was also demonstrated *in vitro* through the 3D culture of isolated mouse primary and secondary follicles on a feeder layer of ovarian stromal cells. This co-culture significantly increased follicle survival and growth.

Study design, size, duration: Pre-antral follicles were isolated from human frozen-thawed ovarian tissue (OT) biopsies and encapsulated in 1% alginate scaffolds. Embedded pre-antral follicles were placed directly on the OSCs feeder layer or bottom of a culture dish for a 7-day *in vitro* culture (control). Follicle viability and growth and hormone production were compared between groups.

Participants/materials, setting, methods: Primordial and primary follicles were isolated from frozen-thawed OT of cancer patients (n = 6). OSCs were isolated from OT of post-menopausal women and cultured as a feeder layer. Follicle diameter was measured on Days 0 and 7 under an inverted microscope. Viability was assessed by staining a proportion of follicles (n = 87) with calcein AM and ethidium homodimer-I. They were classified (V1/V2: healthy/minimally damaged; V3/V4: damaged/dead follicles) using confocal fluorescence microscopy. Estradiol levels were measured by ELISA.

Main results and the role of chance: A total of 379 human pre-antral follicles (primordial=360; primary=19) were isolated and embedded in 1% alginate hydrogels and classified according to their viability. Most follicles (96%) were viable after isolation with a diameter of 40.8 ± 9.9 µm (mean ± SD). At Day 7, pre-antral follicles have grown significantly in size in both culture conditions: on the OSCs feeder layer and without it ($p < 0.0001$ for D0 vs. D7). However, no significant difference ($p = 0.07$) between culture conditions was observed. The mean diameter of follicles cultured on the OSCs layer was 80.6 ± 11.0 µm and without it, 67.3 ± 7.2 µm. Nonetheless, the distribution pattern of different quality grade follicles was significantly different on OSCs (V1: 62%, V2: 25%, V3: 2%, V4: 11%) and follicles without OSCs (V1: 35%, V2: 38%, V3: 0%, V4: 23%; $p = 0.03$). Additionally, more follicles have been activated and reached a higher developmental stage on OSCs (D0 primordial: 184, primary: 7 vs. D7 primordial: 53, primary/secondary: 93) than without stromal cells (D0 primordial: 186, primary: 4 vs. D7 primordial: 84, primary/secondary: 64; $p < 0.001$) with 66 and 43 follicles reaching a secondary stage ($75 < x < 200$ µm), respectively. Estradiol level was significantly ($p = 0.006$) higher in media of follicles cultured on the OSCs, 54.1 ± 14.2 pg/ml vs. 29.9 ± 4.0 pg/ml.

Limitations, reasons for caution: This study was performed only in a short-term culture and no primordial/primary follicles have reached the antral stage. Further *in vitro* studies on follicular developmental capacity, physiology and steroidogenesis in alginate scaffold with human ovarian stromal cells are needed.

Wider implications of the findings: Activation and growth of human primordial follicles during *in vitro* short-term culture to a secondary stage has been a long-lasting challenge. Co-culture with human ovarian stromal cells can help to overcome this limitation.

Trial registration number: not applicable

Abstract citation ID: dead093.759

P-408 The ovarian tissues outside of the radiation field could be damaged by radiation-induced abscopal effects in mice: an experimental study.

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Study question: Could irradiation-induced abscopal effects (RIAEs) induce structural and functional damage in the distal ovary in female mice, and what is its mechanism?

Summary answer: The RIAEs can induce an inflammatory reaction and inhibit the growth and development of ovarian follicles in mice leading to a decrease in ovarian reserve.

What is known already: The irradiated tumor could induce cell and tissue injury in the organs distant from the radiation site, referred to as the radiation-induced abscopal effects (RIAEs). Many pieces of research have shown out-of-field tumor regression effects of radiation therapy. However, RIAEs is a double-edged sword and may cause serious side effects on normal tissues. Premature ovarian insufficiency (POI) is one of the main side effects of radiotherapy, but the relationship between RIAE and POI has not been clarified.

Study design, size, duration: C57BL/6J mice were used to establish a RIAEs model by irradiating the thorax and the abdomens were shielded. Sixteen C57BL/6J female mice were randomly allocated to sham and experimental groups (n = 8/group) according to the presence or absence of irradiation with an 8 Gy X-ray on the local area of the chest every day for three days. After irradiation for twenty-one days, The effect and possible mechanism of RIAEs on non-irradiated ovarian were discussed.

Participants/materials, setting, methods: After irradiation, the estrous cyclicity, serum steroid hormones, and pro-inflammatory factors were compared between groups. Furthermore, RNA-seq was used to detect the expression of transcriptional levels in ovarian tissues in both groups. The differentially expressed genes (DEGs) were screened and analyzed by gene ontology-biological process (GO_BP) between irradiated and sham groups. The expression and localization of spermatogenesis-and oogenesis-specific basic helix-loop-helix-containing protein 1(SOHLH1) and neutrophil elastase (NE) in ovarian tissues were detected by immunohistochemistry(IHC).

Main results and the role of chance: Compared with mice in the sham group, the irradiation group had disordered estrous cycles, reduced primordial follicles ($P<0.001$)] and growing follicles ($P<0.001$), significantly increased atretic follicles ($P<0.001$). Levels of serum estradiol [(70.28 ± 5.27) pmol/L] and AMH [(104.00 ± 6.98) ng/L] in the irradiation group were significantly lower than those in the sham group [estradiol (97.58 ± 7.25) pmol/L, $P=0.016$; AMH [(129.70 ± 8.39) ng/mL, $P=0.046$], but FSH levels in the irradiation group were not significantly different from those in the sham group ($P=0.996$). Compared with the sham group, Serum levels of TNF-α [(488.30 ± 36.20) ng/L vs. (31.61 ± 12.89) ng/L, $P<0.001$] and IL-1β [(62.37 ± 2.50) ng/L vs. (52.75 ± 2.06) ng/L, $P=0.018$)] in the irradiation group were significantly increased. Serum levels of IL-6 in the irradiation group were also increased compared with the sham group, but the difference was not statistically significant ($P=0.301$). The results of GO_BP analysis showed that down-regulated DGEs were mainly involved in the process of follicular development, and up-regulated DGEs were involved in the inflammation process. The IHC results showed that the positive expression area of SOHLH1 in the irradiation group was significantly lower than in the sham group ($P=0.005$). In comparison, the positive expression area of NE was significantly higher than that of the Sham group($P=0.024$).

Limitations, reasons for caution: This study was performed using a short, 3-day irradiation treatment condition. Therefore, the results need to be cautiously interpreted concerning radiation-associated chronic toxicity. Moreover, further studies in mouse tumor models are required to determine the clinical relevance of the role of RIAE.

Wider implications of the findings: Although the precise molecular mechanisms of these distal effects on the genital system are still unclear, the current discovery might pave the way for establishing novel fertility preservation protocols. The research offers hope to women with POI related to cancer

therapy through the modulation of abscopal effects associated with radiotherapy.

Trial registration number: not applicable

Abstract citation ID: dead093.760

P-409 Oocyte cryopreservation following ovarian stimulation for fertility preservation in transgender men: a French case series

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Study question: To evaluate the feasibility and results of a fertility preservation program for transgender men.

Summary answer: Ovarian stimulation outcomes are similar between transgender men and oocyte donation candidates, leading to a satisfactory mean number of cryopreserved oocytes.

What is known already: The reproductive potential of transgender people may be impaired by gender-affirming hormone treatment (GAHT) and is obviously suppressed in case of gender-affirming surgery involving bilateral ovariectomy and/or hysterectomy. The evolution of medical support for transgender people has made fertility preservation strategies possible. Fertility preservation in transgender men mainly relies on oocyte cryopreservation following controlled ovarian stimulation. However, few data are available to date on the subject, concerning small sample sizes, so no reliable conclusions can be drawn about the feasibility and efficiency of fertility preservation procedures in trans men.

Study design, size, duration: This retrospective study reports the results of fertility preservation counselling in 118 transgender men referred to our fertility centre from September 2018 to December 2022. Among them, 16 benefited from oocyte cryopreservation. Ovarian stimulation outcomes were compared with those of cisgender oocyte donors.

Participants/materials, setting, methods: Sixteen transgender men benefited from oocyte cryopreservation following ovarian stimulation and were matched 1:1 to cisgender oocyte donors according to age and body mass index. Antral follicle count and serum AMH levels were systematically measured. Primary outcomes included duration of the stimulation, total FSH dose, peak serum estradiol, oocyte yield, number of mature oocytes, and maturity rate (mature oocytes/total oocytes collected). Results were compared using Fisher's exact or Wilcoxon's rank sum tests.

Main results and the role of chance: One hundred and eighteen transgender men were referred in our centre for fertility preservation counselling. Among them, 95 asked for a medical consultation, and 86 came to the appointment. Following the consultation, only 16 (18.6%) finally decided to benefit from oocyte cryopreservation following ovarian stimulation, including 2 that had already started testosterone therapy. Sixteen presumably fertile oocyte donors were matched based on age and BMI. Mean age of trans' men was 23.9 ± 5.0. Basal ovarian reserve tests showed satisfactory results in trans' men and oocyte donors, with similar AMH (4.5 ± 2.3 vs. 4.4 ± 3.2, respectively, NS) but lower AFC in trans'men than in oocyte donors (13.8 ± 11.0 vs. 26.7 ± 10.1, $p = 0.033$). This result may be explained by a more frequent use of the abdominal approach to assess AFC in the transgender group. Ovarian stimulation outcomes were comparable, with no differences in the duration of stimulation, total FSH dose and peak estradiol levels, leading to a comparable mean number of mature oocyte (15.4 ± 9.9 vs. 17.2 ± 10.6, respectively, NS). Thirteen out 16 transgender men had 10 or more cryopreserved mature oocyte following a single procedure.

Limitations, reasons for caution: Only a low percentage of trans men that were referred to our centre finally chose to perform oocyte cryopreservation, leading to a small sample size. The majority of them had not started any GAHT, preventing us to evaluate the potential consequences of these treatments on ovarian stimulation outcomes.

Wider implications of the findings: While parenthood strategies for transgender people have long been ignored, fertility preservation is an important issue to consider, especially because medical treatments and surgeries may be undertaken in very young adults. Oocyte cryopreservation seems to represent a feasible way for trans men to preserve their fertility for future biological parenting.

Trial registration number: N/A

Abstract citation ID: dead093.761

P-410 First wave of primordial follicle activation during human ovarian tissue manipulation for fertility preservation

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Study question: Does ovarian tissue biopsy, transportation and processing for fertility preservation and restoration trigger primordial follicle activation?

Summary answer: Early manipulation of ovarian tissue is sufficient to trigger follicle activation by stimulating PI3K/Akt and disrupting the Hippo pathway.

What is known already: Primordial follicle recruitment occurs continuously in physiological conditions by modulation of autocrine and paracrine factors, like the PI3K/Akt and Hippo pathways, to ensure follicle growth over time. During fertility preservation and restoration procedures, follicle activation may be disrupted and follow nonphysiological patterns. The ability to control activation dynamics by up- or downregulation of these pathways may enhance fertility restoration outcomes in a number of ways. Indeed, downregulation of follicle activation shortly after transplantation may protect the ovarian reserve from early depletion. Conversely, ovarian tissue in vitro culture may benefit from upregulation of primordial follicle activation to boost further growth.

Study design, size, duration: Fresh ovarian tissue was retrieved from nine women undergoing laparoscopic surgery for benign conditions. Three timepoints were investigated. One-third of collected tissue per patient was immediately fixed in the operating room, without any manipulation (time zero, T0). The remaining tissue was transferred to the laboratory and dissected to remove any surplus medulla. It was then cut into small cortical fragments, half of which were fixed after 25 minutes(T25) and the other half after 90 minutes(T90).

Participants/materials, setting, methods: All cortical fragments were fixed in 4%formaldehyde and embedded in paraffin for histology. In order to explore follicle activation,markers of the PI3K/Akt and Hippo signaling pathways were immunolabeled at each timepoint, targeting: (i) phospho-Akt (p-Akt) in primordial follicles by immunohistochemistry as a marker of early PI3K/Akt pathway activation; and (ii) Yes-associated protein (YAP) cellular localizationin the granulosa cell layer of primordial follicles by immunofluorescence as a marker of Hippo disruption.

Main results and the role of chance: An upturn in p-Akt expression was observed at T25 (22,34 ± 0.13%; $p = 0.0233$) and T90 (39,01 ± 0.22%, $p = < 0.0001$) compared to T0 (2,87 ± 0,03%). In terms of YAP cellular localization, a significant nucleus-to-cytoplasm shift was detected at T25 (1.11 ± 0.09; $p = 0.0428$) compared to T0 (0.97 ± 0.10), while T90 (1.07 ± 0.14) values were similar to T25. Our data prove that ovarian tissue manipulation triggers primordial follicle activation very early, involving both the PI3K/Akt and Hippo signaling pathways, which appear to cooperate in primordial-to-primary follicle transition. Our results indicate that the first stages of any fertility preservation or restoration procedure involving ovarian tissue manipulation contribute to dysregulation of the very mechanisms responsible for the ovarian reserve maintenance and follicle growth. Additional strategies are required to gain the control of follicle activation mechanisms in nonphysiological

conditions (ex vivo ovarian tissue manipulation), in order to exploit ovarian reserve dynamics to serve the need of patients.

Limitations, reasons for caution: Analyses in the study were limited to histology and immunolabeling to acquire a descriptive picture of pathway activation kinetics over time. Further investigations using dynamic experimental models are essential to advance our understanding of signaling pathway synergy in vivo.

Wider implications of the findings: Since dysregulation of follicle activation in nonphysiological conditions appears to be associated with poor oocyte quality, enhancing our ability to control the relevant signaling pathways is crucial to optimizing fertility preservation procedures.

Trial registration number: not applicable

Abstract citation ID: dead093.762

P-411 Cumulative live-birth rate after oocyte vitrification for fertility preservation (FP) in oncologic or benign conditions: a retrospective comparative monocentric study

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Study question: Does cumulative livebirth-rate (CLBR) after reutilization of vitrified oocytes differ between patients having undergone fertility preservation (FP) for oncologic (onco-FP) reason or benign conditions (benign-FP)?

Summary answer: Although CLBR per woman was lower in the onco-FP group, there was no statistically significant association in multivariate analysis between onco-FP and CLBR.

What is known already: Recent ESHRE guidelines reported oocyte vitrification after controlled ovarian hyperstimulation (COH) as an established option for FP. Although age at the time of oocyte vitrification remains the main predictive factor of success, several lines of evidence suggest that the use of COH or in vitro maturation (IVM), as well as the indication of FP, in particular the type of disease, may influence outcomes after devitrification. The present investigation aimed at clarifying this issue.

Study design, size, duration: Observational comparative, monocentric retrospective study including all patients having reused, between January 2014 and December 2021, their oocytes vitrified for oncologic or benign conditions between 2013 and 2021. Women having undergone FP for non-medical indication were excluded.

Participants/materials, setting, methods: Among the 2201 patients having vitrified their oocytes, 94 (4.7%) returned for reutilization. The primary objective was the comparison of CLBR between onco-FP (n = 48) and benign-FP (n = 46) groups. A logistic model was performed. Factors associated with the CLBR in univariate analysis were included in a multivariate model. A secondary analysis was performed, comparing the benign-FP group and 2 groups of onco-FP according to the use of COH (Onco-COH, n = 25) or *in-vitro* maturation (Onco-IVM, n = 23).

Main results and the role of chance: Overall, in comparison with benign FP, women with malignant diseases were younger (median [IQR]: 34.0 y [31.0;37.0] *vs.* 36.5 y [33.2;38.0], p = 0.04) and had fewer oocytes vitrified (median [IQR]: 6.0 [3.0;9.2] *vs.*15.5 [6.2;18.0]), p < 0.001). The CLBR in onco-FP and benign-FP groups were 14.6% (7/48), and 32.6% (15/46), respectively. In univariate analysis, the CLBR was significantly lower in the onco-FP group when compared with the benign-FP group: (OR [95%CI]: 0.35 [0.12;0.94], p = 0.04). However, this difference in the CLBR did not reach significance after multivariate analysis (OR [95%CI]: 0.38 [0.09;1.51], p = 0.18). As expected, age at the time of oocyte vitrification was negatively associated with CLBR (OR [95%CI]: 0.80 [0.67;0.92], p = 0.005), while the number of oocytes inseminated was positively related to CLBR (OR [95%CI]: 1.22 [1.08;1.40], p = 0.002).

Unlike onco-COH, onco-IVM was associated with a significant decrease in CLBR when compared to benign-FP (only COH), in univariate analysis: 8.7% (OR [95%CI]: 0.20 [0.03;0.80], p = 0.04), without significance after multivariate analysis (OR [95%CI]: 0.37 [0.04;2.16], p = 0.29).The livebirth rate (LBR) per oocyte was 2.1% in the onco-IVM subgroup (3 babies for 141 oocytes thawed), 2.7% in the onco-COH subgroup (7 babies for 260 oocytes thawed) and 3.7% (18 babies for 479 oocytes thawed) in the benign-FP group.

Limitations, reasons for caution: The small size of our population was probably responsible for a lack of statistical power. The retrospective and monocentric nature of our study may also be a weakness. Furthermore, LBR per oocyte was analyzed without age-adjustment.

Wider implications of the findings: This study provides actual data on chances of achieving a livebirth after oocyte vitrification, according to the use of a COH or IVM and the context of FP, oncologic or benign conditions.

Trial registration number: not applicable

Abstract citation ID: dead093.763

P-413 Lessons learnt from a long-term fertility cryopreservation program regarding usage-rate of vitrified oocytes and embryos

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Study question: Does woman's age during cryopreservation affect usage-rate of the vitrified oocytes and embryos from fertility preservation cycles?

Summary answer: The usage-rate of vitrified oocytes and embryos from cryopreservation cycles is low. Sub-analysis shows a higher usage-rate with advanced age and increased storage time.

What is known already: previous studies show a 3-9% usage-rate in social oocyte freezing fertility preservation cycles. Studies show that social preservation of oocytes before the age of 39 is not cost effective. To the best of our knowledge, data is scarce regarding the vitrified oocyte and embryo usage-rate according to the woman's age during both social and medical fertility preservation cycles.

Study design, size, duration: A retrospective case series study including all fertility preservation cycles at our IVF unit between Dec 2010 and Jan 2023. During a period of 12 years, 558 cycles in 358 women without a known fertility problem were performed. Vitrification was performed in 535 cycles. A total of 3605 oocytes and 382 embryos were cryopreserved for social and medical reasons (272 and 286 cycles respectively).

Participants/materials, setting, methods: The treatment cycles were divided into 3 groups according to the time-period of cryopreservation, reflecting the length of storage. Group 1: 2010-2016 (54 cycles), Group 2: 2017-2020 (132 cycles), and Group 3: 2021-2023 (349 cycles). We analyzed usage-rate in each time group according to their age at freezing, 17-35 (334 cycles) or 36-44 (201 cycles)

Main results and the role of chance: Average age was 34 ± 5 years (17-44). Overall, 30 women (30/358=8%) thawed their oocytes or embryos cryopreserved in 37 cycles (37/535=7%) of them 16 for social and 21 for medical reasons. In all three time-period groups there was a clear trend of increased usage among older women at cryopreservation (36-44y) compared to younger women (17-35y) : Group 1 [43%(9/21) vs 24%(8/33)], Group 2 [9%(6/64) vs 3%(2/68)] and Group 3 [8%(9/120) vs 1%(3/229)]; respectively. As shown, usage-rates also increased in accordance to length of storage. For thawed embryos positive β-hCG rate was higher for the 17-35 age group compared with 36-44 age groups [53% (10/19) vs 19% (3/16)] and Clinical pregnancy were [37% (7/19) vs 19% (3/16); respectively. For oocyte thawing usage-rates were extremely low; only one woman thawed her oocytes in the young age group and was pregnant and 6 women in the older age group thawed their oocytes and 2 were pregnant. Seven women

transferred their frozen oocytes to another fertility center and the outcome is unknown. Overall usage-rate of vitrified oocytes and embryos from fertility preservation cycles is low. Sub analysis shows a higher usage-rate in correlation with women's age at time of cryopreservation and length of storage.

Limitations, reasons for caution: This is a single center retrospective study.

Wider implications of the findings: Cost effectiveness and success rates should be discussed with women undergoing fertility preservation cycles. Cost benefit should take into account the low usage rate, length of storage and the clinical pregnancy rates reported from oocyte and embryo thawing according to women's age.

Trial registration number: 0029-23-CMC

Abstract citation ID: dead093.764
P-414 Trends in patient age at planned oocyte cryopreservation

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Study question: Did the age of women who underwent Planned Oocyte Cryopreservation (POC) change in the last decade?

Summary answer: Mean age of women presenting for POC decreased from 38.2 ± 2.8 to 35.6 ± 2.7.

Proportion of women <36 increased from 13% in 2011 to 51% in 2022.

What is known already: Mean age of women who had POC reported in the literature to date is 37-8.

Given that efficacy of POC is strongly related to age, some clinicians advice women considering POC to complete the procedure before age 35.

Study design, size, duration: Retrospective, observational multicenter study of all POC cycles from January 2011 to October 2022. Women who had oocyte cryopreservation for other indications were excluded.

Participants/materials, setting, methods: In all, 4021 women had at least one POC cycle in two large private IVF units belonging to the same medical organization in Israel. The main outcome measure was age at first cycle. Total number of women who underwent POC at each year and age at first cycle of every woman were recorded. Data was analyzed using SPSS version 23. ANOVA was used to test whether mean age differed significantly throughout the years.

Main results and the role of chance: Since 2011, when POC was first approved in Israel, until the end of October 2022, 4021 women underwent at least one cycle of POC in our units. The number of women who had POC per year increased gradually from 62 in 2011 to 979 in 2021 (last year with full data).

Mean age declined with each year, from 38.2 ± 2.8 in 2011 to 35.6 ± 2.7 in 2022. The proportion of women aged < 36 increased gradually from 13% in 2011 to 35% in 2019 and further to 51% in 2022 (R = 0.20, p value<0.01).

Limitations, reasons for caution: This study was performed in a high-socioeconomic catchment area of one country. This may limit the generalizability of our findings to other populations. In addition, the usage rate and delivery rates of these patients are not known yet.

Wider implications of the findings: The significant decrease in age of women presenting for POC and the increased proportion of women<36 may present a growing awareness for age related infertility and possibly increased efficacy of POC in younger women.

Trial registration number: not applicable

Abstract citation ID: dead093.765
P-415 Cryostorage of human ovarian tissue: Evaluating the storage and disposal pattern of a 22-year period with 2475 patients

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Study question: What are the characteristics of patients who did ovarian tissue cryopreservation?

Summary answer: Patients store their ovarian tissue in average 5 years, when storage is actively ended about 50% of the patients conceived without using the frozen tissue.

What is known already: In female fertility protection two major options for cryopreservation are available today: cryopreservation of oocytes and ovarian tissue cryopreservation (OTC). If cryopreservation of oocytes is not feasible, OTC remains the only option for individual fertility protection in women, adolescents and especially prepubertal girls.

Study design, size, duration: The relevant parameters of a single university center were revised and digitalized in the period from 2019 to 2021 and were analyzed in the time period between 2000 and 2021.

Participants/materials, setting, methods: 2475 patients with stored ovarian tissue were analyzed. Data extraction was done with MedITEX (CRITEX) and processed with SPSS (IBM). To assess the patient motivation of storage, patients were contacted by letter, e-mail and telephone calls.

Main results and the role of chance: On average, patients were aged 26.8 years, diagnosed with breast cancer (44.8%) and lymphoma (22.4%) in majority. In the active storage group (n = 1320) patients were storing for 5.4 years, patient age was 25 years at time of OTC and indications were breast cancer (37.6%) and lymphoma (26.7%). Analyzing the longtime storage group ≥10 years (n = 148), patient age was 23.8 years at time of OTC, storage duration was 12 years and indications were again mainly breast cancer (27%) and lymphoma (27.7%). When patients deceased (n = 133), patient age was 28.1 years, with a storage duration of 2.7 years and initially diagnosed with breast cancer (39.8%) and sarcoma (17.3%). When storage ended (n = 1155), patients stored for 4.2 years, mean age was 33 years.

Analyzing the mode of storage end we observed that 2.5% had a transplantation on site, 10.3% transferred their tissue to another cryobank while 11.5% deceased. The majority of this group (75.7%) ended their storage due to pregnancy (49.1%), no desire to have children (25.9%), too expensive storage fees (8.9%), death (8.5%), recurrence of cancer (8.5%), no partner (4%) and fear of surgery in the future (3.1%). 6.7% regretted the end of storage retrospectively.

Limitations, reasons for caution: Live birth rate was not part of this paper as our main intention was to explore the storage characteristics. Few patients transferred a part of the samples for retransplantation while still storing tissue on site. The response rate of 28.8% limits the scope for interpretation regarding end of storage.

Wider implications of the findings: The pregnancy rate resulting from ovarian tissue that wasn't removed for OTC (49,1%) supports the approach of removing only 50% of one ovary. The long storage time and the fact that 6.7% regretted the end of storage, shows how important the OTC is, it should be covered by health insurances.

Trial registration number: not applicable

Abstract citation ID: dead093.766
P-416 Age and Oocyte Donation: An Analysis of Pregnancy Outcomes in Women Under and Above 45

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Study question: Do Maternal and Neonatal Risks Increase among oocyte recipients > 45 years compared to younger oocyte recipients?

Summary answer: This study fails to demonstrate increased maternal and neonatal complications among oocyte recipients >45 years. A lower socioeconomic status is strongly related with those risks.

What is known already: Oocyte donation (OD) is an integral part of modern assisted reproductive care, and its prevalence has increased dramatically. Although OD is an effective fertility option for women with advanced age these pregnancies have higher risks of maternal and obstetric morbidity.

Study design, size, duration: This study is a retrospective big data cohort study that utilizes electronic data from Maccabi Healthcare Services, a 2.5-million patient integrated care organization, which represents 25% of the pregnant population in the country. The data used in this study was collected from 2000 through 2018.

Participants/materials, setting, methods: A total of 1433 OD pregnancies were divided into two groups according to maternal age at birth: 946 singleton gestations (335 of women > 45 and 611 of women ≤45) and 487 twin gestations (175 of women > 45 and 312 of women ≤45). The two groups were compared for maternal characteristics, perinatal outcomes including PTB and small for gestational age (SGA), and obstetric outcomes.

Main results and the role of chance: For singletons, mean maternal age at birth was 47.6 for women > 45 and 40.7 for women ≤45 ($p < 0.001$). The women > 45 group had significantly higher BMI and had higher birth weights (26.4 vs 25.6, $p = 0.04$ and 3011g vs 2920g, $p = 0.04$, respectively). The incidence of PTB <37 weeks, PTB <34 weeks and PTB <32 weeks among singletons was higher among the women ≤45 group (24.9% vs 18.5%, $p = 0.05$, 7.9% vs 4.5%, $p = 0.04$ and 4.9% vs 2.5%, $p = 0.05$, respectively). Multivariable logistic regression analysis for PTB <37 weeks and SGA in singletons demonstrated that maternal age <45 and low socioeconomic status (SES) were significant variables. For twin gestations, mean maternal age at birth was 47.3 for women > 45 and 40.2 for women ≤45 ($p < 0.001$). The women > 45 group had significantly lower SES and lower birth weights (6.8 vs 7.2 SES scale, $p = 0.04$ and 2119g vs 2256g, $p = 0.01$, respectively). multivariable logistic regression analysis for PTB <37 weeks in twins demonstrated that low SES and maternal smoking were significant variables. No differences were found between the groups among singletons and twin gestations regarding hypertension, diabetes, intrauterine fetal demise, malpresentation, postpartum hemorrhage, retained placentae and cesarean deliveries.

Limitations, reasons for caution: Underdiagnoses and missing information regarding indications leading to OD may have led to better understanding of the data.

Wider implications of the findings: OD pregnancies should be considered high-risk. Understanding the differences in complications among age groups can help healthcare providers provide more personalized and effective care to women who are considering oocyte donation. Comparative studies between different age groups can contribute to future practices and policies related to oocyte donation.

Trial registration number: 0046-18-BBL

Abstract citation ID: dead093.767
P-417 Biovular follicles are commonly found in prepubertal but not adult ovaries

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Study question: Is the morphology of ovarian tissue from paediatric and adolescent girls similar to that from adults?

Summary answer: The presence of biovular follicles was more frequently observed in paediatric ovary than in the adult ovary.

What is known already: At birth there are approximately 1 million ovarian follicles that reduce to around 380,000 by puberty. Limited information is available on the morphology of paediatric and adolescent ovary but it is thought that, before the age of 6 years, the prepubertal ovary contains around 20% abnormal primordial follicles - more than in the adult ovary. Polyovular follicles are common in some animal species but rarely observed in the human adult ovary. They are thought to contribute to dizygotic twinning but the low frequency suggests they are not the sole origin.

Study design, size, duration: Haematoxylin and eosin stained sections of ovarian tissue were examined by 2 operators blinded to patient age. Follicles were classified according to modified Gougeon criteria. The proportions were compared with Fisher's exact test and the frequency by one- way ANOVA.

Participants/materials, setting, methods: Ovarian cortex tissue was collected from a total of 71 children and adolescents and 85 adults undergoing fertility preservation. Those who had had previous chemotherapy and or pelvic irradiation were excluded. The population was divided into 30 children (<8 years, range 1 - 7.6 years), 25 peripubertal (8 - 14.1 years), 16 with confirmed pubertal status (13.7 - 16.6 years) and compared to 85 adults (18-36 years).

Main results and the role of chance: Biovular follicles were observed in 36.7% (11/30) of the ovarian tissue from children <8 years with, where present, an average frequency of $1/10.6 \times 10^3$ follicles, in 28% (7/25) of the peripubertal girls with an average frequency of $1/11.9 \times 10^3$ follicles, and 18.7% (3/16) of the post pubertal girls with an average frequency of $1/7.7 \times 10^3$ follicles. In contrast, biovular follicles were rarely seen in the adult ovarian tissue examined (3.5%, 3/85) with an average frequency of $1/25 \times 10^3$ follicles. The observed frequency in all of the younger samples was significantly different to the adult ($p < 0.001$ <8 years, $p < 0.001$ peripubertal, $p < 0.05$ post pubertal) but not between the young age groups. In those samples with biovular follicles the frequency was not significantly different between any of the groups.

An antral follicle was observed in only one case <8 years of age (1.4 years) - 3.3%, in 2 in the peripubertal (8%), in 4 of the post pubertal (25%) and in 9 of the adult ovarian cases (10.6%). The higher observed frequency in the post pubertal ovary compared to the <8 year was significantly different ($p < 0.05$).

Limitations, reasons for caution: Only a small sample of the ovary cortex was examined for each individual, which may be more representative of the young ovary than the adult. It is not possible to predict potential function from this analysis.

Wider implications of the findings: Although follicle numbers in the paediatric ovary are high, follicles with structural anomalies such as biovular follicles are also more frequently observed in the paediatric ovary than in the adult. It appears that the changes at puberty may cause the loss of these abnormal follicles.

Trial registration number: Not Applicable

Abstract citation ID: dead093.768
P-418 Medical sperm cryo-preservation in adolescents: what proportion needs long term storage?

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Study question: What proportion of adolescents who have cryo-preserved sperm for medical indications will have normal semen analysis after treatment and no longer require stored sperm samples?

Summary answer: More than half of adolescent patients with cryo-preserved sperm will not need continued storage as sperm parameters return to normal after completion of treatment.

What is known already: Worldwide, about 12,400 young adults are diagnosed with cancer every year. With modern treatment 80% of them will achieve a long-term cure[1]. Lymphomas, brain tumours, sarcomas, and carcinomas account for more than 90% of cases, for which treatment tends to be predominantly gonadotoxic. Sperm cryo-preservation prior to starting treatment is recommended by all clinical guidelines. Although there is evidence that treatment could cause prolonged azoospermia, especially alkylating or

platinum-based agents, several therapy regimes seem to induce only a temporary reduction in sperm count. In some cases spermatagonial stem cells can repopulate the seminiferous tubules after chemotherapy-induced damage[2].

Study design, size, duration: This was a retrospective cohort study on male adolescent who underwent medical sperm cryo-preservation between 1985 and 2014. The data was retrieved from a prospective held secure electronic database. Within 10 years of storage a review was carried out to ascertain if they met criteria for continued storage. A total of 323 teenagers were included in the study.

Participants/materials, setting, methods: In a tertiary fertility preservation centre, 323 adolescents had sperm cryopreservation before undergoing potentially gonadotoxic treatment. All patients were offered repeat semen analysis within 10 years from storage. The indication for discarding sperm within this period were analysed. The primary outcome was the proportion of patients who did not have adverse impact on sperm. Their diagnosis, treatments and outcomes including death were included in the analysis. Statistical analysis was performed using IBM SPSS Statistics.

Main results and the role of chance: According to WHO criteria, at time of cryo-preservation 168/323 (52%) adolescents had a normal semen analysis, 120/323 (37,1%) had oligospermia and 35/323 (10,8%) had azoospermia.

For the 198 adolescents included in the final analysis, median age was 16 (IQR 15-16). Haematological cancer was the predominant group [94/198 (47%)] followed by sarcoma/osteosarcoma [58/198 (29%)]. Outcome analysis revealed that 99/198 (50%) patients had repeated semen analyses. Median time between the pre- and post-treatment analysis was 10 years (IQR 7-10).

Of those who had a subsequent semen analysis, 59/99 (58,4%) showed improvement, 23/99 (22,8%) had no change and 17/99 (16.8%) revealed decline in sperm quality parameters compared to the stored sample.

Post treatment, spontaneous pregnancy was reported by 16 (8%) patients.

Sperm was discarded for patient demise in 35.8% (71/198) of cases and for inability to contact the patient since storage in 13.1% (26/198).

Limitations, reasons for caution: One limitation is the retrospective design and the relatively small number of cases included in the study. A second limitation is that although we have information of semen analysis, we did not have information on pregnancies achieved for patients with a normal semen analysis.

Wider implications of the findings: This is one of the largest case series of outcomes in adolescents. We can anticipate retention of fertility potential in half of the adolescents freezing sperm. A repeat semen analysis facilitates reassurance to the patients and allows discarding sperm when in remission. Patients can refreeze sperm if disease relapse occurs.

Trial registration number: Non applicable

Abstract citation ID: dead093.769

P-419 Oocytes quality in random start protocols for fertility preservation: insights from follicular steroidogenesis.

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Study question: Is follicular steroidogenesis impaired in women undergoing random start protocols for fertility preservation?

Summary answer: The phase of menstrual cycle at initiation of ovarian stimulation does not influence the endocrine microenvironment surrounding oocytes.

What is known already: Cryopreservation of oocytes is the gold standard for fertility preservation in women with cancer. Random start protocols have been introduced for oocytes cryopreservation in these patients to shorten the duration of ovarian stimulation. However, albeit generally reassuring, available evidence is still insufficient to rule out a sub-optimal cycle outcome of random start protocols.

Study design, size, duration: The present study was conducted to provide evidence on the validity of random start protocols by exploring the quality of ovarian steroidogenesis. The primary outcome was comparing levels of steroids in the follicular fluid between women initiating the random start protocol in the luteal phase and those initiating in the follicular phase (considered as controls since equivalent to the conventional protocols). We excluded those requiring concomitant letrozole assumption.

Participants/materials, setting, methods: Seventy-one women with cancer were prospectively recruited during a 24-month period. Thirty-three initiated in luteal phase while 38 in follicular phase. All women were stimulated with recombinant FSH and GnRH antagonists. At the time of oocytes retrieval, follicular fluids were pooled, and a sample was frozen at -80 °C. All samples were assayed concomitantly after thawing, by liquid chromatography tandem mass spectrometry. The concentration of 15 different steroid hormones was determined.

Main results and the role of chance: Baseline characteristics of the two groups were similar. No differences emerged in anamnestic data and ovarian reserve variables. Cycle outcome did not also differ, the two study groups being similar in terms of total dose of gonadotropins, duration of stimulation, number of developed follicles and number of oocytes retrieved. The median [interquartile range] number of frozen mature oocytes was 9 [5-14] and 10 [5-21] in women who initiated in the luteal and the follicular phase, respectively ($p = 0.42$). None of the 15 tested steroid hormones differed. Two subgroup secondary analyses were performed to rule out confounders. First, we excluded women who were on estroprogestins at the time of recruitment (leaving 31 women initiating in the follicular phase and 32 in the luteal phase). Levels of steroids in the follicular fluids were still mainly similar. We observed a significant difference only for androstenedione, levels being higher for women initiating stimulation in the follicular phase. Second, we compared women in the early (up to day 5) and late follicular phase (26 and 12 cases, respectively). Levels of steroids in the follicular fluids did not differ except for cortisone, the concentration being higher in the early follicular phase.

Limitations, reasons for caution: Our study is not randomized, inevitably exposing our results to confounders. Assessment of intraovarian steroidogenesis is an indirect evidence of oocyte quality. Multiple comparisons were done, exposing our findings to type I errors (such as, in our opinion, the differences that emerged in the secondary analyses).

Wider implications of the findings: Random start protocols have become the standard of care for fertility preservation in women with cancer in the absence of robust evidence. Our data supports the validity of random start protocols in terms of quality of ovarian response since none of the tested hormones differed.

Trial registration number: Not applicable

Abstract citation ID: dead093.770

P-420 Involvement of Interluekin-6 (IL-6) in the development of different stages of spermatogenesis in vitro, using spermatogonial cells isolated from normal and busulfan-treated immature mice.

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Study question: Can Interluekin-6 (IL-6) induce spermatogonial cells from seminiferous tubules of normal or busulfan-treated mice to develop spermatogenesis in vitro using methylcellulose culture system (MCS)?

Summary answer: IL-6 induced the development of spermatogonial cells from seminiferous tubules of normal or busulfan-treated mice to premeiotic, meiotic and post-meiotic cells in vitro.

What is known already: Spermatogenesis is a complicated process of sperm generation. During this process spermatogonial cells proliferate and differentiate to meiotic, postmeiotic stages that continue in spermiogenesis to generate mature sperm. This process is under regulation of autocrine and

paracrine factors provided by developed germ cells and somatic cells such as Sertoli, peritubular and Leydig cells. These microenvironmental factors are modified following pathological condition, such as chemotherapy, which may lead to subfertility or sterility. Different in vitro culture systems were used to induce the development of complete spermatogenesis in vitro; however, this was not yet achieved.

Study design, size, duration: Sexually immature mice (7-day-old) were used as normal or were intraperitoneally (i.p) injected with busulfan (45 mg/kg) to isolate cells from their seminiferous tubules (STs). Cells were enzymatically isolated from the STs of the mice and were cultured in methylcellulose culture system (MCS), as a 3-dimensinal in vitro system. Fresh media without (CT) or with IL-6 were added to the cultures from the beginning, and after two weeks. The cultures were determined after 4 weeks

Participants/materials, setting, methods: Isolated cells from the STs were cultured ($2x10^5$/well/0.5ml) in MCS that contained StemPro-34 medium, KSR, rEGF, rGDNF, rLIF, and r-bFGF and in the presence/absence of IL-6 (1,10 or 100 pg/ml) and incubated for four weeks in a CO_2 incubator at 37°C. The developed cells and colonies/organoids were examined microscopically and quantified for the development of cells of the different stages of spermatogenesis and Sertoli cells functional markers by immunofluorescence staining (IF) and/or qPCR analyses.

Main results and the role of chance: Addition of IL-6 to MCS that contained isolated cells from STs of normal immature mice significantly increased the development of premeiotic cells (VASA), meiotic (BOULE) and meiotic/postmeiotic cells (ACROSIN) as examined by specific IF and/or qPCR analyses. Addition of IL-6 also differently affected the expression levels of Sertoli cell functionality markers (androgen receptor, androgen binding protein, transferrin, GDNF, FSHR). Furthermore, addition of IL-6 to isolated cells from STs of busulfan-treated immature mice also increased the development of VASA-, BOULE- and ACROSIN-positive cells (as examined by IF and qPCR analyses). However, the effect of IL-6 was more potent in the development of different stages of spermatogenesis in in vitro cultures that contained isolated cells from STs of normal compared busulfan-treated mice. Also, addition of IL-6 distinctly affected the expression levels of Sertoli cell functional markers in cultures of normal and busulfan-treated mice.

Limitations, reasons for caution: This in-vitro culture used animal models. The bio-activity of the developed post-meiotic needs to be confirmed

Wider implications of the findings: These findings indicate the possible involvement of IL-6 in the development of spermatogenesis in vitro. This effect could be directly on spermatogonial cells and/or through Sertoli cells. Our result could suggest IL-6 as a potential factor to be used in developing future in vitro therapeutic strategies for male fertility preservation

Trial registration number: not applicable

Abstract citation ID: dead093.771
P-421 Return rates and outcome of fertility preservation in women with cervical cancer.

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Study question: What are the return rates and reproductive outcome after fertility preservation (FP) in women with cervical cancer (CC)?

Summary answer: Return rates after FP in women with CC seem to be comparable with those after FP for other indications.

What is known already: Cervical cancer is the fourth most common cancer among women. Approximately 42% of the affected women are under the age of 45, and many of them wish to preserve fertility at the time of diagnosis. Fertility sparing treatment modalities and FP with cryopreservation of oocytes, embryos or/and ovarian tissue can be offered for this purpose to carefully selected patients. These treatments are offered at Swedish academic centers within the health insurance coverage available to all citizens. To date, data on return rates, reproductive outcome and survival in women with cervical cancer undergoing FP are scarce.

Study design, size, duration: Prospective, single center study aiming to report long-term outcomes in women with CC who have versus have not undergone FP at Karolinska University Hospital between January 1st 1999 and September 30th 2018.

Participants/materials, setting, methods: During the study period, 74 women with CC received FP counseling at Karolinska University Hospital, and 52 of them women proceeded to FP. Data on return rates, reproductive outcomes and overall survival were extracted using the clinical registries.

Main results and the role of chance: By January 15th, 2023, 22 of 52 women with FP returned for a new fertility counselling or treatment, mean time between FP and return was 4 years (1-7 years). Among women with cryopreserved ovarian tissue (n = 40), 17 have been in contact with the clinic with fertility wish, 3 of them had re-transplantation of the tissue, but none of them achieved oocyte retrieval yet. Additionally, one woman is planned for re-transplantation in February 2023, and one woman is awaiting the decision. In two cases the decision not to re-transplant ovarian tissue was based on absence of endometrial growth following attempts of hormonal substitutive therapy. Among women with cryopreserved oocytes (n = 5), 1 returned for thawing but no pregnancy has been achieved. Among women with cryopreserved embryos (n = 5), 3 returned during follow-up, 2 proceeded to thawing and transfer, 1 got pregnant and gave birth to a child. Of two women with combination of oocytes and ovarian tissue cryopreserved, one returned for fertility treatment, but no pregnancy has been achieved after thawing and re-transplantation. Eight women died during follow-up because of recurrence of their cancer, 4 of 22 in the group without FP and 4 of 52 in the group with FP.

Limitations, reasons for caution: This study provides much needed data on real-world outcome in women with FP indicated by diagnosis of CC, but it has the limitations related to its descriptive character. The use of gestational carriers is not permitted in Sweden and FP program may differ from those in other countries.

Wider implications of the findings: The study results provide much needed data on real-world reproductive and oncologic outcome following FP in women with CC.

Trial registration number: NTC04602962

Abstract citation ID: dead093.772
P-422 Ovarian tissue vitrification as a low-technology and cost-effective protocol for female fertility preservation: a bovine study

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Study question: Which are the potential effects of vitrification on ovarian tissue, isolated follicles and short-term cultured follicles?

Summary answer: Vitrification-warming protocol using ethylene glycol as a cryoprotectant causes no severe damage to the ovarian tissue or the isolated follicles.

What is known already: Ovarian tissue cryopreservation (OTC) is used for fertility preservation (the only possible approach applicable for prepubertal girls). To date, more than 200 babies have been born from cryopreserved ovarian tissue. However, a slow-freezing protocol has been used most of the time, and only four babies have been born using the vitrification protocol. This suggests that current vitrification protocols may have an unknown effect on ovarian tissue and the developing follicles. Identifying the effects that vitrification may have on ovarian tissue will make vitrification for OTC more available to everyone while reducing high costs and new equipment requirements.

Study design, size, duration: We obtained 19 bovine ovaries from the local slaughterhouse and processed further by separating the medulla from the cortex. Hematoxylin and Eosin stain (H&E) was then used to calculate the follicle pool. Ovarian tissue pieces, where follicles were not identified, were excluded from the study.

Participants/materials, setting, methods: Ovarian cortical tissue pieces were fixed in Bouin's solution and 4% paraformaldehyde and were used for morphological analysis using H&E, Ki67 immunofluorescence and TUNEL assay. Moreover, bulk RNA sequencing was used, and ovarian pieces with RIN>7 were selected for library preparation. Finally, fresh and vitrified ovarian tissue pieces were used for follicle isolation. The isolated follicles were cultured for up to 6 days, followed by a viability test performed after every two days.

Main results and the role of chance: Vitrification and subsequent warming of the ovarian tissue showed no significant effect on the total population of follicles compared with the fresh tissue. However, from bulk RNA sequencing, we observed 165 differentially expressed genes (DEGs) in vitrified tissue (VT) compared with the fresh tissue (FT).

Vitrified cultured (VTC) pieces showed a significant decrease in monolayered and antral follicles. Moreover, a significant increase was observed in the population of atretic follicles in the fresh cultured (FTC) and VTC pieces compared with their non-cultured tissue pieces. Also, culturing fresh and vitrified tissue pieces for six days increased the proliferation of ovarian stromal cells and showed the presence of DNA damage in the stromal cells of both study groups. The RNA sequencing analysis yielded 1,042 and 1,191 DEGs in FTC and VTC pieces, respectively, compared with their uncultured control groups.

Furthermore, approximately 30% of the isolated follicles (152) from vitrified tissue were viable after isolation compared with the number of viable follicles isolated from the fresh tissue (525). The isolated follicles showed no changes in their viability rate for up to 4 days.

We used a Two-way ANOVA with multiple comparisons to analyse the collected data.

Limitations, reasons for caution: The study's main limitation was the lack of possibility to use the slow freezing protocol to compare the effect between the slow freezing and vitrification methods.

Wider implications of the findings: The vitrification and warming of bovine ovarian tissue did not show significant changes in ovarian tissue morphology. The follicle isolation process and subsequent culturing indicate that the vitrification method could be a way to preserve ovarian tissue and its follicles. However, culturing the tissue pieces affects their transcriptomic profile.

Trial registration number: N/A

Abstract citation ID: dead093.773
P-423 Effect of pergafast 201 on in vitro meiotic maturation of pig oocytes

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Study question: Does pergafast 201 (PF201) have harmful effects on selected markers of *in vitro* maturation of pig oocytes?

Summary answer: PF201 affects the course of *in vitro* oocyte maturation, reaching the metaphase II stage, formation of the meiotic spindle and methylation of selected epigenetic markers.

What is known already: PF201 is a chemical used as a color developer and currently serves as a replacement for bisphenol A and S in related paper and other products. Bisphenol A and S are already proven endocrine disruptors that can released into the environment and from there enter the human body, where they disrupt physiological hormonal functions. In the case of PF201, toxicity to aquatic organisms, persistence in the environment and affinity to some proteins associated with non-infectious diseases have already been confirmed. Therefore, further testing of the biological effect of PF201 is necessary to assess the risks to human health and reproduction.

Study design, size, duration: Cumulus-oocytes complexes (COCs) from the ovaries of slaughtered prepubertal sows were used. *In vitro* maturation dynamics of PF201-treated COCs were compared with control COCs ($n \geq 30$). The meiotic spindle was assessed under a confocal microscope after fluorescence labeling of meiotic chromosomes and spindle microtubules. Dimethylation of histone H3 at lysine K4 (H3K4me2) and trimethylation at lysine K9 (H3K4me3) were imaged under a fluorescence microscope after fluorescent labeling, the intensity was subsequently evaluated in the ImageJ software.

Participants/materials, setting, methods: COCs were treated with PF201 in at least five independent experiments using the following concentrations: 300 pM, 30 nM and 3 μM, dissolved in DMSO to a final concentration of 0.05%. A vehicle control was used when COCs were cultured in medium with the same concentration of DMSO. COCs were cultured for 48 hours to metaphase II in 5.0% CO2 at 39 °C.

Main results and the role of chance: PF201 exposure *in vitro* affected meiotic maturation of pig oocytes, their ability to reach metaphase II, meiotic spindle formation, and epigenetic histone modifications. Treatment with 30 nM concentration of PF201 *in vitro* caused a significant accumulation of oocytes in metaphase I stage (35% vs. 7% in controls, $p < 0.05$), consistent with a non-linear effect. Moreover, according to our results, PF201 also disrupts the formation of the meiotic spindle in pig oocytes and probably affects the organization of the meiotic spindle at multiple levels. After 300 pM (30%) and 30 nM (25% vs. 10% in controls, $p < 0.05$) *in vitro* treatment of PF201, a significant increase in abnormalities of the meiotic spindle, most often irregular arrangement of the spindle, reduction of the meiotic figure, faulty attachment of chromosomes to tubulin and elongated poles of the meiotic spindle located far apart. A significant increase in the relative fluorescence intensity of epigenetic markers was also observed after treatment of oocytes with PF201. For histone H3K4me2, a significant increase in relative fluorescence intensity was observed after treatment with 300 pM and 3 μM concentrations, while for histone H3K9me3, a significant increase in relative intensity was observed after treatment with 30 nM and 3 μM concentrations.

Limitations, reasons for caution: As work with human oocytes encounters a number of ethical and legal regulations, a porcine model was chosen, which is physiologically close to the human oocyte. However, it would be great benefit if the effects of PF201 were evaluated in human discarded oocytes that can be obtained from IVF laboratories.

Wider implications of the findings: It is increasingly difficult for the human population to avoid exposure to environmentally toxic substances, and this fact could explain the decline in human fertility in recent decades. The finding that PF201 negatively affects meiotic maturation suggests, that it may reduce fertilization rates, thus requiring a re-evaluation of its use.

Trial registration number: MZE-RO0718

Abstract citation ID: dead093.774
P-424 Effects of in vitro activation vs fragmentation on human ovarian tissue in culture

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Study question: What is the added value of *in vitro activation* (IVA) protocol compared with fragmentation only in human ovarian tissue culture?

Summary answer: Although histological assessment shows that the IVA increases follicle survival and growth, IVA and fragmentation stimulate extensive and nearly identical transcriptomic changes in cultured tissues.

What is known already: Treatments based on activation of the PTEN/PI3K pathway in ovarian tissue *in vitro* have been administrated to refractory primary ovarian insufficiency (POI) patients and resulted in live births following auto-transplantation of the tissue. However, some studies have shown that mere fragmentation of the tissue produces comparative effects, questioning the added value of chemical stimulation protocol which could potentially activate oncogenic pathways.

Study design, size, duration: Thirty-three ovarian cortical biopsies were obtained from consenting women undergoing elective caesarean section at Karolinska University Hospital Huddinge. The samples were fragmented for culture studies. Half of the fragments were exposed to bpV (HOpic) +740Y-P (IVA group) during the first 24 h of culture while half were exposed to vehicle only (fragmentation only group). Subsequently, both groups were cultured for additional 6 days. Media change was performed every other day.

Participants/materials, setting, methods: Effects on follicles were evaluated by counting and scoring follicles before and after the 7-day culture via HE-stained serial sections. Follicle function was assessed by quantification of steroids by UPLC-MS/MS at the end of the culture. Transcriptomic effects were measured by RNA-sequencing (RNA-seq) of the tissue after the 24-h initial culture step. Selected differentially expressed genes (DEGs) were validated by qPCR and immunofluorescence in independent culture experiments.

Main results and the role of chance: Compared to fragmentation only group, significantly higher follicle survival rate, increased secondary follicle number and sizes were found in IVA group. No significant differences were detected in levels of steroids in culture media on day 7 between the two groups. In our RNA-seq data, when comparing the IVA group to fragmentation only group, only 110 DEGs were found with the relaxed cutoff of FDR<0.1. The signaling pathways affected by IVA involved follicle growth but also inflammation and DNA damage. However, we found that the gene expression was profoundly affected in both IVA and fragmentation only groups compared to the fresh tissue: in total 3,676 and 4,223 DEGs were found (FDR<0.001), respectively. The top enriched gene sets in both groups included several pathways that are known to modulate follicle growth, e.g. PI3K/AKT, MTORC1. We also found that classical steroidogenesis genes (e.g. *CYP11A1, CYP17A1*) were significantly downregulated in both groups after 24-h culture. Moreover, upregulation of genes related to glycolysis, a process that was shown to promote activation of primordial follicles, and its upstream regulator were shown in the RNA-seq data. This effect was validated by qPCR (*ENO1*, *PKM*, *LDHA*, $p < 0.0001$). Future validation will be performed using western blot and immunofluorescence.

Limitations, reasons for caution: The study was performed in an *in vitro* model where tissue was isolated from the regulation of hypothalamic-pituitary-ovarian (HPO) axis. And thus, further experiments with xeno-transplantation may be needed to explore the effect of IVA *in vivo* in the future.

Wider implications of the findings: The impact of 24-h culture on gene expression in ovarian tissue far exceeds the effect of IVA. Yet, follicle growth was stimulated by IVA, which may suggest effects on specific cell populations that are diluted in bulk transcriptomics. Cell type-specific impacts need further studies to conclude about effectiveness of IVA.

Trial registration number: Not applicable

Abstract citation ID: dead093.775

P-425 Biosilk and ovarioids: recombinant silk as a new tool for establishing a 3D-culture system for human ovarian primary cells

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Study question: Can Biosilk be used as scaffold for establishing a 3D-culture system to support attachment and growth of primary cells derived from adult ovarian biopsies?

Summary answer: The use of recombinant spider silk-based scaffold allowed the formation of 3D-structured ovarioids from both cortex and medulla isolated from 5 different patients.

What is known already: Fertility in women is adversely affected by several factors, such as age, environmental pollutants and diseases requiring gonadotoxic treatments. Understanding ovarian cell composition and organization may pave the way to novel fertility preservation methods with the ultimate goal of being applied to clinics. So far, there are no clinically established methods for in vitro growth and maturation of human ovarian follicles leading to mature competent oocytes. Therefore, exploring new 3D-culture systems for *in vitro* reconstruction of ovarian somatic cell niche could lead to development of novel tools to support growth of patient-specific follicles.

Study design, size, duration: Ovarian tissue was collected from gender reassignment patients (GRP) after informed written consent at Karolinska University Hospital Huddinge from 2019 to 2022. After separation of cortex and medulla, the samples were mechanically and enzymatically dissociated into single-cell suspensions and used to evaluate 3D- and 2D-culture methods. Freshly fixed biopsies from cortex and medulla (3x3x1 mm^3) were used as control for the transcriptomic analysis and RNA-FISH assay.

Participants/materials, setting, methods: Tissue was obtained from five patients aged 23-31 years. Dissociated primary somatic cells seeded on Biosilk-foam scaffolds were kept in culture for 2 weeks, followed by detachment, equal division of the foams and suspension culture for additional 4 weeks (BioSilk-Ovarioids, BSO). BSOs were harvested in PFA and RNAlater (n = 6/patient, respectively) for morphological and transcriptomic analysis. Protein ZO1, and cell type-specific marker genes (*AMHR2*, *PDGFRa*, *CLDN5*, *GJA4*) were evaluated via immunodetection and RNA-FISH assay, respectively.

Main results and the role of chance: The sizes of BSOs from both cortex and medulla ranged between 0.5-1 mm at the end of the culture, appearing highly compacted under optical microscope. HE-stained BSO sections revealed that cells were distributed throughout the foams, showing good attachment and distribution. Marker genes were selected for specific cell types [*AMHR2*-granulosa, *PDGFRa*-stroma, *CLDN5*-endothelial, *GJA4*-perivascular cells (Wagner et al. 2020)] and used to study the representation of different somatic cells in the BSOs. The RNA-FISH analysis confirmed the presence of all cell type-specific marker genes, with predominating presence of stromal cells (*PDGFRa*). Newly formed ZO1-specific gap junctions were detected in both cortex- and medulla-derived BSOs, appearing mainly in the outer part of the structures. Interestingly, cell layers surrounding the original Biosilk scaffold were observed, especially in medulla-BSOs. Transcriptomic profiling of the samples showed clear separation to three main clusters by principal component analysis: freshly fixed tissue, 2D-cultures, and BSO. Moreover, clustering analysis of differentially expressed genes (DEGs) showed the presence of gene clusters in fresh tissues affected by both 3D/2D-cultures. Further analyses will focus on identification of significantly affected gene ontologies and pathways, which will further guide the optimization of the BSO culture system.

Limitations, reasons for caution: The tissue was derived from GRPs who always receive androgen treatments prior to surgery. As hormonal treatment may affect the ovarian environment, further trials with untreated patient samples will be needed to generalize the model to fertility preservation patients.

Wider implications of the findings: The establishment and development of the BSO 3D-culture system may enable the construction of patient-specific clinically significant tools for *in vitro* folliculogenesis. This would open new avenues for fertility restoration in patients who cannot receive auto-transplants and treatment of infertility in premature ovarian insufficiency if residual follicles remain in tissue.

Trial registration number: Not applicable

Abstract citation ID: dead093.776
P-426 In vitro and in vivo toxicity of carboplatin and paclitaxel regimen on BRCA-mutated ovarian tissue fragments

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Study question: Does carboplatin and paclitaxel regimen have a toxic cumulative impact on the ovarian reserve of young breast cancer patients with germline *Breast Cancer* (*BRCA*) mutation?

Summary answer: Based on follicle classification and survival through apoptosis and DNA repair mechanism recruitment analyses, gonadotoxic but no cumulative effect was observed in *BRCA*-mutated ovarian tissue.

What is known already: Ovarian function recovery and fertility issues remain major concerns for young patients diagnosed with cancer. Breast cancer is the first cancer diagnosed among women under 40 years and around 10% of them are *BRCA* germline mutation carriers. Beside their predisposition to breast and ovarian cancers, *BRCA* carriers might be more sensitive to chemotherapy-induced gonadotoxicity due to the role of *BRCA* in DNA repair mechanism. Current therapies for *BRCA*-mutated breast cancer patients often include carboplatin and paclitaxel, drugs that are both considered as moderately gonadotoxic. However, the effect of combined therapy in *BRCA*-mutated ovarian tissue is unknown.

Study design, size, duration: Ovarian tissue cryopreserved for fertility preservation before chemotherapy and donated for research by breast cancer patients with or without *BRCA1/2* mutation (< 35 years at diagnosis) were used in this study. Two models were investigated: 3 days *in vitro* culture with carboplatin (10 µg/mL) and/or paclitaxel (1 µM) and ovarian tissue xenograft into mice followed by 3 weeks injections of carboplatin (50mg/kg/week) and paclitaxel (10mg/kg every 3 days). Control conditions were performed for each model.

Participants/materials, setting, methods: Ovarian tissue from 3 *BRCA*-mutated and 3 non-mutated patients were thawed and exposed *in vitro* and *in vivo* to chemotherapy. Follicle density (number of follicles/mm^2) as well as ratios of quiescent/activated and healthy/atretic follicles were evaluated following haematoxylin-eosin staining. Apoptosis and DNA repair mechanisms were analysed using TUNEL/GDF9 co-staining and phosphorylated H2A histone family member X (γH2AX)/ZP-3 co-staining, respectively. Genes expression level will be evaluated on isolated follicles to assess the activation process.

Main results and the role of chance: First, the impact of *in vitro* culture and *in vivo* xenotransplantation was evaluated by follicle counting and classification. A decrease of follicular density (5 to 95% irrespective of patient, model, nor condition) and an increase of activated and atretic follicles was observed after both *in vitro* and *in vivo* experiments in all conditions. However, a slight increase of quiescent follicle pool was observed in the *in vitro* control condition, with no difference between mutated and non-mutated fragments. Then, immunofluorescence staining was performed to evaluate follicle damage. For quantification, 30 to 150 follicles per patient were counted within each condition and classified as positive if at least one cell was stained. Apoptosis was assessed with TUNEL/GDF9 co-staining. In both models, the ratio of positive follicles was higher in fragments treated with chemotherapy (40-60%) compared to control conditions (15-20%). Recruitment of DNA repair complex was analysed using γH2AX/ZP3 co-staining. In both models, an increase in positive follicles ratio was observed in treated fragments (15-40%) compared to control conditions (5-10%). Interestingly, the positive follicles ratio was lower in *BRCA*-mutated (15-18%) than in non-mutated fragments (25-40%) after 3-days *in vitro* culture, irrespective of the treatment used.

Limitations, reasons for caution: Reasons of caution include the high inter-variations between patients as well as intra-variations in one patient regarding follicular density within ovarian tissue. Moreover, *BRCA*-mutated patients included (27.67 ± 2.08) are younger than control ones (33.67 ± 1.53). Ongoing experiments with additional patients should support the results already obtained.

Wider implications of the findings: Carboplatin and paclitaxel combined exposure *in vitro* or *in vivo* does not seem to have a cumulative deleterious impact on ovarian reserve. Ovarian tissue with *BRCA*-mutation did not appear to be more sensitive to chemotherapy exposure. Further investigations focusing on DNA repair mechanisms will be performed in both models.

Trial registration number: Not applicable

Abstract citation ID: dead093.777
P-427 Control of in vitro follicle activation by inhibition of YAP/TAZ activity in mouse ovaries

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Study question: Does verteporfin, an inhibitor of YAP/TAZ activity, prevent from the impacts of chemotherapy exposure on signaling pathways governing follicle activation and survival?

Summary answer: Verteporfin treatment prevents from the chemotherapy-induced impairments of the Hippo pathway but also of PI3K and survival pathways.

What is known already: The Hippo pathway is a crucial regulator of the ovarian reserve. Disruption of this pathway occurring in non-physiological contexts, such as ovarian processing during ovarian tissue preservation or after chemotherapy exposure, leads to massive uncoordinated follicular growth and depletion of the ovarian stockpile. To prevent from the harmful impact of chemotherapy exposure, several studies assessed the potential of inhibition of the PI3K pathway, the major signaling pathway involved in physiological follicle activation. However, the protective effect of this inhibition appeared to be moderate in vitro, suggesting the involvement of other disrupted mechanisms, as Hippo pathway.

Study design, size, duration: This study was performed on post-natal day 3 mouse ovaries. Half-cut ovaries were first treated with 0, 0.2, 1.5 and 3 µM Verteporfin (VERT) during 3 hours in vitro culture to establish the efficient dose of VERT. Whole ovaries were then cultured for 24 and 48 hours and exposed to 10 µM 4-hydroperoxycyclophosphamide (4HC) and/or 3 µM VERT to assess the effect of this inhibitor on follicle activation and survival pathways. (N = 3-5)

Participants/materials, setting, methods: To assess the impacts of sectioning and VERT treatment on follicle activation, gene expression analyses (RT-qPCR) were used to assess Hippo, PI3K/AKT/mTOR and apoptosis signaling pathways. The potential preventive effect of VERT co-treatment with 4HC exposure was evaluated by gene (RT-qPCR) and protein expression (western blot) analyses of these three pathways, and completed with histological staining for DNA damages (TUNEL).

Main results and the role of chance: Following exposure to increased concentration of VERT for 3 hours, a significant impact of the inhibitor was observed at 3µM on *Ccn2* ($p = 0.003$) and *Cmyc* ($p = 0.045$) expression levels compared to control, without impacting on apoptosis genes expression. Exposure to 10 µM 4HC induced a higher YAP/pYAP protein ratio and *Ccn2* expression level compared to control after 24 and 48 hours of culture, confirming the Hippo pathway disruption. VERT co-treatment was able to prevent from this increase of protein levels and gene expression, although the impact on *Ccn2* expression was moderated after 48 hours of culture. Assessment of PI3K signaling revealed an induction of mTOR signaling following 4HC exposure at both time of culture as shown by the higher pRPS6/RPS6 ratio compared to control. Surprisingly, VERT co-treatment significantly

decreased this level compared to 4HC alone at 24 and 48 hours of culture. This result suggests an indirect impact of this inhibitor on the PI3K pathway. Despite *Bax* and *Bcl2* gene levels remained stable among conditions, ovaries exposed to 4HC had a significant higher level of DNA damages at both culture timepoints compared to control. Notably, VERT co-treatment was able to decrease the 4HC-induced gonadotoxicity at 48 hours of culture.

Limitations, reasons for caution: This study evaluated the impact of an inhibitor to control follicle activation in mouse model, and was limited to the assessment of two main signaling. Although we completed our study with apoptosis analyses, the results should be interpreted with caution as other pathways may be involved into the activation process.

Wider implications of the findings: Our results suggest that verteporfin is a promising inhibitor to control acute signaling pathway impairments under unphysiological conditions. Moreover, this study sustains the presence of a potential interaction between the two main signaling pathways regulating follicle activation, PI3K and Hippo pathways.

Trial registration number: Not applicable

Abstract citation ID: dead093.778
P-428 Impact of chemotherapy exposure using 4-hydroperoxycyclophosphamide on a male germ cell line (GC1-spg) and testes from prepubertal mouse

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Study question: What are the dynamics of the toxicity from 4-hydroperoxy-cyclophosphamide (4-HC) on male germ cell line and prepubertal mouse testes?

Summary answer: Chemotherapy has dose- and time- dependent harmful effects on genes regulating apoptosis and survival in the two different models.

What is known already: Fertility preservation strategies offered to prepubertal boys during oncological treatment are limited. The development of a noninvasive pharmacological protection of spermatogenic cells during oncological treatments would be a major advancement in the field. Studies in the female gonad have shown that chemotherapy exposure alters the expression profile of miRNAs and that miRNA-mimics administration could be used as an innovative tool to protect germ cells during gonadotoxic treatment. This project aims to assess the acute effect of 4-HC exposure on apoptosis and proliferation in spermatogonia in order to evaluate the differential miRNAs expression profile in male germ cells following gonadotoxic insult.

Study design, size, duration: GC-1 spermatogonial (GC1-spg) cells were cultured for 48h and exposed during the last 24h to different doses of 4-HC (20, 30 and 50µM) before assessing apoptosis and proliferation to evaluate the gonadotoxic dose of this agent. Cells were then exposed to 4-HC-50µM and apoptosis/proliferation were evaluated after 1,4,8,12,16,20 and 24h to establish the acute timepoint of gonadotoxicity. Similar experiments were then conducted on post-natal day 3 mouse testes during a culture of 24h. (N = 3)

Participants/materials, setting, methods: Histological analyses were performed to detect early apoptosis within spermatogonia cells using DDX4 and Cleaved Caspase 3 (CC3) co-immunostaining. DNA damages were evaluated on testes using TUNEL assay. A large screening of apoptosis gene expression was performed on cells with Taqman array (RT-qPCR). Apoptosis and cell proliferation were further assessed in both models through gene expression analyses of CC3 and Ki-67, respectively.

Main results and the role of chance: After 24 hours of in vitro exposure, a significant increase of apoptosis was observed in the cells treated with 30 µM of 4-HC and 50 µM of 4-HC, reflected by a higher level of CC3 expression in those two conditions compared to the control and the 4-HC-20 µM conditions. The panel screening of apoptosis gene expression performed on cells exposed to 20µM 4-HC during 24h showed no significant difference of expression between the control and the treated condition, sustaining the absence of harmful effect of 4-HC at this concentration in our model. The different timepoint analyses on cells using 50µM 4-HC have shown that chemotherapy-induced CC3 expression occurs approximatively 20 hours after the initiation of the treatment ($p = 0.014$). Similarly, there is a significant increase of apoptosis in prepubertal mouse testes following exposure to 50 µM of 4-HC compared to control, while no significant effect was observed at 20 µM 4-HC exposure. In both models, no change in proliferation through Ki-67 gene expression analyses was observed between non-treated and treated conditions. Finally, histological analyses performed on newborn mouse testes previously exposed or not to 4-HC revealed an increase of DNA fragmentation in the treated testes compared to control.

Limitations, reasons for caution: The use of a mouse cell line and *in vitro* cultured mouse testes is a limitation because of the species barrier. The direct exposure to chemotherapy (compared to *in vivo* models) can influence the level of toxic damage.

Wider implications of the findings: Those data characterize the dynamics of the toxic effect of 4-HC on spermatogonia. They will precise the optimal temporal window to detect the modifications in miRNA profiles by sequencing, to address the expression alterations and provide new targets to reduce cyclophosphamide gonadotoxic effects on the testicular germ cells.

Trial registration number: not applicable

Abstract citation ID: dead093.779
P-429 Transcriptomic heterogeneity within cortical ovarian follicle pool in child and adult

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Study question: What are the transcriptomic differences between child and adult ovarian follicles in the human cortex?

Summary answer: Our results suggest that human ovarian cortical follicles show marked differences before and after puberty, with the greatest differences being present in the secondary stage.

What is known already: The ovaries and their main functional structures, follicles, are important for determining the reproductive health. Follicle growth and maturation are controlled by multiple mechanisms, including the hypothalamus pituitary ovarian axis and local paracrine signalling. Although dormant primordial follicles are activated to grow even in infancy, all growing follicles before the first menarche are bound to undergo atresia. Follicle growth activation in infancy and adulthood remains poorly understood. Additionally, child and adult follicles behave differently during *in vitro* culture suggesting differing biology, raising many questions e.g., about their suitability for fertility preservation.

Study design, size, duration: Ovarian cortical tissues from children were collected through fertility preservation program at the Helsinki Children's hospital (Finland). Families of underage patients signed an informed written consent. Adult ovarian tissue was collected from gender reassignment patients at the Helsinki University hospital and Karolinska university hospital after informed written consent. Follicles at different maturation stages were isolated from ovarian cortex for single follicle transcriptomic analysis. Freshly-fixed cortical biopsies (3x3x1 mm^3) were used for validation of transcriptomic analysis.

Participants/materials, setting, methods: Single viable follicles (n = 120, 60/age group) were mechanically and enzymatically isolated from frozen-thawed ovarian tissue from four adults (age >18 yrs) and five children (age 1-

11 yrs) and processed for RNA-sequencing. Individual follicles were lysed, prepared for RNA libraries following Smart-Seq2 protocol and sequenced using NextSeq 500 platform. After quality assessment, 109 follicles were analysed (Adult n = 54, Child n = 55). DDX4, AMH, FOXL2 and DAZL transcripts and proteins localization were evaluated via immunodetection and RNA-FISH assay.

Main results and the role of chance: Principal component analysis (PCA) of all sequenced follicles demonstrated an expected timely progression of their stages based on maturation, and an overall similarity between child and adult. Interestingly, PCA displayed two distinct clusters of follicles in both adult and child samples. The first group contained only growing follicles and, after differentially expressed gene (DEG) analysis, demonstrated lower expression of traditional oocyte markers (group 1). The second group consisted of primordial and growing follicles and had lower expression of traditional granulosa marker (group 2). Group-related differential expression of main oocyte (*DDX4*, *DAZL*) and granulosa (*FOXL2*, *AMH*) marker genes were validated in tissue sections with RNA-FISH.

Downstream analyses focused on group 2 follicles. When child and adult follicles were compared to each other, the highest number of DEGs were found in the secondary stage (primordial n = 32, primary n = 14, secondary n = 387). Pathway analysis of DEG displays enrichment of oestrogen-related related pathways in adult secondary follicles. Furthermore, we analysed DEGs between the follicular stages per age group, finding high number of DEGs between primordial and primary follicles (Adult n = 1663, Child n = 1914). The majority of those genes are unique to the age group (common=512).

Limitations, reasons for caution: Adult ovarian tissue is obtained from gender reassignment patients, who receive androgen treatments prior to surgery. Child ovarian tissue was collected from fertility preservation programme where some patients were exposed to chemotherapy prior to tissue collection.

Wider implications of the findings: The differences between child and adult follicles encourage further investigation of the suitability of child ovarian tissue in current fertility preservation protocols. Understanding transcriptomic changes during early folliculogenesis and reasons for follicle degeneration can help tailor protocols for *in vitro* growth of follicles.

Trial registration number: not applicable

Abstract citation ID: dead093.780
P-430 Characterization of secondary follicles from cultured cryopreserved-thawed human ovarian cortical tissue

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Study question: Can we obtain secondary follicles from cultured cryopreserved-thawed human cortical tissue?

Summary answer: We obtained a comparable percentage of secondary follicles after culture of cryopreserved-thawed human cortical tissue to that reported after culture of fresh human cortical tissue.

What is known already: The complete in vitro maturation of oocytes starting from primary oocytes (present in unilaminar follicles) until mature MII oocytes has been achieved previously by two different multi-step culture protocols. These protocols were applied to culture fresh ovarian cortical tissue from adult cisgender donors. However, the efficiency of these two protocols for growing unilaminar follicles to secondary follicles starting from cryopreserved cortical tissue from cisgender patients has not been investigated. As the ovarian tissue available for fertility preservation is cryopreserved, it is important to understand the potential of cryopreserved unilaminar follicles to mature in vitro.

Study design, size, duration: Cryopreserved ovarian cortical tissue from 4 cisgender adult donors was used for in vitro culture. According to the existing culture protocols, cortical ovarian tissue was cultured either for 8 days using the first step medium (Telfer's medium) reported by M.McLaughlin 2018 (doi: 10.1093/molehr/gay002) or for 7 and 21 days using the first step medium (Xu's medium) reported by Xu 2021 (doi: 10.1093/humrep/deab003).

Participants/materials, setting, methods: Ovarian cortical tissue obtained from adult cisgender donors undergoing oophorectomy for fertility preservation purposes was cryopreserved before chemotherapy. The cryopreserved ovarian cortical tissue was thawed and cut into small pieces. Several pieces were fixed immediately (day 0), the others were either cultured in Telfer's medium or Xu's medium. After culture, follicle survival, growth and morphology were assessed by histology and immunofluorescence.

Main results and the role of chance: We performed quantification of the different follicular stages (primordial, primary, secondary and atretic) after culture and observed that the percentage of secondary follicles increased independently of the culture media used. However, the ovarian cortical tissue cultured using Telfer's protocol resulted in a higher percentage of secondary follicles and lower percentage of atretic follicles compared to that using Xu's protocol. After culture, the ovarian cortical tissue was further immunostained for TUNEL, PCNA, COLLIV, STAR, AMH and KRT19. We observed that secondary follicles present in ovarian cortical tissue cultured in Telfer's medium showed more proliferative (PCNA+) FOXL2+ granulosa cells and less apoptotic (TUNEL+) stromal cells. By contrast, ovarian cortical tissue cultured in Xu's medium showed less proliferative (PCNA+) FOXL2+ granulosa cells and more apoptotic (TUNEL+) stromal cells. Moreover, the expression of AMH was high in granulosa cells of secondary follicles present in ovarian cortical tissue cultured in Telfer's medium and low in Xu's medium. Finally, in both Telfer's and Xu's medium cultures the expression of KRT19 was low in granulosa cells of secondary follicles present in ovarian cortical tissue.

Limitations, reasons for caution: The number of donors was limited and the study lacked fresh ovarian cortical tissue as control. Only the first step in Telfers' and Xu's protocol was performed, hence further culture is required to determine whether complete maturation of oocytes in vitro is possible starting from cryopreserved human cortical tissue.

Wider implications of the findings: Our study showed evidence of follicular growth in cryopreserved-thawed human ovarian cortical tissue after a period of in vitro culture. This is an important first step to achieve in vitro maturation of oocytes from cryopreserved-thawed human ovarian cortical tissue that could be used for clinical applications.

Trial registration number: NOT APPLICABLE

Abstract citation ID: dead093.781
P-431 Effect of biphasic CAPA-IVM on ovarian tissue oocytes of transgender men

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Study question: Can CAPA-IVM (Biphasic in-vitro maturation) with C-type natriuretic peptide (CNP), followed by in vitro maturation (IVM) improve ovarian tissue oocyte (OTO) maturation in transgender patients?

Summary answer: Biphasic CAPA-IVM did not show any significant difference in maturation competence and genetic variations when compared with in-house IVM.

What is known already: OTO-IVM is a method of fertility preservation in patients where prior ovarian stimulation is undesired. It has resulted in live births in cancer and polycystic ovarian syndrome (PCOS) patients. CAPA-IVM has further improved the OTO competency in these patients. Similarly,

OTO could be collected from transgender men without ovarian stimulation during gender reassignment surgery. Even having adequate survival and maturation, OTO-IVM in transgender men has exhibited decreased fertilization rate and severely compromised developmental competency probably due to long testosterone treatment before surgery. For this reason, we sought to investigate whether this compromised potential can be rescued through biphasic CAPA-IVM.

Study design, size, duration: Patients were recruited from July 2022 to December 2022. Ovaries were collected from 4 transgender patients (age= 18-26 years, mean age=21 years) who underwent gender reassignment surgery after testosterone treatment (mean length= 32 months). All ovaries were collected in cold medium (4°C) and manipulation was performed within 30 minutes of the collection for the retrieval of cumulus oocyte complexes (COCs).

Participants/materials, setting, methods: COCs collected during ovarian manipulation were cultured either in in-house IVM medium for 48 hours or in biphasic CAPA-IVM for 54 hours. Following maturation, these oocytes were analyzed for Calcium (Ca^{2+})-releasing potential and developmental competency after ICSI. *In vitro* matured GV (germinal vesicle), MI (metaphase I) and *in vivo* matured oocytes with clusters of smooth endoplasmic reticulum (SERa) served as controls. Genetic analysis was performed on subsequent embryos to detect chromosomal abnormalities in all groups.

Main results and the role of chance: A Total of 133 COCs were collected (44 in CAPA-IVM and 53 in in-house IVM) and both showed a similar maturation rate i.e. 55%. After culture period, the survival rate tended to be higher in the in-house IVM compared to CAPA-IVM (78% vs. 68%, $p = 0.184$). Following ICSI, 8/11 CAPA-IVM and 8/13 in-house IVM oocytes were normally fertilized, comparable to controls (5/5) ($p = 0.195$ and $p = 0.103$ respectively). Blastocyst rates were lower in both CAPA-IVM (1/8) and in house IVM (2/8) compared to controls (3/5), however not significant ($p = 0.071$ and $p = 0.207$ respectively). Ca^{2+}-releasing potential of oocytes was determined as a product of amplitude and frequency, in arbitrary units (AU). The average values for CAPA-IVM (1.61) and in-house IVM (1.58) were similar ($p = 0.97$). Shallow whole genome sequencing (Shallow WGS) of developed embryos showed that 3/5 in PMC group, 3/7 in in-house IVM group were chromosomally abnormal.

Limitations, reasons for caution: The number of included patients is low, so obtained results should be interpreted with caution.

Wider implications of the findings: To the best of our knowledge, this is the first study on the use of biphasic CAPA-IVM for transgender OTO-IVM oocytes. Further investigation on the added value of CAPA-IVM together with the effect of testosterone treatment length might help shape more accurate guidelines for the fertility preservation of transgender men.

Trial registration number: not applicable

POSTER VIEWING
REPRODUCTIVE EPIDEMIOLOGY

Abstract citation ID: dead093.782
P-433 Male infertility information on Swedish fertility clinics' websites: An evaluation of readability, suitability, and quality

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Study question: The aim of the study was to examine online information regarding male infertility on private clinics or university infertility clinics' websites in Sweden.

Summary answer: The results of the present study indicate that there is a lack of fertility information directed at men on the Swedish fertility clinics' websites.

What is known already: Previous research has shown that male infertility can be perceived as a threat to the masculinity and identity. Therefore, online forums are perceived as highly valuable by men seeking health-related information on the Internet, however, there is always a risk of obtaining information that is not evidence-based. For individuals seeking fertility-related information, studies have shown that the quality of Internet websites varies; online information seldom meets standards of truthfulness, reliability, and navigability. There is also a suboptimal quality across medical websites regarding evidence-based information and they have reading levels most appropriate for individuals with at least a high school education.

Study design, size, duration: A cross-sectional design was used to evaluate 22 fertility clinics' websites focusing on male infertility.

Participants/materials, setting, methods: By using standardized methods for evaluating printed and electronic resources, with established reliability and validity, we evaluate how suitable the text on the fertility clinics' websites is for patient purposes that affect both readability and comprehension. The Suitability Assessment of Materials (SAM), and the DISCERN instrument was used.

Main results and the role of chance: Results disclosed that Swedish fertility clinics websites has focus on information concerning women's infertility. Information regarding male infertility was scanty. The suitability as established by SAM, was 59% for the private clinics and 66% for the government clinics. The highest suitability scores for the private clinics were items related to the cultural image, layout, subheadings, and chunking. For the governmental clinics, items were related to summary and review and cultural image. Items rated low were content about interactions for the private clinics, and interactions, writing styles, cover graphic, type of illustrations and motivations for the governmental clinics. The mean readability SAM score for the entire sample was 57%, meaning that the content was geared to readers at least 5th grade, demonstrating that the websites scored lower than the recommended 8th grade level readability, which made the information easy to understand for most visitors. For the DISCERN, one website met the criteria for "excellent" quality, nine were "good", one "fair", one "poor", and five "very poor". Items that scored lowest were clarity of sources, description of risks of treatment, and what would happen without treatment, benefits of each treatment, and affect quality of life.

Limitations, reasons for caution: The focus was to assess the quality of Swedish online information about male infertility on both private and university clinics' websites, regulated by Swedish laws regarding fertility treatments. This may affect the information available on the websites and therefore may not be applicable to websites in other countries.

Wider implications of the findings: Men affected by infertility needs to make various choices from the information published on the fertility clinics websites. The responsibility of the website's information lies with health-care staff. Therefore, the information must be reliable, evidence-based, and up to date so that men can base their choices on reliable facts.

Trial registration number: non applicable

Abstract citation ID: dead093.783
P-434 Anonymous sperm recipients in Spain. What are their perspectives?

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Study question: What are the perspectives regarding sperm donor anonymity among Spanish recipients?

Summary answer: 56.6% of the patients who undergo treatment with donated sperm in a Spanish population would like to choose between the type of anonymity.

What is known already: Spain is one of the few European countries that still maintains the donor anonymity. Spain is the main gamete provider for all European countries. Among other reasons (cross border legislation, donor limitations in other countries, etc.), the Spanish Society of Fertility defend that the anonymity of their donors is the main reason for such high demand from other countries, as well as within the Spanish patients. However, information regarding patient preference and intention to disclose is scarce and contradictory.

Study design, size, duration: A prospective study performed at the only public hospital with own sperm bank within a large territory in Spain. From September 2021 to October 2022. A questionnaire was provided to all women who required sperm donor. A total of 106 women responded the questionnaire.

Participants/materials, setting, methods: The questionnaire included socio-demographic characteristics, as well as their perspectives towards donor anonymity, donor selection, intention to disclose towards the child and opinion on the support received by professionals and relatives/inner circle. Data were analysed using IBM SPSS Statistic programme (V26). $P < 0.05$ was considered significant.

Main results and the role of chance: A total of 46/106 (43.4%) patients were intended solo mothers, 50.9% (54/106) had a female partner and 5.7% (6/106) had a male partner. Mean age was 35.9, 34.3 and 28.8, respectively. There was no statistical difference between level of education among groups. Only 34.9% of the patients live in Barcelona city, whereas 65% have to travel a mean of 1.15 hours to reach the Hospital. Overall, 56.6% of the patients would like to choose between the type of anonymity. Among them, 58.7% are solo mothers, 57.4% female couples and 33.3% heterosexual couples. A total of 76.4% of the patients would like to choose their donor. More than half (61.3%) do not wish to have any relationship with the donor. The rest of them would only want contact if the child asked for it or in the case of a genetic disease. Most patients (96.2%) have intention to disclose the genetic origin of their child, and more than half (58.5%) intend to do so during the age of 0 to 5 years old. Fifty patients (47.2%) feel a lack of support during the process and only 6.6% perceive a negative impact on their reproductive project from the Covid-19 pandemic.

Limitations, reasons for caution: The main limitation is that the study is from a single, public centre and that the questionnaire was self-administered. Also, the questionnaire was limited to women patients, excluding the perspective of donors, men, children and other relatives.

Wider implications of the findings: The ethical, psychological and economical aspects of requiring gamete donation have an important impact on our societies. Some claim that the era of donor anonymity has reached an end, because of direct to consumer DNA testing. Others believe that anonymity protects donors and allows recipients to fulfil their reproductive project.

Trial registration number: Not applicable

Abstract citation ID: dead093.784
P-435 HPV and infertility in couples requiring Assisted Reproduction Techniques (ART).

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Study question: How frequent is Human Papilloma Virus (HPV) infection in both partners of infertile couples referring to our ART center and whats its impact?

Summary answer: Only 6% of infertile couples are positive for HPV and infection has no impact on embryologic parameters.

What is known already: There is limited and conflicting evidence about the relationship between HPV infection in male partners and infertility. Notably, most of the previous studies did not assess the presence of the infection in both partners in the context of ART. Furthermore, even if considered, the influence of the infection of the female partner may be underestimated because only data on adverse pregnancy outcomes were evaluated. Elucidating the HPV-positive status of the two individuals belonging to the couple could open up new scenarios.

Study design, size, duration: This prospective cohort study was performed on 81 couples. The analysis was conducted on semen samples of male patients and on cervical samples of female partners who underwent assisted reproduction techniques (ART) treatment at the infertility unit of Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico of Milan from February 2022 to November 2022.

Participants/materials, setting, methods: The female partecipants were recruited before the beginning of the controlled ovarian stimulation (COS) while male participants were recruited on the day of the female ovum pick-up (OPU). The technique used to assess the presence of the HPV virus was the Real Time PCR (RT-PCR). Personal characteristics, lifestyle habits and sexual habits were investigated as possible favoring elements for the insurgence of HPV infection.

Main results and the role of chance: Among 81 couples recruited, 12 men and 22 women tested positive for high- and low risk HPV viruses. This streins corrisponds to a prevalence of about 15% (95% CI: 9-24%) for males and 27% (95% CI: 19-38%) for females. Among these positive couples, only 5 cases (6%, 95% CI: 3-14%) simultaneously tested positive for both partners: in two pairs they were positive for the same viral strains, in two other pairs there was no concordance between the viral strains found, and in one pair there was only a partial concordance. McNemar's test (a dichotomous evaluation test for data paired aimed at assessing possible associations) was not significant. No differences in embryo developmental failure, "top-quality" embryo formation and "top-quality" blastocyst formation were found between the two groups (positive vs negative couples).

Limitations, reasons for caution: A possible limitation of the study is the small sample size. Additionally we mainly focused on the impact of infection by HPV on embryological variables, without considering developmental pregnancies and live birth rate.

Wider implications of the findings: The very low concordance observed suggests that infection follows intricate pathways, far beyond the trivial view of a ping-pong effect between partners of the couple. These results suggest that HPV infection probably develops independently in the two individuals of the couple, as if there is not a mutual influence.

Trial registration number: NOT APPLICABLE

Abstract citation ID: dead093.785
P-436 Non-Essential Trace Elements in women's biofluids are associated with worse IVF outcomes in euploid single embryo transfer cycles.

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Study question: What is the non-essential trace element exposure impact on the reproductive outcomes of women undergoing IVF treatment?

Summary answer: Increased blood and urinary non-essential trace element concentration were associated with worse IVF outcomes.

What is known already: Lower fertility rates have been observed in recent decades in industrialized regions, increasing the need for assisted reproductive

techniques. Factors responsible for this decline in human fertility include exposure to non-essential trace elements. Since these elements have no known biological functions, they are considered detrimental to the normal function of the organism. The most common elements evaluated so far have been those traditionally considered toxic, such as heavy metals and metalloids (mercury, lead, cadmium and arsenic), for which inconsistent negative associations with different IVF treatment variables have been described.

Study design, size, duration: 51 women who underwent an euploid single-embryo transfer (SET) after PGT-A analysis, were included. Nine non-essential elements (barium (Ba), strontium (Sr), rubidum (Rb), arsenic (As), tin (Sn), cesium (Cs), mercury (Hg), lead (Pb), antimonium (Sb)) were measured in follicular fluid (FF), whole blood (B) and urine (Urine-VOR), all collected at vaginal oocyte retrieval day (VOR), and urine collected at transfer day (Urine-T). These measurements were correlated with IVF clinical outcomes.

Participants/materials, setting, methods: Quantification of non-essential elements in the four biofluids were performed by inductively coupled plasma mass spectrometry (ICP-MS). Urine concentrations were normalized by creatinine, quantified by Jaffe reaction. Generalized linear models were employed to explore ovarian response variables and embryological IVF outcomes (associations, estimated as percentile 20th to 80th increase (95% confidence intervals (CI)). Associations with IVF clinical outcomes were estimated by odds ratio (95%CI). Both unadjusted and age-BMI-race-smoking-adjusted models were applied.

Main results and the role of chance: Participants had a median age of 39 years [Inter Quartile Range (IQR): 31.37, 36.50] and BMI of 22.97 kg/m2 [IQR: 20.63, 25.12], and 50% had never smoked. In adjusted models, significantly negative associations were found between blood Hg concentration and relative frequency of fertilized embryos [0.73 (0.56,0.96), $p = 0.024$], blastocyst arrival [0.64 (0.44,0.93), $p = 0.019$], and euploid embryos [0.60 (0.37,0.98), $p = 0.044$]. We also found negative associations for urine-VOR Sn and blastocyst arrival [0.72 (0.53,0.98) $p = 0.038$] and euploid embryos [0.55 (0.39,0.77), $p < 0.001$]. In the case of urine-T, higher concentrations of St were associated with lower number of retrieved oocytes [0.71 (0.57,0.90), $p = 0.006$], relative frequency of mature oocytes [0.75 (0.62,0.90), $p = 0.003$], fertilized embryos [0.72 (0.62,0.83), $p < 0.001$], blastocyst arrival [0.68 (0.56,0.84), $p < 0.001$] and euploid embryos [0.83 (0.69,1.00), $p = 0.048$]. urine-T Cs was associated with lower proportion of mature oocytes retrieved [0.65 (0.42,0.99), $p = 0.046$] while both Rb and Cs was associated with lower proportion of euploid embryos [0.066 (0.44,0.99), $p = 0.048$; 0.55 (0.34,0.89), $p = 0.019$, respectively]. Regarding IVF clinical outcomes, in fully adjusted models, our results suggest that higher concentrations of As in urine-T were associated with lower probability of live birth [0.03 (0.00,0.36); $p = 0.038$] and reproductive goal (live birth/all participants) [0.03 (0.00,0.36); $p = 0.038$].

Limitations, reasons for caution: Further studies are needed to confirm this association in greater populations, including the measurement of different element species.

Wider implications of the findings: Exposure and circulating levels of these non-essential elements have a significant impact on IVF outcomes. These data highlight the need to further study non-essential trace elements to identify and characterize how may be affecting IVF.

Trial registration number: not applicable

Abstract citation ID: dead093.786

P-437 Breaking the taboo on infertility and access to medically assisted reproduction through better information: a desire shared by young European adults

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Study question: What are the social representations of young Europeans of infertility and medically assisted reproduction (MAR)?

Summary answer: Young people generally expressed a desire for better representation of infertility and access to MAR in their country in order to normalise this subject.

What is known already: There is a lack of research on the representations, knowledge and opinions of people not directly concerned with infertility issues on infertility and the use of assisted reproductive technologies. However, other research with those concerned already shows the strong taboo that this subject represents in various societies and the difficulties that accompany these pathways, to which the young people interviewed refer.

Study design, size, duration: The European Project B^2-InF aims to analyze, from a sociocultural, gender and legal perspective, the information, representations and expectations in the field of MAR in 8 European countries (Albania, Belgium, Spain, Italy, Kosovo, Northern Macedonia, Slovenia, Switzerland), by interviewing young adults (18-30 years old) on their knowledge and expectations regarding MAR and by analyzing the information provided by fertility centres.

Participants/materials, setting, methods: The B^2-InF team conducted and analysed 98 interviews with young people living in 8 European countries (10 to 15 interviews per country), using thematic analysis software (NVivo and AtlasTi). Their selection is based on an intentional sampling to obtain a diversity of profiles in terms of gender, sexual orientation, conjugal status, geographical origin in the country. The interviews were conducted in the national language by members of the research team before being translated into English.

Main results and the role of chance: Young people representations of infertility and medical assisted reproduction (MAR) are often similar regardless of the country they come from.

They identify infertility as a social taboo, especially for women and in some rural areas. For this reason, according to them, recourse to MAR techniques remains largely unmentionable, an act that remains in the intimate sphere and cannot be shared.

However, there are differences between countries, with a greater or lesser focus in the discourse on the traditional family or the reproductive rights of LGBT people.

But whatever the form of parenthood around which the denunciation of a taboo is formulated, it always generates the desire for greater national communication around infertility and access to MAR techniques. The challenge for the interviewees is thus to promote the normalisation of these subjects, which would help to facilitate use of MAR.

In their view, this objective should be achieved through greater information and transparency about existing knowledge on infertility and MAR, that is with the dissemination of less technical and commercial information than that conveyed by the clinics. They expect governmental information campaigns, including dedicated websites, social networks, dedicated courses from school to university, as well as informative leaflets in different health centres.

Limitations, reasons for caution: Few interviews were conducted per country (10-15) and the disparity of profiles is not always homogeneous between these countries. Nevertheless, this difference also provides information on the different countries concerned, particularly in terms of the representativity of specific social groups (rural, transgender or homosexual people for example).

Wider implications of the findings: The analysis of these interviews from a gender, legal and sociocultural perspective allowed the B^2-InF team to propose national and international guidelines for policy makers and medical centres in order to improve information on infertility and MAR.

Trial registration number: not applicable

Abstract citation ID: dead093.787

P-438 Does artificial endometrial preparation for frozen embryo transfers have poorer outcomes compared to other endometrial preparation?

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Study question: Do artificial thaw cycles increase miscarriage rates compared to Letrozole and natural thaw cycles?

Summary answer: After adjusting for potential confounders, artificial thaw cycles had twice the risk of miscarriage compared to both natural and Letrozole thaw cycles.

What is known already: In frozen embryo transfers, miscarriage rates have been shown to be higher with artificial cycle regimen than with natural cycles whereas there is inconsistent evidence regarding live birth rates between the two types of endometrial preparation. Studies comparing artificial to Letrozole thaw cycles also gave inconsistent data as to whether one had better outcomes than the other. There is lack of studies comparing these three types of endometrial preparation with regards to pregnancy, miscarriage and live birth rates.

Study design, size, duration: This is a retrospective study on 712 frozen embryo transfers from August 2019 to March 2021 for women between 23 to 45 years old. The endometrial preparation distribution is as follow: 288 Letrozole cycles, 200 artificial cycles and 224 natural cycles. Only single blastocyst transfers were included. Exclusion criteria included women with endometrial cancer/hyperplasia, recurrent pregnancy loss, Mullerian anomalies or sexual dysfunction, cycles with preimplantation genetic testing and use of donor gametes.

Participants/materials, setting, methods: Anonymised data of patients undergoing frozen embryo transfers were obtained from the database in a fertility centre in Singapore. Primary outcome is miscarriage rate and secondary outcomes are pregnancy and live birth rates. Data was analyzed with Stata. Continuous variables of the different groups were compared using Student's T-test while chi-square test was used for dichotomous variables. Logistic regression analysis was performed to assess the effect of independent variables on the outcomes.

Main results and the role of chance: Artificial thaw cycles had the highest miscarriage rate at 43.8%, compared to 25.3% in natural thaw and 33.0% in Letrozole thaw cycles ($p = 0.028$). Clinical pregnancy rate was highest in artificial thaw cycles at 48.0%, compared to 40.6% in natural thaw and 34.7% in Letrozole thaw cycles ($p = 0.013$). Live birth rate was highest in natural thaw cycles at 30.3%, followed by 27.0% in artificial thaw and 23.2% in Letrozole thaw cycles ($p = 0.028$). After adjusting for confounding factors, artificial thaw cycles have approximately twice the risk of miscarriage compared to Letrozole thaw ($p = 0.007$) and natural thaw cycles ($p = 0.032$). The chance of pregnancy was 1.5 times higher in artificial compared to Letrozole thaw cycles ($p = 0.027$) but similar to that in natural thaw cycles. The chance of a live birth in natural thaw cycle was 1.6 times higher than that in Letrozole thaw cycle ($p = 0.027$) but similar in artificial thaw cycle. The mean age of participants in the artificial group was significantly lower at 33.9 years, compared to 35.3 years and 35.5 years in the Letrozole and natural group respectively ($p < 0.001$). However in multivariate analysis, increasing age did not have a significant impact on either miscarriage ($p = 0.907$) or live birth rate ($p = 0.066$).

Limitations, reasons for caution: The retrospective nature of this study may have resulted in selection biases and unrecognized confounders may influence the results of this study.

Wider implications of the findings: A possible hypothesis which can explain higher miscarriage rates in artificial thaw cycles may be insufficient progesterone support due to the lack of corpus lutea unlike in natural or Letrozole thaw cycles. Optimizing progesterone support in women with low progesterone levels undergoing artificial thaw can be further explored.

Trial registration number: not applicable

Abstract citation ID: dead093.788

P-440 Equity in reproductive health: A systematic review of factors influencing higher maternal mortality among Black Women in the United Kingdom

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Study question: What factors influence maternal mortality (MM) among women of black origin in the UK?

Summary answer: There are multiple biomedical and systemic factors affecting black maternal mortality including late antenatal booking, poor communication, pregnancy complications and medical comorbidities.

What is known already: One of the key factors impacting global equity in reproductive health is maternal mortality. A maternal death is defined as a death occurring during pregnancy, childbirth or within six weeks postnatally. Black women in the United Kingdom (UK) are over three times more likely to suffer a maternal death than white women. In the UK, the maternal mortality ratio is 10.9 women per 100,000. This has significant implication for fertility and reproductive health equity. There is currently no systematic review of the factors contributing to maternal mortality in black women in the UK.

Study design, size, duration: A systematic literature review was conducted searching Medline, Embase, Global Health, Maternity and Infant Care, and Web of Science databases from their conception till September 2022. Only papers in English language covering a study period from 1970-2018 were included in this study. All peer-reviewed manuscripts irrespective of language or study design were reviewed. The reviews were conducted according to Preferred Reporting Items for Systematic Reviews (PRISMA) guidelines.

Participants/materials, setting, methods: Participants were black women with experiences of maternal care in the UK. Peer-reviewed articles that presented original data which investigated influences primarily on MM in UK women of black descent and, factors that may increase MM risk were included. Papers which included black women without delineating them from other groups of women were excluded. The methodological quality of the studies was assessed using Critical Appraisal Skills Programme and National Institutes of Health checklists.

Main results and the role of chance: A total of 14 studies were included. Biomedical and systemic factors increase black MM risk at the individual, provider (healthcare professionals (HCPs)) and system (the National Health Service) level. Commonly cited biomedical factors including increased risk of pregnancy complications and increased prevalence of medical comorbidities. Systemic factors included late antenatal booking and communication barriers between black women and HCPs.

Qualitative findings from black women and HCPs centred on a theme of 'deficit' including mutual trust, basic maternity care and individualised care. Black women's negative experiences in previous pregnancies often impacted trust levels and subsequently affected receptiveness to messages from HCPs. Regarding deficits in basic maternity care needs, black women spoke on the contrast in their care between the antenatal and postnatal period, viewing their postnatal care more negatively.

However, it is difficult to gauge the full influence of these factors without increased investigation into the other systemic and structural factors that affect black MM including institutional racial biases, stereotypes and microaggressions.

Limitations, reasons for caution: An important limitation was the exclusion of grey literature given the relatively limited evidence base for the proposed research question. However, inclusion may have increased the validity of the conclusions. A further limitation is the studies included tended to lack differentiation between nationality and ethnicity when identifying black women.

Wider implications of the findings: This review is the first to synthesise the literature on factors that influence black MM in the UK. The multiplicity of factors identified highlights the need to incorporate lived-experiences of black

women, improve the cultural competence of HCPs, improve fertility and reproductive health care for black women and reduce MM.

Trial registration number: N/A

Abstract citation ID: dead093.789

P-441 Frequency of sexual intercourse and fecundability among women trying to conceive in Japan

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Study question: To what extent is the frequency of sexual intercourse associated with fecundability?

Summary answer: Frequency of sexual intercourse was the most influential factor in achieving pregnancy but was low among women trying to conceive in Japan.

What is known already: The Japanese National Fertility Survey showed that 39% of married couples had experienced some fertility problems. A recent national survey revealed that 47% of married couples were sexless. The effect of intercourse frequency on time-to-pregnancy (TTP) has not been well investigated in Japan despite the high prevalence of fertility problems and sexless marriages.

Study design, size, duration: We used a social research panel in Japan to conduct an internet-based preconception cohort study of pregnancy planners who were not undergoing fertility treatment. In total, 3,796 women were enrolled in February 2021 and followed up for six months. We used data from 2,371 (62%) participants who discontinued contraception.

Participants/materials, setting, methods: Participants were married women between the ages of 25 and 39 who planned to conceive within a year but were not pregnant at the time of enrolment. Those receiving fertility treatment or trying to conceive for more than six months were excluded from the recruitment. Participants reported frequency and timing of intercourse along with sociodemographic and lifestyle factors. TTP was measured in months, and discrete-time Cox regression was used to estimate fecundability odds ratios (FORs).

Main results and the role of chance: Of the 2,371 women, 31% had intercourse "less than once in a few months" (hereafter, the Lowest Frequency), 54% had intercourse "several times per month" (hereafter, the Middle Frequency), and 15% engaged in intercourse "several times or more per week" (hereafter, the Highest Frequency). Higher frequency groups were younger, were married for a shorter period, and were less likely to have kids (all *Ps* for trend<0.05). Adjusted FORs of the Middle and the Highest Frequency groups were 2.20 (95% confidence interval [CI]: 1.56—3.10) and 3.41 (95% CI:2.29—5.08), respectively, resulting in a shorter TTP compared to the Lowest Frequency group. Covariates showing significant associations with fecundability were timed intercourse of fertile window (adjusted FOR=1.76, 95% CI: 1.37—2.27), folic acid and/or multivitamin use (adjusted FOR=1.44, 95%CI: 1.12—1.84), and participant age (adjusted FOR=0.96, 95% CI: 0.92—0.99), whereas the other variables, such as partner age, sociodemographic factors, health indicators (e.g., body mass index, cycle regularity), or lifestyles (e.g., smoking, physical activity), did not show significant associations.

Limitations, reasons for caution: Intention to conceive could be a confounder, although we used timed intercourse as a proxy measure. Misclassification may have occurred due to self-reported information.

Wider implications of the findings: Occasional intercourse among pregnancy planners might be linked to the high prevalence of infertility in Japan through a direct reduction in fecundability before the age-related fertility decline. Informing couples about the frequency of intercourse and TTP might help them achieve pregnancy earlier.

Trial registration number: NA

Abstract citation ID: dead093.790

P-442 Establishment of prediction model of pregnancy rate and live birth rate in vitro fertilization-embryo transfer

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Study question: Establishment of prediction model of pregnancy rate and live birth rate in vitro fertilization-embryo transfer

Summary answer: the calibration degree of the prediction model of live birth rate is good, and it is a relatively ideal prediction model of live birth outcome.

What is known already: The prediction model of clinical pregnancy rate has some differences between the average prediction probability of clinical pregnancy and the actual probability of occurrence of the IVF-ET patients, but the calibration degree of the prediction model of live birth rate is good, and it is a relatively ideal prediction model of live birth outcome.

Study design, size, duration: statistically significant influencing factors were obtained by multivariate logistic regression analysis. The influencing factors were used as covariables, and the prediction models of clinical pregnancy rate and live birth rate were constructed by using whether clinical pregnancy was obtained and whether live birth was obtained as dependent variables. The study population data (n = 2021) were randomly divided into the training set and the verification set according to the ratio of 6:4.

Participants/materials, setting, methods: Independent sample t test and nonparametric test were used for univariate analysis, and $\chi 2$ test was used to compare rates.Logistic regression analysis was used for multivariate analysis. The fit degree of the model was evaluated from the two aspects of differentiation degree and calibration degree respectively. Hosmer-Lemeshow x2 was used to test the degree of agreement between the two

Main results and the role of chance: The prediction model of clinical pregnancy rate was established with female age, basal FSH, progesterone level on HCG day, endometrial thickness on HCG day and embryo transfer as predictive variables.

P=exp(1.669-0.069xfemale age -0.056x basal FSH-0.545xprogesterone level on HCG day+

0.063x endometrial thickness on HCG day +0.807x transfer two cleavage embryos +0.803x transfer one blastocyst embryo)

/[1 + (1.669-0.069x female age -0.056x basal FSH-0.545xprogesterone level on HCG day+ 0.063x endometrial thickness on HCG day +0.807x transfer two cleavage embryos +0.803x transfer one blastocyst embryo)]

The prediction model of live birth rate was obtained with female age, basal E2, protocol of controlled ovarian hyperstimulation, progesterone level on HCG day, endometrial thickness on HCG day and embryo transfer as predictive variables.

P=exp(0.135-0.074x female age +0.003x basal E2 + 1.110x ultra long protocol +0.768x long protocol +0.623x antagonist protocol -0.544xprogesterone level on HCG day+0.075xendometrial thickness on HCG day +0.771x transfer two cleavage embryos+0.750x transfer one blastocyst embryo)

/[1 + (0.135-0.074x female age +0.003x basal E2 + 1.110x ultra long protocol +0.768x long protocol +0.623x antagonist protocol -0.544xprogesterone level on HCG day+0.075xendometrial thickness on HCG day +0.771x transfer two cleavage embryos+0.750x transfer one blastocyst embryo)]

Limitations, reasons for caution: The predictive model has not yet been translated into clinical application software

Wider implications of the findings: we hope to use the prediction model to give patients an expected value of assisted pregnancy outcome before treatment, so that patients can have an objective and correct understanding of their own conditions and assisted pregnancy outcome, reduce their psychological burden, and increase the confidence and compliance of treatment.

Trial registration number: not applicable

Abstract citation ID: dead093.791

P-443 Resumption of ovulation during lifestyle intervention in anovulatory women with PCOS and obesity is associated with reduction of serum AMH and androgens concentrations

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Study question: Are there differences in AMH and androgens concentrations between women with PCOS who resume ovulation (RO+) and those remain anovulatory (RO-) during lifestyle intervention?

Summary answer: RO+ women showed significant decrease in AMH and 11β-hydroxyandrostenedione concentrations at three months, and increase in SHBG concentrations at six months compared to RO- women.

What is known already: Lifestyle interventions have been shown to reduce clinical and biochemical hyperandrogenism in women with PCOS. Weight loss of 5-10% may reverse anovulatory status, thereby increasing natural conception rates. However, the underlying mechanisms why some women with PCOS remain anovulatory and others resume ovulation after weight loss are unclear. Reproductive characteristics at baseline and a greater degree of change in endocrine and metabolic features with lifestyle intervention may be crucial for ovulatory response.

Study design, size, duration: We used data and samples originating from an RCT which examined the efficacy of a six-month lifestyle intervention prior infertility treatment compared to prompt infertility treatment on live birth rate in women with obesity. In total of 577 women with obesity were randomized between 2009 and 2012. Anovulatory women with PCOS who were allocated to the intervention arm of the original RCT (n = 97) were included in the current analysis.

Participants/materials, setting, methods: We defined women as ovulatory during or after intervention based on the following criteria either: (1) spontaneous pregnancy; Or (2) Treatment strategy after lifestyle intervention; expectant management; (3) or IUI treatment in natural cycles. The steroid hormones were measured using LC-MS/MS. Multilevel analysis with the adjustment of baseline measurements was used to examine differences in changes in AMH and androgens concentrations between RO + (n=34) and RO- groups (n = 61) at three and six months after intervention.

Main results and the role of chance: At baseline, the mean age was 27.5 ± 3.6 in RO+ group and 27.9 ± 4.1 years in RO- group (p = 0.65). The mean weight was 101.2 ± 9.5 and 105.0 ± 14.6 kg, respectively (p = 0.13). AMH concentrations showed significant differences between RO+ women and RO- women (median and IQR 4.7 [3.2; 8.3] in the RO+ group and 7.2 [5.3; 10.8] ng/mL in the RO- group, p = 0.03). Androgen concentrations did not differ between the two groups. During and after lifestyle intervention, RO+ women had significant decrease in AMH (mean difference: −1.6 ng/mL, 95%CI: −3.0 to −0.20, p = 0.03) and 11β-hydroxyandrostenedione (mean difference: −1.7 nmol/L, 95%CI: −3.1 to −0.41, p = 0.01) concentrations than RO- women at three months. SHBG concentrations were significantly increased in RO+ compared to RO- women at six months (mean difference: 11 nmol/L, 95%CI: 1.1 to 22, p = 0.03). Changes in 11-ketotestosterone (three months mean difference: −0.15 nmol/L, 95%CI: −0.49 to 0.20, p = 0.41; six months: −0.39 nmol/L, 95%CI: −1.3 to 0.57, p = 0.43) and testosterone (three months mean difference: 0.13 nmol/L, 95%CI: −0.32 to 0.57, p = 0.58; six months: −0.29 nmol/L, 95%CI: −0.81 to 0.23, p = 0.27) did not differ at three or six months between groups.

Limitations, reasons for caution: The indirect parameters to confirm ovulatory cycles at the end of lifestyle program and small sample size may limit the robustness of the results.

Wider implications of the findings: Reduction of serum AMH and androgen concentrations during lifestyle intervention is associated with recovery of ovulatory cycles. When our results are confirmed in other studies, serum AMH and androgens concentrations could be monitored during lifestyle intervention to provide individualized recommendations on resumption of ovulatory cycles in anovulatory women with PCOS.

Trial registration number: NTR1530

Abstract citation ID: dead093.792

P-444 The use of socioeconomic deprivation as an explanation of variation in IVF success rates between clinics

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Study question: Can differences in the live birth rate (LBR) between clinics with similar standard operating procedures (SOPs) be explained by variations in socioeconomic deprivation?

Summary answer: Socioeconomic deprivation does not appear to explain differences in LBR between clinics with similar SOPs in this cohort

What is known already: IVF success rates vary markedly between clinics. Numerous factors determine whether an IVF cycle is likely to be successful. It has been suggested that more socioeconomically deprived women are significantly less likely to achieve a live birth per cycle than less deprived women. In the UK, there is considerable variation in the levels of socioeconomic deprivation between different cities.

Study design, size, duration: We undertook a multi-center, retrospective review of prospectively collected data. We included all women undergoing their first IVF/ICSI cycle between January 2016 and December 2020 in eight different clinics belonging to the same IVF group. Clinics were located across England: three were situated in 'very deprived' cities, two in 'predominantly affluent' cities and the remaining cities were 'neither very deprived nor very affluent' i.e. their distribution was not skewed.

Participants/materials, setting, methods: We included all women undergoing their first IVF/ICSI cycle involving transfer of a single, fresh embryo. SOPs for medical and laboratory aspects of treatment were similar across all eight clinics. Socioeconomic deprivation was assessed using the Index of Multiple Deprivation (IMD) determined by the residential postcode of each woman undergoing treatment. Patients were categorized into quintiles according to IMD score. Live birth rates across IMD quintiles were calculated. Statistical analysis was performed using SPSS 22.

Main results and the role of chance: In total, across the 8 clinics, 17171 women underwent an IVF/ICSI cycle. The LBR ranged from 17.9 - 35.7% (p < 0.001) with both upper and lower extremes in clinics in cities classified as 'neither very deprived nor very affluent'. The overall LBR ranged from 23.7-26.0% across the IMD quintiles. This difference was neither linear nor statistically significant. Similar trends were noted whether the treatment was NHS (n = 4992) or self-funded (n = 12179). The proportion of women in each IMD quintile varied significantly (p < 0.001) across the different clinics: in the most deprived quintile this ranged from 3.2 - 22.8% and in the least deprived quintile this ranged from 19.1 - 30.8%. The LBR in each IMD quintile also varied significantly (p < 0.001) across the different clinics. The LBR in the most deprived quintile ranged from 7.1 - 46.1% and in the least deprived quintile ranged from 19.4 - 22.9%.

Limitations, reasons for caution: Our results require caution as the distribution of deprivation amongst women undergoing IVF was not representative of the cities in which clinics were located with a skew towards greater affluence seen. Further unknown variables appear responsible for the significant variation in LBRs between clinics rather than IMD categorization alone.

Wider implications of the findings: The distribution of deprivation amongst women undergoing IVF was not representative of the cities in which clinics were located. Whilst not unexpected due to the cost of IVF, ethical questions regarding access to fertility care are highlighted and efforts to promote inclusion should be prioritized.

Trial registration number: Not applicable

Abstract citation ID: dead093.793

P-445 Prevalence of medically assisted reproduction use and its social and demographic determinants - findings from a German representative survey

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Study question: What percentage of people use types medically assisted reproduction (MAR) in Germany? Is MAR use unequally distributed by social categories and stage of treatment?

Summary answer: The lifetime prevalence of MAR use in Germany is 11.4%. Treatment use varies by social categories and stage of treatment.

What is known already: Increasing postponement of births to higher ages in most European countries contributes to higher risk of infertility and seeking assisted reproductive technologies (ART) or broader MAR. Studies indicate several non-medical factors associated with MAR use. Demographic and socio-economic factors, reproductive history, and attitudes are associated with MAR use. Country context is crucial for understanding MAR use patterns because access and reimbursement schemes differ widely among nations. Retrospective and longitudinal studies suggest the benefit of conceptualizing MAR use as a sequential process. The social and demographic determinants of MAR use differ across treatment stages.

Study design, size, duration: This study uses pre-release data from the first wave of the newly established FReDA panel survey (Family Research and Demographic Analysis), a large-scale representative survey of German residents 18 to 49 years. Information on infertility and MAR use was included in sub-wave W1A, therefore most of the data come from wave W1A, and other sub-waves as needed. We will present results from the official data release at the conference.

Participants/materials, setting, methods: For the lifetime indicator of MAR use participants were categorized into the highest category that they indicated: (0) no treatment, (1) see a doctor, (2) receiving medication only, (3) MAR services (insemination, operations, In-Vitro-Fertilisation, Intracytoplasmatic Sperm Injection). A Brant test shows that the parallel regression assumption for ordinal regression is not met. The less restrictive partial proportional odds model is estimated, allowing coefficients for gender, age, parity, and ever infertile to vary across stages.

Main results and the role of chance: The sample consists of 15,263 persons. The lifetime-prevalence of any MAR use is 11.4%. 3% saw a doctor, 3.1% had medication only and 5.2% used further MAR services.

Women are more likely than men to use MAR at all stages. Those 30-34 years have the highest odds when comparing no treatment to seeing a doctor or higher treatment categories, and when it comes to being in the treatment category of receiving medication or higher. The odds for being in the highest treatment category increase from youngest (<=29 years) to oldest (45+ years) age group. Having children increases the odds of seeing a doctor or higher treatment category, but not the odds for being in any higher treatment category. Having ever experienced infertility is a strong predictor of MAR use at all stages.

Coefficients of the following variables are the same across treatment categories. Having ever been married increases MAR use considerably. The odds of help-seeking are lower in suburban areas compared to cities. Those who evaluate their economic situation as good are more likely to use MAR. Positive child orientation of both partners increases MAR use. Migration background, religion, education, and subjective health are not linked to MAR use.

Limitations, reasons for caution: Characteristics of individuals are based on self-reports that cannot be validated externally. Use of a lifetime-indicator of MAR use could imply that temporal order of explanatory variables and the outcome are inconclusive No further information on duration of infertility, diagnosis, and the timing of help-seeking was available.

Wider implications of the findings: Seeking MAR is a multifaceted process influenced by many social and demographic factors. The use of lower levels of services prior to the use of more invasive services such as ART is common. More studies should focus on MAR, not just ART, to understand inequities in use.

Trial registration number: not applicable

Abstract citation ID: dead093.794

P-446 Sperm Donors' Identity Disclosure: Is It REALLY Crucial? For Whom?

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Study question: What is the actual preference of sperm donors with identity disclosure vs. anonymous donors among recipient patients? Does this selection affect clinical outcomes?

Summary answer: Identity disclosure donation is important for certain sections, while almost half of the patients used anonymous donation. An Identity disclosure-only policy should be considered carefully.

What is known already: A major shift over the last decades focuses on sperm donation identity disclosure. Opposed to historic anonymity, related to heterosexual parents' tendency to avoid conception disclosure to their children, the growing proportion of single women and same-sex lesbian couples has raised this issue to high priority. Recipients' perceptions of anonymity vs. identity disclosure are influenced by family structure.

Study design, size, duration: Israeli regulations permit two sets of sperm donation - local and imported. Israeli sperm donors is anonymous only, while imported sperm donors may be anonymous donors or identity disclosure. This retrospective study included only patients who chose imported sperm donors during 2017-2021.

Participants/materials, setting, methods: This retrospective study included patients who chose imported sperm donation during 2017-2021. 526 and 43 patients who used autologous eggs and egg donation, respectively. The primary endpoint was the type of chosen donor – ID vs. AD. We examined the tendency towards identity disclosure according to demographic parameters and the theoretical impact of donor type selection on reproductive outcome and compared patients who performed autologous treatments vs. egg donation.

Main results and the role of chance: Among patients who used autologous eggs, 385 (73.2%) were single (368 never married and 17 divorced) while 57 (10.8%) and 84 (16%) had same-sex and heterosexual relationships, respectively. 270 (51.3%) patients chose ID compared to 256 (48.7%) who selected AD. Single women had a significantly higher probability to choose ID compared to heterosexual couples (55.6% vs. 33.3%, OR 2.5, 95 CI 1.52-4.11, $p < 0.001$). Same-sex couples were also more likely to choose ID (49.1%) compared to heterosexual couples with a statistical trend (OR 1.93, CI 95% 0.97-3.85, $p = 0.06$). 2501 vials were imported, 698 IUI and 812 IVF cycles were performed, respectively, resulting in 283 pregnancies without differences between patients who chose ID vs. AD. To assess donor type selection stability over time we explored 105 patients' selection who changed their donor. 81.9% of patients preserved their initial preference (46 and 40 who chose ID and AD, respectively, $p > 0.05$). ID selection among ED patients was 44.2% compared to 51.3% among autologous patients without reaching statistical significance.

Limitations, reasons for caution: Religious and cultural aspects, which differ from societies and countries, contribute to patients' perspectives and preferences. Implementation of current findings to other societies may be challenging. Research retrospective design may be attributed as an additional limitation.

Wider implications of the findings: An Identity disclosure only policy may result in a global sperm donations shortage which will impact a substantial fraction of recipients who do not select or seek contact with ID. A double path policy, seems like an optimal and preferable solution in this era.

Trial registration number: not applicable

Abstract citation ID: dead093.795

P-447 Gestational weight gain in relation to time-to-pregnancy and conception by assisted reproductive technology

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Study question: Does the gestational weight gain trajectory differ across levels of time-to-pregnancy (TTP) for spontaneously conceived pregnancies and pregnancies conceived by assisted reproductive technology (ART)?

Summary answer: Women with ART pregnancies had lower weekly weight gain during the third trimester compared with women with spontaneous conceptions with TTP ≤3.

What is known already: Studies suggest that both prolonged TTP and conception by ART may increase the risk of pregnancy complications and affect perinatal outcomes such as birthweight. Suboptimal gestational weight gain might further increase the risk of adverse pregnancy outcomes. However, it remains unclear whether there may be differences in gestational weight gain according to TTP and ART, which contribute to the subsequent risk of adverse pregnancy outcomes.

Study design, size, duration: We studied 69,121 singleton planned pregnancies contributed by 60,847 women participating in the Norwegian Mother, Father and Child Cohort Study. Participants completed questionnaires at 15 weeks' gestation (study entry), 30 weeks' gestation, and six months postpartum on sociodemographic, reproductive, and behavioral factors. Participants reported their TTP, and up to four weight measurements and corresponding gestational age from preconception through delivery. We identified use of ART in the Medical Birth Registry of Norway.

Participants/materials, setting, methods: We fitted gestational weight gain trajectories using mixed-effects linear regression models and included an interaction term between time (gestational weeks) and the exposure groups, TTP ≤3 (reference); TTP 4-6; TTP 7-11, TTP ≥12 months, and ART, to assess differences across levels of TTP and conception by ART. The analyses were adjusted for maternal age at start of pregnancy, pre-pregnancy height, educational attainment, pre-pregnancy smoking, parity, and gestational age at birth.

Main results and the role of chance: The adjusted average weekly weight gain was -15g (95% CI: −19; −12) during the first trimester, 620g (95% CI: 615; 624) during the second trimester, and 491g (95% CI: 489; 494) during the third trimester. When we compared differences in average weekly weight change during the first trimester, we observed little difference between the exposure groups. During the second trimester, compared with TTP≤3 months women with TTP 4-6 months, TTP 7-11 months or TTP ≥12 months gained on average 7g (95% CI: −18; 3), 19g (95% CI: −33; −5) and 15g (95% CI: −29; −1) less per week, respectively, whereas women with ART pregnancies gained 11g more per week (95% CI: −13; 34). However, the results for TTP 4-6 and ART pregnancies were imprecise. During the third trimester, average weekly weight gain did not differ according to TTP for spontaneous conceptions, while women who conceived after ART gained 35g (95% CI: −49; −22) less than women with TTP≤3 each week.

Limitations, reasons for caution: Because TTP and gestational weight gain were self-reported, non-differential misclassification may have influenced our results.

Wider implications of the findings: Decelerated weight gain during the third trimester in ART pregnancies compared with women who conceived spontaneously within 3 months might be associated with adverse pregnancy outcomes. Although our findings are imprecise and need to be replicated, monitoring gestational weight gain trajectories might support identification of pregnancies at increased risk.

Trial registration number: Not applicable

Abstract citation ID: dead093.796

P-448 The impact of assisted reproductive technology for second births: a follow-up study

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Study question: What is the probability and the modality of conception of a second birth according to the modality of conception of the first one?

Summary answer: Assisted reproductive technology (ART) plays a minor role in the achievement of second births and the fulfilment of the desired family size.

What is known already: Although fertility rate has been declining and is currently at 1.30, the number of desired children of Italian couples is reported to be at two. Moreover, the desire for children is high even after the age of forty. Recent evidence suggests that ART mainly helps women to obtain their first child, rather than satisfying the desired family size. However, evidence regarding the rate of women who underwent ART and returned to ART centres for other children is very limited and the role of ART in fulfilling couples' reproductive intentions is a challenging issue to investigate.

Study design, size, duration: We conducted a population-based study based on administrative data from regional healthcare databases of Lombardy Region, including 431,333 first deliveries conceived naturally and 16,837 first deliveries occurred after ART between January 1st, 2007 and December 31st, 2017. The probability of a second live birth was longitudinally assessed up to 2021.

Participants/materials, setting, methods: The probability of a second delivery after a natural or medically induced pregnancy, stratified by maternal age at first birth, was estimated with the Kaplan-Meier method. Cox proportional hazard models were fitted for estimating the hazard ratio (HR) and 95% confidence interval (CI) of the association with the mode of conception of the first pregnancy. Right censorship occurred if a woman moved out of region, died, or did not deliver by the end of follow-up.

Main results and the role of chance: The probability of a second birth after a first pregnancy with ART was half of that observed in women with a previous natural pregnancy, being 32.1% and 58.6%, respectively (HR = 0.68, 95%CI:0.66-0.70). The probability to have a second natural pregnancy in women who conceived naturally was 59.3%, while 1.1% had ART. However, among women who conceived with ART the probability of a new spontaneous pregnancy was also higher (25.2%) compared to those who had a baby with ART (11.5%). Natural pregnancy after a first successful ART attempt was overall less frequent than a second natural conception in fertile women. Thus, a consistent proportion of infertile women did not achieve the most common intended number of children. Determinants of this results could not be reliably assessed with our study.

Limitations, reasons for caution: We have no information on women who underwent ART and failed; this may underestimate of the actual return rate to ART. Furthermore, we do not know if women changed the partner between the first and second child.

Wider implications of the findings: In the ART field, more attention should be given to satisfy the desired family size, as the main goal is to not only reduce childlessness. Studies are warranted to reveal the determinants behind the infrequent return to ART. Identifying possible solutions could help women achieving their intended number of children.

Trial registration number: not applicable

Abstract citation ID: dead093.797

P-449 Live birth rates after breast cancer among women who desired a child: a French regional study

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Study question: What are the live birth rates after breast cancer (BC) among women who desired a child?

Summary answer: In our cohort, after treatment for a BC, a live birth is achieved for 36.2% of woman who desire a child.

What is known already: In France, BC is the most common cancer among women under the age of 40. From 38 to 70% of women have not fulfilled their parental plans at the time of diagnosis. The gonadotoxicity of the treatments and the follicular physiological decline linked to age can become an obstacle to this project. Many studies on the subject do not take into account the desire for pregnancy or are interested in the pregnancy rate rather than the birth rate.

Study design, size, duration: In our retrospective, descriptive and observational study, 386 patients treated for BC at the regional BC treatment center (CGFL) between January 2011 and December 2018 (at least 4 years after their treatments), were identified. A total 192 patients met the inclusion criteria (aged 18-39 years old and histologically proven breast cancer). We excluded metastatic cancers, cancer *in situ* and pregnant patients at diagnosis. In January 2022, eligible participants were contacted.

Participants/materials, setting, methods: A total of 124 patients agreed to participate in the study. The included patients filled out a self-questionnaire concerning information about their desire for pregnancy after the breast cancer treatments, live births after breast cancer treatments and the place of fertility preservation. Data were collected from the patient's electronic medical and therapeutic records. The primary endpoint of this study was the live birth rate.

Main results and the role of chance: The patient participation rate was 64.6%. The mean age at diagnosis was 33.7 years old. Fertility was preserved by oocyte cryopreservation in 13.8% of patients (17/124).

Among women who desired a child after BC and in whom pregnancy had been approved, the overall rate of live births was 36.2% (21/58). Most achieved pregnancies were spontaneous (90.5%). No factor was significantly associated with the absence of obtaining birth (i.e. the BC biological subtype or the different BC treatments received). Of these 21 patients, 3 had cryopreserved their oocytes but none used them.

Among 23 patients who had a fertility preservation consultation, 17 preserved *via* oocyte cryopreservation. Only one patient (5.9%) used her preserved oocytes resulting in a miscarriage.

When patients were medically allowed to start a pregnancy the median time to conception in patients who received chemotherapy was 8 months [1.0 - 60.0] *vs* 2 months [1.0 - 7.0] in women who did not receive chemotherapy.

Limitations, reasons for caution: Even though our BC-patients' cohort is a good reflection of the all-French population of one region (Burgundy) since all patients are treated almost exclusively at the CGFL, the main limitations are the retrospective nature, the declarative character, and a short follow-up for the last included patients.

Wider implications of the findings: The non-negligible proportion of live births following spontaneous pregnancy after breast cancer allows us to be reassuring for patients. However, the emergence of new chemotherapy protocols whose consequences on long-term gonadotoxicity are still not well known requires further studies and prompts the promotion of fertility preservation as a precautionary measure.

Trial registration number: sans objet

Abstract citation ID: dead093.798

P-450 Prediction models for estimating treatment independent conception in unexplained infertility – a systematic review

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Study question: What are the best prediction models which can estimate the chance of natural conception in couples with unexplained infertility?

Summary answer: The best quality clinical prediction models were those generated by Hunault et al (2004), Van Eekelen et al (2017) and McLernon et al. (2019).

What is known already: Although couples with unexplained infertility have a chance of natural conception, a formal estimate of prognosis is not part of routine clinical decision making in most settings. In the UK, NICE recommends IVF after two years of unexplained infertility in all couples, regardless of their individual chances of conception without treatment. This approach risks overtreatment for some whilst delaying access to IVF for others. Since the last systematic review on this topic 10 years ago, several models which aim to predict natural conception in infertile couples have been published, but few are in routine clinical use

Study design, size, duration: We searched OVID MEDLINE, OVID EMBASE, and OVID PsycINFO systematically for primary articles published between 1978 and 2022, reporting on the development and/or validation of models in predicting spontaneous conception, pregnancy, or live birth. No language or any other restrictions were applied.

Participants/materials, setting, methods: We included couples with unexplained infertility / those with no major barriers to natural conception. Women who underwent any form of fertility treatment immediately after the initial diagnostic work up were excluded. The methodological quality of the included papers was assessed using criteria within the CHARMS checklist. Risk of bias was evaluated using the PROBAST tool while discrimination and calibration results, which rate the performance of the prediction model, were also reported.

Main results and the role of chance: Eighteen publications reported on 23 prediction models with natural conception, pregnancy, ongoing pregnancy and live birth as outcomes. Of 11 studies involving model development, internal and external validation were reported in 4 and 2 publications respectively . Two studies published extended versions of the same model (Hunault et al, 2004) while 3 studies focussed on independent validation of existing models. The methodological rigour of the models has improved over time, as demonstrated by accuracy measures including discrimination and calibration. Three models (Hunault et al, 2004, Van Eekelen et al, 2017, and McLernon et al, 2019) had low risk of bias. The static Hunault model can be used to determine the chance of conception at a single point in time – usually at the conclusion of the initial fertility work up, whereas the Van Eekelen and McLernon models are dynamic models that can estimate chances of conception at different time points. The discriminatory ability of models ranged between 0.59-0.64 in the internal validation and external validation studies. The calibration slope for the Hunault's static model was 0.6 to 1.0 and for the dynamic models it ranged from 0.62 to 1.01 for Van Eekelen's model and 0.65to 1.06 for McLernon's model.

Limitations, reasons for caution: The quality of prediction models which predated the CHARMS checklist and PROBAST tool could not be adequately assessed. The population with most models went beyond unexplained infertility and included other couples with a chance of natural conception e.g. mild male infertility and minimal endometriosis.

Wider implications of the findings: Of the 3 models which are of high quality, the Hunault model can only be used once but the Van Eekelen and

McLernon models have the potential to be used more flexibly, following further external validation in different settings and larger populations.

Trial registration number: not applicable

Abstract citation ID: dead093.799
P-451 Fertility awareness among Tunisian university students: A state-of-knowledge survey

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Study question: To investigate the beliefs and knowledge of university students regarding fertility.

Summary answer: University students are aware of the impact of unhealthy lifestyle on fertility. There are however misconceptions about the ideal age for childbearing and treatment limits.

What is known already: The gradual decline in fertility is currently well documented. Furthermore, the mean age of first-time mothers is increasing, especially in groups with higher educational levels. However, the level of awareness (sensitization) of couples to infertility factors such as female age and environmental factors is not well-known.

Study design, size, duration: A 12 items-questionnaire on infertility aspects « 12QSI » was previously developed and validated. The questionnaire is divided into 3 sections: (i) epidemiology of infertility, (ii) causes and risk factors for infertility, and (iii) treatment of infertility. A seven-month online survey of a random sample of Tunisian students was conducted. Data collection was carried out via Google Forms® by sharing the link on social media platforms and groups.

Participants/materials, setting, methods: The survey was completed by 800 respondents. We retained 775 responses according to the inclusion criteria: complete response, being of childbearing age and attending university education. Statistics were obtained using SPSS® software.

Main results and the role of chance: Participants were 24.6 ± 5 years of age on average. Almost 57% were women. The average knowledge score was 56.7 ± 28%. The lowest scores were found for the questions on infertility treatment (only 15% of correct answers) with an overestimation of the chances of success of Assisted reproductive technology (ART). An underestimation of the impact of age on both male and female fertility was reported. The mean score was significantly better for the group of respondents studying medicine than for the rest (63 ± 32% vs. 48 ± 26%; $p < 0.001$), and for respondents who have faced an infertility problem compared to those who have not (65 ± 28% vs. 56.3 ± 29%; $p < 0.001$).

Limitations, reasons for caution: The representativeness of the sample is not ensured. Survey distribution only through social networks could be a limit.

Wider implications of the findings: Over-optimism regarding the importance of age and the effectiveness of infertility treatments is noted, which can lead to unintended infertility. Educational programs should be developed to address erroneous beliefs and to increase the population's knowledge of fertility.

Trial registration number:

Abstract citation ID: dead093.800
P-452 Economic impact of antagonist stimulation protocol vs progestin primed stimulation protocol in oocyte donor program A retrospective study

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Study question: Is the cost per oocyte lower in antagonist ovarian stimulation protocol vs progestin primed ovarian stimulation protocol in oocyte donation program?

Summary answer: The cost per oocyte is lower in progestin primed stimulation protocol in oocyte donation program

What is known already: 1. The use of progesterone during ovarian stimulation is effective in blocking the LH surge, and it does not affect the number of oocytes collected or the quality of the embryos obtained. Progestins can be used orally and are less expensive than GnRH analogues. These new regimens enable more flexibility and are interesting but their medical and economic significance remains to be demonstrated.

Study design, size, duration: Calculation of cost-effectiveness ratios (cost per mature oocyte achieved) using data derived from retrospective clinical practice in a large egg donor program of a single University Hospital between 2011 and 2021 5677 egg donor cycles identified from the electronic medical records database were eligible.

Participants/materials, setting, methods: Donors fulfilled national legal and medical-guide requirements, 18- 35 y.o, regular menstrual cycles, BMI 18-30 Kg/m2, no relevant medical history and normal karyotype. They were divided into 2 groups(4subgroups): Antagonist FSH stimulation group(subcategory recombinant and biosimilar FSH) and Progestin primed FSHstimulation group(subcategory recombinant and biosimilar FSH). Demographic data were collected and compared between groups. The cost per unit of gonadotrophin, progestin and antagonist was calculated according to the manufacturer's sale price.

Main results and the role of chance: The mean age of women recruited into the study was 26,5 years. The distribution into the different study groups was as follows:

1.1. 3155 (55,6%) in recombinant FSH antagonists group (FSHrA), Mean days stimulation 9,17-antagonists 4,91; mature eggs:13,62
2.2. 1489 (26,2%) in biosimilar recombinant FSH antagonist group (FSHrbA), Mean days: stimulation 9,17-antagonists 5,28; mean mature eggs: 13,37
3.3. 187 (3%) in recombinant FSH progestin primed group (FSHrPP), Mean days stimulation 9,26-desogestrel 23; mean mature eggs: 12,54
4.4. 846 (14,9%) in biosimilar recombinant FSH progestin primed group(FSHrbPP). Mean days stimulation 9,07-desogestrel 24; Mean mature eggs: 13,78

According to clinical outcomes in oocyte donor, we did not find statistical differences neither in the mean of days of stimulation nor in mature eggs collected in each treatment group but there is a higher rate in the range of 0-4 mature eggs collected in Progestin primed group.

The costs for each drug were as follows: 0,33-0,382€ per unit of recombinant FSH; 0,299-0,33€ per unit of recombinant biosimilar FSH; 25,85€ per unit of Ganirelix; 0,14 per unit of Desogestrel.

In conclusion, cost savings can be achieved using FSH progestin primed treatment. The cost per mature oocyte is 52,59€ compared with 58,25€ per oocyte in FSH antagonist group.

Limitations, reasons for caution: Cancellations before reaching pickup are not contemplated,they may increase the total cost of the cycle.

Although the days of stimulation are equivalent in the different groups, the cost of visits including the costs of nursing, infrastructure, patient care time and consumables might be different between the groups.

Wider implications of the findings: Progestins can present an effective option for egg donation programs in terms of cost

Trial registration number: not applicable

Abstract citation ID: dead093.801
P-453 Bacterial semen infections of North-African men: A 10-year retrospective study of 10.386 semen samples between January, 2013 and December, 2022

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Study question: What is the situation of bacterial semen infections in North African men between 2013 and 2022?

Summary answer: The proportion of positive-bacterial-semen-cultures of North-Afrian men was of 5.9%: Tunisian-patients positive rate was of 5.8%. Algerians of 7.9% and of Lybian was of 4.7%.

What is known already: Numerous bacteria can influence spermatogenesis process at different levels and disrupt spermatozoa development, maturation, and transport. Presence of pathogenic bacteria in the male-genital-system has been mainly associated with poor sperm function, leading to infertility. Bacterial semen infections such as infections by Chlamydia-trachomatis (CT), Mycoplasma-hominis (MH) and genitalium (MG), Ureaplasma urealyticum (UU) and parvum (UP), Escherichia coli (EC), Enterococcus-faecalis (EF), Staphylococcus aureus (SA) and haemolyticus (SH), Helicobacter pylori, Streptococcus agalactiae, Gardnerella-vaginalis, Anaerococcus, Neisseria gonorrhoeae (NG), and Pseudomonas aeruginosa are among the most common isolated bacteria, affecting semen quality and interfering with male fertility. (Wang et al., 2021; Farsimadan and Motamedifar, 2020).

Study design, size, duration: Retrospective study including 10.386 Sperm-culture-analyzes performed between January, 2013-December, 2022 of patients from North-Africa (Tunisians, Algerians and Libyans) aged between 19 and 73 years-old (y.o) (average-age: 43.2 y.o ± 7.16). Semen samples were divided in two main groups, Group-1: Bacterial-Semen-Infected-samples and Group-2: Semen without bacterial-infection. The investigation of the situation of North-African men' semen-bacterial-infections was based on the types of bacteria analyzed, the years from 2013 to 2022 (year by year) and the nationalities of the patients.

Participants/materials, setting, methods: 10.386 North-African-patients composed of 79% of Tunisian-patients (n = 8201), 11% of Algerian-patients (n = 1142) and 10% of Libyan-patients(n = 1038) presented to the Laboratory for Semen-infection-diagnosis. Semen-samples were analyzed according to World-Health-Organization(WHO)-guidelines. Real-time-polymerase-chain-reaction (RT-PCR) and biochemical-identification using Vitek2®ID-cards (BioMérieux) were used for bacterial-semen-infections' detection such as with atypical-bacteria, aerobic/anaerobic bacteria. Statistical-analyzes were performed using SPSS22.0 for Windows-software. Kolmogorov–Smirnov-test for normality-analysis and comparisons by Student-t-test/Mann–Whitney-U-test, as appropriate. Pearson/Spearman' tests for correlations were used as appropriate, *P-value* <0.05 was considered as significant.

Main results and the role of chance: A total of 10.386 semen cultures were performed with 613 positive-bacterial-semen-cultures, showing an annual variation rate of 0.7% from the first year to the last year of the study. The proportion of positive bacterial semen cultures was of 5.9% and was stable throughout the study period (4.8% to 7.3%) in except of 2018 (9.5%) and 2022 (12.5%). Leucocytes concentration which is positively correlated to bacterial-semen-infections in our study ($r = 0.165$; $p < 0.001$) have shown a significant increase in its average levels between 2019 and 2021 in comparison with the other years of the study. UU was the most present atypical bacteria in Goup-1 with 270 infected-samples, UP was the second with 134 infected-samples and then MH with 71 infected-samples and CT with 19 infected-samples and MG with 13 infected-samples. For aerobic and anaerobic bacteria in Group-1, EC was present with 17.3%, EF with 10.5%, SH with 5.7%, SA with 5.2% and NG with 2.2%. Positive semen cultures of Tunisian patients in our study presented a rate of 5.8%. Algerian positive bacterial semen cultures' rate was of 7.9% and of Lybian patients was of 4.7%. Our study may represent an update on bacterial-semen-infections of North-African men over the past decade.

Limitations, reasons for caution: Our study is a retrospective-statistical-survey that included patients presenting to the laboratory for bacterial-semen-infection-diagnosis due to a pathology and/or inflammation of the genital tract or for a simple fertility-diagnosis. Meta-analysis studies in addition to more prospective-randomized-controlled-trials in collaboration with other Microbiology/Andrology laboratories are necessary to confirm or deny our results.

Wider implications of the findings: These results should interest epidemiologists, reproductive biology fundamentalists, urologists, microbiologists, gynecologists, and embryologists who want to improve the investigations on the semen bacterial infections in North-African men with or without fertility problems.

Trial registration number: Not applicable

Abstract citation ID: dead093.802
P-454 Assisted reproduction techniques, ovulation induction cycles and spontaneous pregnancies: are there differences in perinatal outcomes?

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Study question: Are perinatal outcomes different in pregnancies resulting from frozen embryo transfers (FET), fresh embryo transfers (IVF-ET), ovulation induction (OI) cycles and spontaneous pregnancies (SP)?

Summary answer: Assisted reproduction techniques (ART) pregnancies are associated with differences in perinatal outcomes when compared to SP, even when different socioeconomic scenarios were considered.

What is known already: Earlier reviews have suggested that IVF-ET pregnancies are associated with higher gestational risks. However, there have been recent advances in the way ART is performed and studies show controversy as to whether IVF-ET and FET singletons are associated with higher perinatal risks and which are these risks. The objective of this study was to analyze if there are differences when comparing perinatal outcomes in pregnancies resulting from FET, IVF-ET, OI cycles and SP.

Study design, size, duration: Retrospective cohort study performed at two medical centers in southern Brazil. Data refers to a period from 2013 to 2022 and was collected from electronic records.

Participants/materials, setting, methods: Data from 6705 singleton pregnancies, divided into four groups: G1, FET (n = 54), G2, IVF-ET (n = 471), G3, OI (n = 121) and G4, SP (n = 6059). Sample included patients from a private reproductive medicine center (G1,G2,G3) and public health hospital (G4) in Brazil. Variables regarding neonatal outcomes were compared between groups and were expressed as mean ± SD/median[IIQ] or n(%). Anova and Kruskal-Wallis tests were applied, considering $p < 0.05$.

Main results and the role of chance: Comparing G1 vs. G2 vs. G3 vs. G4, the following results found, respectively, mean ± DP: maternal age (35.8 ± 5.2 vs. 35.0 ± 3.7 vs. 33.3 ± 3.6 vs. 26.1 ± 6.8, $p < 0.001$); newborn's weight, kg (3364.7 ± 553.4 vs. 3115.6 ± 565.9 vs. 3195.9 ± 506.3 vs. 3165.1 ± 651.5, $p = 0.043$); and length, cm (48.6 ± 2.4 vs. 48.1 ± 2.7 vs. 48.2 ± 2.8 vs. 50.4 ± 1.1, $p = 0.971$); median[IIQ]: gestational age, weeks.-days (39[33-41.3] vs. 38.5[22-41] vs. 39[28-41.5] vs. 39 [25-46], $p = 0.809$); n%: prematurity (11.3 vs. 13.8 vs. 9.8 vs. 8.9, $p = 0.006$); Apgar 5thmin≥7 (100 vs. 98.4 vs. 99.1 vs. 96.4, $p = 0.020$); macrosomia (9.2 vs. 3.1 vs. 3.2 vs. 6.0, $p = 0.028$); C-section (88.6 vs. 91.0 vs. 85.2 vs. 32.0, $p < 0.001$). It's important to highlight Brazil's health care differences between public and private services, as public's poor assistance could explain lower Apgar index and C-sections (performed only if obstetric indication). Even so, higher macrosomia and preterm rates were found after ART.

Limitations, reasons for caution: Women who deliver in public hospitals in Brazil usually have a lower socioeconomic status and less access to adequate prenatal care, which could bring bias in some analysis. Also, there is a sample size discrepancy between groups and no data regarding fertile status in the SP patients.

Wider implications of the findings: The higher prematurity rates and, in FET cases, macrosomia, reinforces previous studies findings on this subject. As infertility is a worldwide growing condition, comparing SP and ART is essential for better understanding the implantation processes that could improve ART's perinatal results in the future.

Trial registration number: not applicable

Abstract citation ID: dead093.803

P-455 Transnational Gestational Surrogacy During Covid-19 pandemics and War in Ukraine. A qualitative, interdisciplinary study of Danish infertile couples' experiences in a world of change

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Study question: How do permanently infertile couples experience surrogacy in cross border reproductive care (CBRC) and which considerations do they have in relation to this?

Summary answer: The couples find surrogacy treatment in CBRC satisfying but feel forced to use CBRC, and estranged from own country, missing reproductive body, and home

What is known already: Permanently infertile couples in Denmark must go abroad if they want a genetically related child, as health care professionals are not allowed to assist in a surrogacy process. Divergent legislation on surrogacy worldwide often creates a demography of permanently infertile couples, moving around depending on which country permits gestational surrogacy. Only few academic studies have described how external circumstances as Covid-19 pandemics and the current war in Ukraine affect the world of surrogacy and even fewer studies have focused on the infertile couples' situation.

Study design, size, duration: Semi-structured qualitative interviews, including a total of fourteen couples, were conducted with permanently infertile couples across Denmark from May 2022 to September 2022. The couples chose place, time and if they preferred to be interviewed individually or together. Eleven couples chose their own home, two preferred online interviews and one interview was held in another place. Each interview lasted for an average of 93 minutes.

Participants/materials, setting, methods: Fourteen infertile couples (N = 23 participants), majority heterosexual, in different stages from planning to use a gestational carrier to having two children through surrogacy were interviewed. Interview themes included "history of infertility", "experiences with surrogacy", "openness about one's own situation", and "considerations about surrogacy". The majority used/had used Ukraine as destination of surrogacy. The interviews were recorded, anonymized, and transcribed. Data were analysed using Malterud's method of systematic text condensation.

Main results and the role of chance: Most couples had a relative in Denmark who offered to be a gestational carrier, but all except one couple declined this offer as they felt very unsecure about the legal situation not only for them, but also for the carrier, and a potential child. Instead, they decided to go abroad, mainly to Ukraine, to have an enforceable transparent contract, professionals to advise and the possibility of using the intended mother's eggs. They didn't feel it as a "choice" but more as the only option to have the longed-for child. Many felt that Danish authorities on purpose tried to obstruct their way home with the new born which contributed to this sentiment of estrangement.

According to Danish legislation if the gestational carrier was reimbursed, the intended mother cannot be granted legal motherhood at any time. This lack of acknowledgement of motherhood was not only a practical issue for the couple, but deeply affected the intended mothers of not being "worthy mothers".

The empirical realities of these couples within the context of a pandemic, a war, and borders that open and close are a confrontation and clear illustration of the different legislations on surrogacy in Denmark and worldwide.

Limitations, reasons for caution: The study participants had all chosen to be part of this study after advertisement in targeted internet forums. Hence, the results may not be directly transferred to all permanently infertile couples regarding attitudes towards being infertile and using surrogacy.

Wider implications of the findings: This study contributes to the understanding of being permanently infertile and the implications of the Danish and international legislation on surrogacy. Findings from our study will be useful to form the academic basis of a near-future revision of the existing surrogacy legislation.

Trial registration number: Institutional Review Board at Aarhus University, Denmark (HE 2022-001)

Abstract citation ID: dead093.804

P-456 Economic evaluations of assisted reproductive technology in high income countries - a systematic review

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Study question: In high-income country settings, what is the evidence supporting economic evaluations of in vitro fertilisation (IVF) interventions?

Summary answer: Most interventions associated with IVF are high cost for minimal improvement in effectiveness, leading to excessively high acceptability thresholds.

What is known already: The economic burden of infertility, and approaches to reduce costs and increase access have been identified in the top 10 research priorities for future infertility research. Meanwhile, there has been exponential growth in use of ART for infertility, especially in high-income countries. Therefore, it is important to map the landscape of economic evaluations in IVF research to inform health policy and future research.

Study design, size, duration: A systematic review of studies of an IVF intervention together with an economic evaluation conducted in high income countries between 2011 and 2022 was completed. Seven electronic databases were searched for eligible studies

(MEDLINE, PUBMED, EMBASE, COCHRANE, ECONLIT, SCOPUS or CINAHL).

Participants/materials, setting, methods: Studies assessing a component of IVF with a cost effectiveness, cost benefit, cost utility or cost minimization assessment were included. Studies were examined to assess their

chosen outcome measure, perspective, completeness of reporting and cost effectiveness of

the intervention studied. Cost data were converted to USD, taking inflation into consideration.

Main results and the role of chance: Of the 40 included studies, 62% evaluated an unselected population and 68% used livebirth as the effectiveness outcome measure. Modelling studies predominated (65%) over those alongside trials. Most (80%) spoke from a health funder perspective.

There were 11 IVF interventions assessed together with an economic evaluation. The incremental cost effectiveness ratio was greatest for those interventions where the intervention did not improve efficacy significantly. The intervention of PGT-A was assessed as either less effective and more expensive, or more effective, but costing up to $600,000 USD for each additional livebirth. Likewise, intracytoplasmic sperm injection for non-male factor infertility, conferred minimal benefit but cost up to $72000 USD per additional livebirth.

Choice of agents for ovarian hyperstimulation were assessed in 15 included papers. Recombinant FSH is more effective and slightly more expensive than biosimilars.

The direction of effect of the cost effectiveness findings was the same for the groups of studies assessing IVF, ICSI, PGT-A and embryo transfer number, increasing confidence in the findings across different settings and funding models.

Limitations, reasons for caution: Systematic reviews of economic evidence rely on assumptions of evidence in included studies. The data on health outcomes (e.g. pregnancy rates) were sometimes optimistic compared with findings in evidence from large trials or systematic reviews. There is variable

reporting quality which might increase uncertainty around the cost effectiveness results.

Wider implications of the findings: Economic evaluation of IVF interventions is important for state funders and individuals who pay for treatment. Awareness of the real price per outcome allows consideration for funding to be applied more effectively. Multi-faceted decision making is needed. Cost effectiveness alone should not champion interventions that may otherwise have adverse consequences.

Trial registration number: not applicable

Abstract citation ID: dead093.805
P-457 A biosimilar FSH is a cost-effective option for women undergoing IVF/ICSI treatment in France

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Study question: Is Bemfola® a cost-effective treatment option compared to Gonal-f® for women undergoing IVF/ICSI treatment in France based on real-world evidence?

Summary answer: Bemfola® is a cost-effective treatment option, based on real-world evidence, demonstrating lower gonadotropin costs per live-birth following one ovarian stimulation cycle compared to Gonal-f®.

What is known already: Since Bemfola®'s first launch in 2015, real-world data has been collected in France, comparing it with the originator Gonal-f®, in the REOLA study (Barriere P, *et al*., J Gynecol Obstet Hum Reprod. 2023;52:102510). This study demonstrated comparable clinical outcomes according to follicle stimulating hormones (FSH) starting dose between the two FSH, in terms of oocytes retrieved, cumulative live-birth rate (CLBR), and total FSH dose per cycle. Previous health economic studies comparing the cost-effectiveness of Bemfola® against Gonal-f® utilise clinical trial data and therefore leave ambiguity over the cost-effectiveness as the results may not reflect what happens in clinical practice.

Study design, size, duration: We developed a decision-tree cost-effectiveness model with a one-year time horizon based on the gonadotropin costs and CLBR per ovarian stimulation for Bemfola® and Gonal-f®. The output was gonadotropin cost per live-birth following one ovarian stimulation cycle. CLBR per ovarian stimulation data were taken from the REOLA real-world study while acquisition costs were taken from publicly available databases in France. Model structure and methodology were based on previous publications and validated by clinical experts.

Participants/materials, setting, methods: The model used clinical data from REOLA, a non-interventional, retrospective, real-world study conducted in 17 French Assisted Reproductive Technology (ART) centres including data from 2,319 Bemfola® and 4,287 Gonal-f® ovarian stimulation cycles for IVF/ICSI grouped according to starting dose. Data was collected for ovarian stimulations between the dates of 01/01/16 and 28/02/17 and CLBR included a follow-up to live-birth of all pregnancies following both fresh and frozen embryo transfer within 12 months of oocyte retrieval.

Main results and the role of chance: The REOLA study data utilised within the model comparing Bemfola® vs Gonal-f® grouped according to FSH starting dose (< 150 IU, 150 - 224 IU, 225 −299 IU and ≥ 300 IU) are the median total FSH used - 1100 IU, 1500 IU, 2250 IU and 3300 IU vs 1008 IU, 1500 IU, 2250 IU and 3300 IU, and the CLBR - 30.5%, 25.4%, 21.4% and 12.3% vs 27.0%, 27.3%, 19.6% and 12.0%, respectively. The gonadotropin cost per live-birth for Bemfola® was €2,302.12 compared to €2,856.26 per live-birth for Gonal-f®, saving €554.15 per live-birth. The main reasons for this cost saving was the lower acquisition costs of Bemfola® of €489.01 compared with Gonal-f® costs of €597.36 on average per ovarian stimulation. As this analysis is based on real world data as opposed to results from clinical studies it can be used to estimate potential gonadotrophin cost savings in clinical practice in France. With 27,861 babies born annually in France using ART it can be estimated that if all had been born following Gonal-f® usage then a change to all being born following Bemfola® usage could result in savings sufficient to fund gonadotrophins for an additional 31,572 ovarian stimulation cycles.

Limitations, reasons for caution: The model assumed equal distribution of Bemfola® and Gonal-f® across the starting dose categories and have assumed that the number of fresh and frozen cycles required following ovarian stimulation would be equivalent irrespective of the gonadotropin used.

Wider implications of the findings: Bemfola® is cost-effective compared to Gonal-f® for IVF/ICSI treatment which would potentially enable more women to be treated with the same healthcare budget for the French health system. This might apply for other countries where Bemfola® is available and benefit from lower acquisition costs than Gonal-f®.

Trial registration number: not applicable

Abstract citation ID: dead093.806
P-458 Egg donation in the Czech Republic: Responsibility of ART clinics in the provision of information to potential egg donors

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Study question: Do ART clinics' websites provide sufficient information for potential egg donors regarding all aspects related to donation?

Summary answer: There is an imbalance of information provided online by ART clinics. The benefits exceed the potential side effects and risks associated with donation.

What is known already: There is high demand for egg donors in the Czech Republic due to the extreme interest of international couples in ART procedures, e.g. because of the affordability of the treatment, the lack of a waiting list or the extended age limit for recipients up to 49 years. Thus, for a population of approximately 10.5 million, there are more than 40 clinics specializing in reproductive medicine in the country.

Study design, size, duration: In this research, all information available on ART clinics' websites for egg donors was analyzed. Each clinic has a different advertising and marketing strategy, and in this work, we will compare the information provided by public and private clinics regarding the egg donation process. Five themes have emerged for the analysis: 1. General information 2. Legislation, 3. Benefits, 4. Risks, 5. Advertisement. The duration of this research was 4 months.

Participants/materials, setting, methods: A quantitative-qualitative analysis of recruitment strategies of IVF centers in the Czech Republic (29 unique clinic websites, 12 of which were specifically designed for marketing purposes) based on 16 criteria reflecting different donor needs.

Main results and the role of chance: In the case of donor recruitment, there are registered websites with predominantly suggestive slogans, emotive photos, and only positive stories of donors highlighting psychological, health, or financial benefits. The exact amount of remuneration (20.000 - 33.000 CZK) was specified by 89.7 % of clinics. The lower and upper limit are not regulated by law in the Czech Republic. ART clinics set the limit themselves to attract more donors by offering higher financial compensation and other benefits. This causes a huge imbalance between bigger and smaller ART clinics causing donor recruitment to become the focus of marketing strategies. The potential risks associated with donation were found in only 51,7 % of clinics' websites. Not only is this an important question of legal noncompliance, but the misinformation may also cause disappointment and feelings of betrayal among donors. It may also result in the emergence of anti-campaigns, impacting the number of donors and devaluing donation in general. Therefore, there is a need to find a compromise between marketing strategies and providing a

complete, transparent, and truly informative picture for potential donors in advance of the donation program.

Limitations, reasons for caution: We analyzed all ART providers that presented their facility online, focussing on both private and public providers. Public providers had very little information available about ART treatments in general let alone donor programs, so minimal information was available.

Wider implications of the findings: We believe that it's crucial to inform all donors not only about benefits associated with donation but also of potential risks, so they can make an informed decision about donation and also about their reproductive health and future parenthood.

Trial registration number: TL05000144

Abstract citation ID: dead093.807

P-459 Sexually transmitted infections in women and fecundability – A prospective cohort study

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Study question: Does a previous infection with *Chlamydia trachomatis* (CT) or genital herpes simplex virus (HSV) affect fecundability, and does recency of infection play a role?

Summary answer: Previous infection with CT or HSV in women was not appreciably associated with fecundability. However, a recent CT infection was associated with slightly lower fecundability.

What is known already: Worldwide, 1 million people contract sexually transmitted infections (STIs) every day. CT and genital HSV infections are among the most common STIs. Untreated CT in women can cause pelvic inflammatory disease (PID), which can lead to scarring and narrowing of the fallopian tubes and the lower uterus, thus complicating the ability to conceive. Also, an association between genital HSV in women and PID has been suggested. However, it is uncertain to what extent CT and HSV infections in women are associated with fecundability, defined as the per-cycle probability of conception, and whether recency of the infection plays a role.

Study design, size, duration: We conducted a prospective cohort study of female participants included in two Danish preconception cohorts: SnartGravid.dk ('Soon Pregnant', 2007-2011) and SnartForældre.dk ('Soon Parents', 2011-ongoing). At enrollment, participants had to be non-pregnant, aged 18-45 years, in a relationship with a male partner, not using contraception or fertility treatment, and trying to conceive. Participants completed a baseline questionnaire and bimonthly follow-up questionnaires until conception or up to 12 months after study entry.

Participants/materials, setting, methods: We analyzed data from 10475 women. At study entry, participants reported if they had ever been diagnosed (including year of first diagnosis) with CT or HSV. Fecundability ratios (FRs) were calculated using proportional probability regression models and 95% confidence intervals (CIs). The analyses were adjusted for age, income, educational attainment, other STIs, and smoking, reported at study entry. The reference groups were either women with no CT or no HSV diagnosis.

Main results and the role of chance: At study entry, 2603 (24.9%) of participants reported a history of infection with CT, and 766 (7.3%) with HSV. Using life table methods, 84.7% of participants conceived within 12 cycles of follow-up. Compared with the reference group, the adjusted FR for women ever diagnosed with CT was 0.98 (CI: 0.93-1.04) and 0.96 (CI: 0.88-1.06) for women ever diagnosed with HSV. Compared with the reference group, a recent CT infection was associated with slightly lower fecundability: adjusted FRs for women diagnosed 0-2 years, 2-5 years, and more than 5 years before study entry were 0.84 (CI: 0.71-0.99), 1.01 (CI: 0.90-1.12), and 1.00 (CI: 0.93-1.07), respectively. The association between recency of a HSV infection compared to the reference group was less evident: adjusted FRs for 0-2 years, 2-5 years, and more than 5 years since study entry were 0.93 (CI: 0.72-1.21), 0.97 (CI: 0.77-1.22), and 0.91 (0.75-1.09), respectively.

Limitations, reasons for caution: Misclassification of exposure is possible, as CT and HSV infections are often asymptomatic and hence undiagnosed. In addition, self-report may have resulted in misclassification of infection type and year. Further, we lacked information on severity or frequency of infections.

Wider implications of the findings: Women diagnosed with STIs often experience psychological distress concerning their future ability to conceive. Our finding of no overall association between previous diagnoses of CT or HSV may reassure women wanting to conceive. Still, testing for STIs remains important because untreated infections can damage the female reproductive system.

Trial registration number: Not applicable

Abstract citation ID: dead093.808

P-461 Patients need fertility specialists to improve communication depth, quality and frequency in order to promote transparency, understanding and empathy with the patient

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Study question: What are the key pain-points that patients experience during fertility treatments? Based on patient perception, how can fertility clinics improve their standard of care?

Summary answer: Most patients would like to receive regular updates and more detailed explanation from their doctor, yet this happened in only half of the cases.

What is known already: IVF treatments have an emotional, psychological and economical toll on patients. On previous studies, half the women reported that infertility is the most upsetting experience of their lives, approximately 20% of the males and females had dysfunctional emotional distress or personal difficulties. Patient's needs, pain points, expectations and education, therefore, require further research.

Study design, size, duration: An electronic survey was dispensed via the Embie mobile application or e-mail to women registered to Embie who have given their consent for communication. 98 women completed the survey from December 26th 2022 to January 15th 2023. The respondent profile was: White, Well-educated, from USA, 70% aged 31-40, 44% aged 31-35, heterosexual, tech oriented (100% Embie users), no kids, middle and upper middle class.

Participants/materials, setting, methods: The survey contained questions on demographics, IVF history, understanding of how patients choose their fertility clinic, how they educate themselves on their treatment options, how they communicate with their healthcare providers, awareness of AI, how they fund their treatment, their expectations for the clinic's equipment, usage of new advanced technologies and level of involvement in their fertility care.

Main results and the role of chance: Pricing is a pain-point for IVF patients, yet it does not affect their clinic choice, which is primarily linked to clinic location and doctor reputation. Most patients are willing to pay for modern technology. 58% of respondents state that the doctor discusses with them their chances of having a successful pregnancy, yet only 16% of women confidently know their chances of taking home a healthy baby. >90% of patients state that they would like to receive regular updates and detailed explanation from their doctor, yet this happened in only half of the cases. "Transparency of the process and decision making" is the most important tool for patients to empower themselves, followed by "answering my questions in detail". While 60% feel comfortable approaching the doctor with

questions, 40% state they search for an answer prior to asking the doctor. Almost all patients expand their knowledge before and after discussions with the doctors by Internet and by approaching experienced peers. Patients expect high-tech in clinics and are ready to pay for that. 92% of patients describe at least 1 pain point with their clinic: primarily communication: inconsistent communication (39%), lack of transparency (17%), lack of understanding (12%) and lack of empathy (4%).

Limitations, reasons for caution: The respondents mostly came from the USA and represented a non-diverse group. These findings may not generalize to other geographies or socio-economic groups.

Wider implications of the findings: The more the healthcare provider shares and involves the patient, the better the education they receive, and the less likely they will seek alternative information elsewhere which may be less medically accurate. Healthcare providers must educate themselves on the latest innovative technologies to meet patient expectations.

Trial registration number: n/a

Abstract citation ID: dead093.809

P-462 The current status of female reproductive health and preconception behaviour in the UK: cross-sectional data from over 135,145 Hertility users

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Study question: We sought to characterise the reproductive health of a large cohort of UK-based women to assess the lifestyle of those who were trying to conceive

Summary answer: The vast majority were experiencing one or more symptoms and a substantial number of women who were trying to conceive were leading an unfavourable lifestyle.

What is known already: Data regarding the prevalence of female reproductive health conditions within the UK is outdated, heterogeneous and often collected from cohorts unrepresentative of the current background population. Recent evidence suggests 31% of UK-based women experience at least one severe reproductive health symptom annually (Public Health England, 2018), however this data was also collected from a small population.

Furthermore, although guidance regarding the optimisation of fertility in those trying to conceive (TTC) is available, the uptake of lifestyle changes to preconception behaviour regarding smoking, recreational drug use, alcohol consumption, body weight and physical activity is unknown.

Study design, size, duration: A cross-sectional study was conducted on 135,145 women who were >18 years old and completed a health assessment for Hertility, an at-home women's health testing service available in the UK, between September 2020 and January 2023.

Participants/materials, setting, methods: Data was collected from women who completed a free virtual health assessment (VHA) on Hertility's website consisting of up to 24 questions assessing the user's demographics, lifestyle and medical history. The number of questions presented to the user differed depending on their archetype and only the most recent VHA data was analysed from registered users who completed it multiple times. Counts, means and percentages of completed VHAs were calculated using R software.

Main results and the role of chance: Of 135,145 users, the most common pre-existing reproductive health conditions reported were polycystic ovary syndrome (12.0%, n = 16,181), endometriosis (4.0%, n = 5,393), fibroids or uterine polyps (3.0%, n = 4,092), pelvic inflammatory disease (1.8%, n = 2,387) and premature ovarian insufficiency (0.2%, n = 311). The majority of all users (80.5%, n = 108,833) reported currently experiencing one or more symptoms indicative of an underlying reproductive or thyroid health condition, most commonly: fatigue (48.5%, n = 65,593), irritability (35.1%, n = 47,426), acne (30.4%, n = 41,071), feeling cold often (30.4%, n = 41,071) and irregular periods (26.7%, n = 36,108).

Of the 26.2% (n = 35,352) who reported they were actively TTC, 45.6% (n = 12,920) were consuming alcohol on a weekly basis, 28.1% (n = 9,667) were regular or occasional smokers and 8.4% (n = 2,876) were regular or occasional recreational drug users. Additionally, 3.9% (n = 1,351) and 38.9% (n = 13,541) had a body mass index (BMI) of < 18.5 or > 30 kg/m2 respectively and 12.1% (n = 4279) were not physically active.

Limitations, reasons for caution: As all data was self-reported, there is a risk of recall bias and false reporting. As the users who completed the VHA were seeking a reproductive healthcare service, there is likely a bias in the selection criteria of study participants. Additionally, users were given limited answer options to select from.

Wider implications of the findings: UK-based women are receiving inadequate symptom management and preconception counselling, evidenced by the majority of users experiencing one or more active symptoms of a reproductive or thyroid health condition and a substantial number TTC who have BMIs and/or lifestyle behaviour(s) associated with decreased conception and live birth rates.

Trial registration number: not applicable

Abstract citation ID: dead093.810

P-464 Childbirth after cancer treatment among young women in Denmark

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Study question: Do young women with cancer have children after cancer treatment comparable to the background population?

Summary answer: Results show differences between young women with cancer and those without cancer including that young woman with cancer more often have children prior to diagnosis.

What is known already: Each year approximately 900 young women are diagnosed with cancer in Denmark. Due to advanced cancer therapy many of them face long-term survival after cancer. A well-known consequence of cancer treatment is the risk of reduced fertility. Also, cancer types in the reproductive organs or diagnosis affecting the hormone-system are accountable for infertility in this population. The last decades have entailed great development in fertility preservation such as cryopreservation of oocytes, embryos or ovarian tissue and therefore increased the possibilities of having children in the future, however, international cohort studies still report less pregnancies and childbirth compared to control populations.

Study design, size, duration: This is a national, register-based study based on the DANAC II cohort. Women diagnosed with cancer as adolescents and young adults (aged 18-39) were registered in the Danish Cancer Register from 1978–2016. According to diagnosis onset, the young women with cancer were randomly age-matched with 60 undiagnosed women from the background population at the time of diagnosis. The primary outcome was childbirth. The women were followed until end of study, death and migration (31/12-2017).

Participants/materials, setting, methods: Both childbirth and IVF treatment before and after cancer treatment were compared with the aged-matched comparison group in all statistical analysis. Cancer diagnoses were divided into sub-groups. We adjusted for having children prior to cancer diagnosis in the statistical analysis. Death was also incorporated as a competing risk in all analyses.

Main results and the role of chance: The study population consisted of 21.596 young women diagnosed with cancer between 1978-2016 and 1.295.760 young women in the age-matched comparison group (CG). The study population consisted of a haematological subgroup (n = 1110), median age at diagnosis 29,7 (IQR 24,0-35,4) and an oncology subgroup (n = 20.486), median age at diagnosis 33,7 (IQR 29,1-37,2). The five-year mortality for haematological cancer was 26% and for oncological cancer 17%.

Among all women diagnosed with cancer 57% had children prior to cancer diagnosis compared to 47% in the age-matched CG. After cancer diagnosis, 19% of women with cancer had children compared to 30% in the CG.

Including children before and after diagnosis, 23% of women with cancer had no children vs 23% in the CG; 20 % had 1 child vs 16% in the CG; 39% had 2 children vs 39% in the CG and 17% had 3 or more children vs 22% in the CG.

Among women with hematological cancer 38% had no children during the observation-period, with a corresponding percentage for oncological cancer of 22%.

In the hematological and oncology sub-group seeking of ART-treatment before cancer diagnosis was 3%. After diagnosis, 5% in the hematological subgroup, 4% in the oncology subgroup sought ART-treatment.

Limitations, reasons for caution: Our study is based on registry data. Information about fertility preservation prior to cancer treatment, desire to- or deliberate opt-out of children after cancer treatment could have provided more insights. Also, we have in these preliminary results not yet adjusted for level of education.

Wider implications of the findings: A cancer diagnosis during the reproductive lifespan can affect childbearing substantially and result in fewer children. Although women diagnosed with cancer had similar levels of childlessness and seeking of ART treatment, vulnerable groups such as women with hematological cancer should be prioritized.

Trial registration number: NA

Abstract citation ID: dead093.811
P-466 The effect of SARS-CoV-2 infection or vaccination on controlled ovarian stimulation and in vitro fertilization: A multicenter retrospective cohort study

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Study question: Are the ovarian response to controlled ovarian stimulation (COS) and embryo development after in vitro fertilization (IVF) affected by SARS-CoV-2 infection and immunization?

Summary answer: Both SARS-CoV-2 infection and SARS-CoV-2 mRNA or inactivated vaccinations do not affect ovarian responsiveness to COS or embryo development following IVF.

What is known already: SARS-CoV-2 infects the target cell by binding to angiotensin-converting enzyme 2 (ACE2) via its surface spike protein. ACE2 receptors are present in the vagina, uterus, fallopian tubes, and ovaries of the female reproductive system. They regulate ovarian steroidogenesis, follicular development, oocyte maturation, and follicle atresia. The virus downregulates ACE2 levels, resulting in decreased conversion of Angiotensin II (ANGII) to Ang (1-7) and increased accumulation of (ANGII). This may impair reproductive fertility. However, initial studies conclude that both SARS-CoV-2 infection and vaccination would not affect reproductive outcomes, but it is necessary to develop new studies that confirm their applicability.

Study design, size, duration: A retrospective multicentric cohort study of 428 patients who underwent COS for oocyte donation between January 1st, 2018 and July 31th, 2022 were screened for eligibility and followed up to September 18th, 2022. Oocyte donation cycles were utilized because this model is an excellent choice for controlling known female characteristics

Participants/materials, setting, methods: Eligibility criteria were donors between 18 to 32 years. Patients were categorized into the vaccinated group if they had received SARS-CoV-2 vaccines (BNT162b2 mRNA Pfizer-Biontech, mRNA-1273 Moderna or inactivated SARS-CoV-2 vaccine Sinovac). The control group was chosen from medical records before March 2020 ensure they were neither infected or vaccinated. Demographic, cycle characteristics, and laboratory outcomes were compared. Normally, distributed data were compared across groups by univariate ANOVA. All P-values were considered significant at < 0.05.

Main results and the role of chance: In total, 428 patients were included in the study, with 12 recovering from SARS–CoV-2 infection (Group 1), 92 reporting vaccination (Group 2), and 324 patients who underwent COS before March 2020 assigned to the non-exposed group (Group 3).

The mean time from recovery to oocyte aspiration was 122 days (range 39-315 days) and 213 days from the first vaccine dose to oocyte aspiration (range 17–249 days).

The study's participants had an average age of 24.8 years. Age was found to be normally distributed and did not differ significantly between the three groups (P-value 0,953). Similarly, ovarian reserve markers, as measured by AFC and AMH, did not differ between groups (P-values of 0,787 and 0,423 respectively).

No significant differences were observed regarding stimulation duration and total gonadotropin dose. The number of retrieved oocytes, MII oocytes, fertilized oocytes, and good-quality day 5 embryos were also similar between the three groups. Comparing oocyte retrieval rate, mature oocyte rate, normal fertilization rate, and blastocyst formation rate consistently revealed no statistically significant differences.

Limitations, reasons for caution: The main limitation is its retrospective design. Other limitations include the small sample size of recovered patients, the absence of sperm analyses, and data of live birth rates and neonatal outcomes.

Wider implications of the findings: SARS-CoV-2 infection and vaccination seem unrelated to detrimental effects on ovarian response or embryo development. These findings contribute to the growing body evidence that supports vaccination in women trying to conceive. Further prospective studies with larger cohort sizes and longer follow-ups are warranted to validate our conclusion.

Trial registration number: NCT05562479

POSTER VIEWING
IMPLANTATION AND EARLY PREGNANCY

Abstract citation ID: dead093.812
P-467 Early (day 1–4) post-treatment serum hCG level changes predict single-dose methotrexate treatment success in tubal ectopic pregnancy

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Study question: How effective is the change between day 1 and 4 post-treatment serum hCG level at predicting single-dose methotrexate treatment success in tubal ectopic pregnancy (tEP)?

Summary answer: Any fall in day 1–4 serum hCG signifies an 85% (95%CI 76.8–90.6) likelihood of treatment success for tEP managed with single-dose methotrexate.

What is known already: For those with tEP managed by single-dose methotrexate, current guidelines advocate intervention if day 4–7 hCG fails to fall by $\geq$ 15%. The trajectory of hCG over day 1–4 has been proposed as an early indicator that predicts treatment success, allowing early reassurance for

women. However, almost all prior studies of day 1–4 hCG change have been small or retrospective.

Study design, size, duration: This was a prospective cohort study of women with tEP (pre-treatment hCG of ≥ 1000 IU/L and ≤5000 IU/L) managed with single-dose methotrexate. The data were derived from a UK multicentre randomised controlled trial of methotrexate and gefitinib versus methotrexate and placebo for treatment of tEP (GEM3). For this analysis, we include data from both treatment arms.

Participants/materials, setting, methods: Participants were categorised by single-dose methotrexate treatment success or failure. Treatment success for this analysis was defined as complete tEP resolution to serum hCG <30 IU/L following single-dose methotrexate treatment only. Patient characteristics between groups were compared. Changes in day 1–4, 1–7 and 4–7 serum hCG were evaluated as predictors of treatment success through receiver operating characteristic (ROC) curve analysis. Test performance characteristics were calculated for percentage reduction thresholds including optimal thresholds.

Main results and the role of chance: A total of 322 women with tEP and hCG ≥1000 IU/L and ≤5000 IU/L were treated with single-dose methotrexate. The overall single-dose methotrexate treatment success rate was 59% (n = 189/322). Day 1–4, 1–7 and 4–7 serum hCG change predicted single-dose methotrexate treatment success with ROC area under curve 0.80 (95% CI 0.74–0.85), 0.86 (95% CI 0.81–0.91) and 0.89 (95% CI 0.85–0.93) respectively. Any fall between day 1 and day 4 hCG predicted single-dose methotrexate treatment success with a positive predictive value of 85%, negative predictive value 57%, sensitivity 58%, specificity 84%, positive likelihood ratio 3.6 and negative likelihood ratio 0.5. A less than 18% rise in day 1–4 serum hCG was identified as an optimal classification threshold and predicted treatment success with 82% positive predictive value, 69% negative predictive value, 79% sensitivity, 74% specificity, positive likelihood ratio 3.0 and negative likelihood ratio 0.3.

Limitations, reasons for caution: Our findings may be limited by intervention bias resulting from existing guidelines which influences evaluation of hCG changes reliant on day 7 serum hCG levels.

Wider implications of the findings: This large prospective cohort demonstrates the value of day 1–4 serum hCG changes in predicting single-dose methotrexate treatment success in tEP. We recommend clinicians provide early reassurance to women who have fall or modest (<18%) rise in day 1–4 serum hCG that their treatment will likely be effective.

Trial registration number: This study is a secondary analysis of the GEM3 trial (ISRCTN Registry ISRCTN67795930).

Abstract citation ID: dead093.813

P-469 Body mass index impacts ectopic pregnancy during in vitro fertilization: are we jumping the gun? An analysis of 42,362 clinical pregnancy cycles

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Study question: Does female body mass index (BMI) have impact on ectopic pregnancy (EP) in women undergoing *in vitro* fertilization (IVF) and embryo transfer (ET) treatment?

Summary answer: EP rate is higher in underweight patients in fresh cycles. However, BMI is not a risk factor for EP after controlling confounders.

What is known already: It has been shown that tubal factor infertility, stage of embryos transferred (cleavage stage or blastocyst stage), high estrogen levels during ovarian stimulation, and endometrial thickness, have impact on EP rate in IVF cycles. In addition, other potential factors including ovarian stimulation protocols, number of embryos transferred, and type of embryos (fresh or frozen thawed) are also seemed to be associated with EP rate. However, BMI has interactions with these confounding factors mentioned above.

Study design, size, duration: This was a retrospective cohort study. From January 2010 to June 2022, patients between the ages of 20 and 45 years who had their first fresh/frozen thawed ET cycles, and reported as clinical pregnant were included. BMI was categorized into three groups: underweight (< 18.5 Kg/m2), normal weight (18.5–23.9 Kg/m2), overweight and obesity (≥ 24 Kg/m2).

Participants/materials, setting, methods: A total of 42,362 cycles were included in the final analysis. The overall ectopic pregnancy rate was 2.57% (1,089/42,362). Firstly, possible factors affecting ectopic pregnancy were screened in both fresh and frozen thawed ET cycles. Then, multivariate logistic regression analysis was used to adjust confounding risk factors.

Main results and the role of chance: EP rates in fresh, and frozen thawed transfer cycles were 2.43% (672/27,600) and 2.82% (417/14,762), respectively. In fresh ET cycles, the ectopic pregnancy rate was significantly higher in underweight patients when compared with that in normal and overweight/obesity patients (3.29% vs. 2.29% vs. 2.54%; P = 0.029). However, the ectopic pregnancy rate was not differ among patients with different BMI (2.72% vs 2.76% vs 2.96%; P = 0.782) in frozen thawed cycles. In fresh ET cycles, secondary infertility, tubal infertility, elevated estrogen level, thin endometrial thickness, and cleavage stage embryo transfer were risk factors with ectopic pregnancy. However, female BMI was not associated with ectopic pregnancy (adjusted OR: 0.98, P = 0.894, for BMI 18.5-23.9 Kg/m2; adjusted OR: 0.89, P = 0.205, for BMI ≥ 24 Kg/m2. Reference = BMI < 18.5 Kg/m2). In frozen thawed embryo transfer cycles, only thin endometrial thickness, and cleavage stage embryo transfer were risk factors with ectopic pregnancy. Female BMI was not predictable for ectopic pregnancy, either (adjusted OR: 1.15, P = 0.513, for BMI 18.5-23.9 Kg/m2; adjusted OR: 1.24, P = 0.367, for BMI ≥ 24 Kg/m2. Reference = BMI < 18.5 Kg/m2).

Limitations, reasons for caution: This is a retrospective study with 12 years period. New embryo culture methods and embryo transfer strategy emerged. It is possible that not all confounders have been controlled. Meanwhile, the definition of tubal infertility and pelvic disease need more caution as the lack of laparoscopic examination in some cases.

Wider implications of the findings: Understanding the impact of BMI on EP during IVF treatment may be useful for predicting results. At the first glance, underweight patients are more likely to encounter with EP during fresh ET. However, it is the high estrogen level, but not low BMI, that contributes to this phenomenon.

Trial registration number: N/A

Abstract citation ID: dead093.814

P-470 Prediction of the cumulative live birth rate of IVF using a human embryonic stem cells-derived trophoblastic spheroid implantation model

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Study question: Can the attachment rate of a human embryonic stem cell-derived trophoblastic spheroids (BAP-EB) onto endometrial epithelial cells predict the cumulative live birth rate of an IVF cycle?

Summary answer: The BAP-EB attachment rate offers modest prediction of the cumulative live birth rate of women aged ≥35 years undergoing IVF.

What is known already: Implantation failure is one of the major causes leading to a low IVF success. There is no reliable tool for assessing endometrial receptivity in IVF.

Study design, size, duration: Prospective observational study of 240 infertile women from 2017 to 2021.

Participants/materials, setting, methods: Infertile women with regular cycles attending IVF centres were recruited. Endometrial aspirates were collected from a natural cycle one month before IVF for the BAP-EB attachment rate. The cumulative live birth rate of a stimulated cycle and its derived frozen embryo transfer cycles within 6 months of ovarian stimulation were obtained.

Main results and the role of chance: The BAP-EB attachment rate in women with a cumulative live birth was similar to those who failed to have a

live birth. When women were stratified by age into <35 years and ³35 years, the BAP-EB attachment rate was significantly higher only in women aged ³35 years who had a live birth when compared to those without a live birth. The receiver operating characteristic (ROC) curve analysis of BAP-EB attachment rate in predicting cumulative live birth showed the area under the curve (AUC) of 0.559 (95% CI 0.479 – 0.639, p = 0.151), 0.448 (95% CI 0.310 – 0.585, p = 0.461) and 0.613 (95% CI 0.517 – 0.710, p = 0.026) for all ages, age <35 and ≥35 respectively.

Limitations, reasons for caution: BAP-EB and the isolated endometrial epithelial cells may not fully represent the in vivo developed human blastocysts and endometrial cells, respectively. The in vitro nature of the experiments and the limited sample size in this study may also limit the interpretation of the data.

Wider implications of the findings: Implantation failure is one of the major reasons contributing to the low success rate of IVF. The BAP-EB-endometrium attachment assay could potentially be used by reproductive medicine specialists to better counsel the couples who fail in IVF treatment.

Trial registration number: NCT02713854

Abstract citation ID: dead093.815

P-471 Impact of endometrial receptivity analysis on pregnancy outcomes In patients undergoing embryo transfer: A systematic review and meta-analysis

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Study question: To analyze the influence of endometrial receptivity analysis (ERA) on embryo transfer (ET) results in patients undergoing in vitro fertilization (IVF) treatment.

Summary answer: We identified thirteen studies, including 14396 patients. No differences were observed between patients undergoing ERA test and those not undergoing ERA test prior to ET.

What is known already: Previous studies studying ERA's efficacy and safety have provided conflicting results. Therefore there is an urgent need to provide a quantitative and comprehensive pool data.

Study design, size, duration: A systematic review was performed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses 2009 guidelines. The primary endpoint was live birth. Secondary endpoints included positive pregnancy test, biochemical pregnancy, implantation rate, clinical pregnancy and miscarriage. A total of 13 studies including 14396 patients were selected and included in this systematic review and meta-analysis.Electronic search was performed on MEDLINE and Embase databases (from inception to December 2022).

Participants/materials, setting, methods: Eligible studies were: (1) studies including patients for IVF treatment; (2) investigations comparing the use of ERA test vs non-test; (3) availability of pregnancy outcome data. For dichotomous outcomes, the odds ratios (ORs) with 95% confidence intervals (CIs) were calculated from the available data and trial-specific ORs were combined with the DerSimonian and Laird random effects model with the estimate of heterogeneity being taken from the Mantel-Haenszel model.

Main results and the role of chance: Of the 1492 citations screened, 7 were excluded as they were considered non-relevant, 1240 were excluded because of a preclinical design (no ERA test was used to assess endometrial receptivity), 3 because of different clinical outcomes. 14 studies were notachieved on humans, 19 included other diseases and 122 for others reasons. Therefore, a total of 13 studies including 14396 patients were selected and included in this systematic review and meta-analysis.

No differences were observed between patients undergoing ERA test and those not undergoing ERA testprior to ET in terms of live birth (OR 1.00, 95% CI 0.63-1.58, I2=92.7%), clinical pregnancy (OR 1.23, 95% CI 0.93-1.63, I2=85.4%), biochemical pregnancy (OR 0.83, 95% CI 0.46-1.49, I2=87%), positive pregnancy test (OR 0.99, 95% CI 0.80-1.22, I2=0%), miscarriage (OR 0.91, 95% CI 0.62-1.34, I2=67.1%) and implantation rate (OR 1.27, 95% CI 0.57-2.88, I2=89.9%).

Two studies had a randomized design, three had a prospective cohort design and eight studies had a retrospective cohort design. All studies included patients undergoing ET in blastocysts stage with a Gardner grade BB or higher except one that took in consideration also cleavage stage. In two studies fresh and frozen ET was performed, in the remaining studies only frozen embryos were transferred.

Limitations, reasons for caution: Our study should be interpreted in light of some limitations. This is a study-level meta-analysis providing average treatment effects. The lack of patient-level-data prevents us from assessing the impact of baseline clinical characteristics and other changes in therapeutic strategies on treatment effects. However, all-stratified analyses are combined with meta-regression analyses.

Wider implications of the findings: In our investigation, after pooling data from 13 studies and 14386 patients; the risk of live birth, positive pregnancy test, biochemical pregnancy, miscarriage, clinical pregnancy and implantation rate did not differ between patients undergoing ERA test and those not undergoing ERA. Therefore, the utility of ERA should be revisited.

Trial registration number: CRD42022332891

Abstract citation ID: dead093.816

P-472 Endometrial stimulation with culture medium containing granulocyte macrophage colony-stimulating factor promotes embryo implantation after vitrified-warmed blastocyst transfer

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Study question: Does endometrial stimulation with culture medium containing granulocyte macrophage colony-stimulating factor (GM-CSF) promote implantation potential of vitrified-warmed blastocysts?

Summary answer: Endometrial stimulation with culture medium containing GM-CSF prior to transfer of vitrified-warmed blastocysts may promote embryo implantation and improve pregnancy continuation.

What is known already: Human embryonic development and implantation *in vivo* is precisely regulated by various cytokines, including growth factors. GM-CSF is a cytokine that plays an important role in reproductive function. We reported the possibility that the use of culture medium containing GM-CSF in cleavage stage vitrified-warmed embryo transfers (ETs) promotes embryo implantation (ESHRE2018). However, it has been reported that endometrial stimulation with culture supernatant up to blastocyst stage (SEET method) improves embryo implantation, but the effect of endometrial stimulation with culture medium containing GM-CSF (G-SEET method) on embryo implantation is not clear.

Study design, size, duration: This study was conducted at a single *in vitro* fertilization (IVF) center from June 2020 to February 2022. The G-SEET procedure was performed at each patient's request. All study participants provided informed consent, and the study design was approved by the ethics committee of IVF Nagata Clinic, Fukuoka, Japan.

Participants/materials, setting, methods: We examined 702 cycles of vitrified-warmed blastocyst transfer. Warmed blastocysts were incubated in recovery culture for several hours and transferred on day 5 (control group). For the G-SEET method, only culture medium containing GM-CSF was injected into the uterus two days before ET, which was performed as in the control group (G-SEET group). The human chorionic gonadotropin (hCG) positive, clinical pregnancy, miscarriage, and ongoing pregnancy rates were compared between the two groups.

Main results and the role of chance: Of the 702 cycles of single vitrified-warmed blastocyst transfers, the control and G-SEET groups had 459 and 243 cycles, respectively. The G-SEET group had a higher patient age (37.5 ± 4.2 vs. 36.2 ± 3.8, p < 0.01), a thinner endometrial thickness (10.3 ± 1.9 vs. 10.9 ± 2.0, p < 0.01), a lower percentage of good blastocyst transfers (65.4% vs. 79.5%, p < 0.01), and a higher cumulative number of

implantation failures (3.6 ± 2.5 vs. 1.9 ± 1.9, p < 0.01) than the control group. The G-SEET method was performed at the patient's request, so the number of cycles and patient background varied. Multivariate analysis was performed using female age, male age, number of miscarriages, endometrial thickness, presence of good blastocysts, and cumulative number of implantation failures as confounding factors. Compared with the control group, the G-SEET group exhibited the following: hCG positivity rate [odds ratio (OR): 1.14, 95% confidence interval (CI): 0.79–1.64, p = 0.481], clinical pregnancy rate (OR: 1.81, 95% CI: 1.21–2.71, p = 0.003), miscarriage rate (OR: 0.735, 95% CI: 0.30–1.76, p = 0.491), and ongoing pregnancy rate (OR: 1.79, 95% CI: 1.20–2.69, p = 0.004). Clinical pregnancy and ongoing pregnancy rates were significantly higher in the G-SEET group compared with the control group.

Limitations, reasons for caution: The study was limited by the study size and lack of data about live birth rates after ET. In addition, intrauterine infusion of GM-CSF-free culture medium should be used as a control, but this was difficult in the private clinic setting.

Wider implications of the findings: This study suggests that endometrial stimulation with culture media containing GM-CSF prior to transfer of vitrified-warmed blastocysts promotes embryo implantation and enhances the ongoing pregnancy. The G-SEET method may be clinically useful as a new ET method.

Trial registration number: not applicable

Abstract citation ID: dead093.817
P-473 The reproductive performance of euploid blastocysts declines after each consecutive embryo transfer: The pendulum is swinging back?

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Study question: the impact of euploid blastocysts on reproductive performance by association between the number of previous failed IVF cycles and live birth rate per transfer

Summary answer: Our findings suggest a negative association between the number of previous failed IVF cycles per euploid transfers. implantation rates decreased following each consecutive FE-ET cycles

What is known already: In assisted reproductive technologies, implantation failure presents a challenging clinical dilemma since its underlying etiology is unknown. Implantation is a very complex process which involves a variety of factors related to the embryo, endometrium, and immune system (Fox et al. 2016). Therefore, implantation failure is also a complex pathological condition that involve both embryonic defects and/or endometrial dysfunctions (Das et al.2012). This situation poses a therapeutic dilemma. Hence implantation failure etiologies and treatments remain unresolved. which results in ongoing debate over whether embryonic chromosomal aneuploidy is a major contributor or within the endometrium itself.

Study design, size, duration: This observational cohort study included 387 patients undergoing euploid embryo transfer between January 2015 and until October 2022.

Participants/materials, setting, methods: Participants: Women with an anatomically normal uterus who underwent autologous vitrified-warmed euploid blastocyst transfer (Frozen Euploid Embryo Transfer: FE-ET) cycles were included in the study.

Setting: Fertility center at a university-affiliated public hospital.

Intervention(s): Trophectoderm biopsy and comprehensive preimplantation 24-chromosome analysis using Next Generation Sequencing (NGS).

Main Outcome Measure(s): Cumulative outcomes from these cycles were analyzed. A logistic regression model was used to assess the differences in outcomes between the first, second, and third FE-ET.

Main results and the role of chance: The mean age of the patient population under investigation was 33.4 ± 5.3 years. Maternal age at oocyte retrieval was negatively associated with the mean euploidy rate per cohort of biopsied blastocysts from each patient (m-ER). The m-ER did not show any association with the number of previous failed IVF cycles (0/1-2/≥3) among different ranges of maternal age at oocyte retrieval (≤30/31-35/36-39/≥40). The transfer of the first single vitrified-warmed euploid blastocyst was associated with an implantation rate (IR) of 47%, a miscarriage rate of 17.9% and a live birth rate (LBR) of 33.9%. Miscarriage rate and LBR were sensitive to the number of previous failed IVF cycles, showing statistically significant differences between women with no previous and ≥3 failed IVF cycles (11.3% versus 27.8%, P = 0.04) and (40.7% versus 26.3%, P = 0.05) respectively. Sustained implantation rates of the first, second, and third FE-ET were 47%, 40.5%, and 30% per transfer, respectively. The sustained IR was sensitive to the number of consecutive euploid blastocyst transfers (P = 0.03).

Limitations, reasons for caution: The retrospective nature of the study. The data should also be verified using a multicenter approach, to increase the sample size.

Wider implications of the findings: After experiencing multiple unsuccessful IVF cycles, transferring euploid blastocysts leads to improved clinical outcomes, but these improvements eventually level off. Additional information beyond embryo euploidy is needed to further enhance clinical outcomes.

Trial registration number: not applicable

Abstract citation ID: dead093.818
P-474 Association between subchorionic hematoma's size and miscarriage rate in IVF/ICSI patients

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Study question: What is the risk factor for miscarriage in patients undergoing IVF/ICSI with subchorionic hematoma(SCH) in the first trimester?

Summary answer: The size of SCH is a risk factor for miscarriage in patients undergoing IVF/ICSI. Miscarriage rate increases as the size of SCH grows.

What is known already: SCH is a frequently observed pathology during routine ultrasound examinations in early pregnancy. Its clinical significance has been unclear. Some studies believed that SCH found in first trimester was not correlated with adverse pregnancy outcome; Other studies believed that SCH in early pregnancy can cause a significant increase in the incidence of late pregnancy complications, such as preterm labor, preeclampsia, and postpartum hemorrhage. Besides, what are the risk factors for miscarriage in pregnant women with SCH? Answers to these questions are urgently needed.

Study design, size, duration: Retrospective cohort study with 646 patients undergoing IVF/ICSI at a tertiary care university hospital, between February 2017 and November 2020.

Participants/materials, setting, methods: All patients who underwent IVF/ICSI from February 2017 to November 2020, achieved pregnancy and underwent transvaginal ultrasound at our center during first trimester were included. Risk factors for miscarriage in patients with SCH were explored. Miscarriage rates in patients with different SCH sizes which was expressed as its' ratio to the area of the gestational sac were compared.

Main results and the role of chance: Patients with SCH who had more embryos transferred (*P*=0.01), earlier detection of IUH (*P*=0.003), a smaller GS area (*P*=0.03) was prone to miscarry. The size of SCH is a risk factor for miscarriage (P = 0.016). Miscarriage rate increased with increasing size of SCH (P for trend: 0.003).

Limitations, reasons for caution: The main limitation of this study is its retrospective nature and relatively small sample size.

Wider implications of the findings: Extra clinical attention needs to be paid to the miscarriage risk of in IVF/ICSI patients with large SCH. There is urgent need for future research on SCH affecting pregnancy outcome and the underlying pathophysiological mechanisms.

Trial registration number: Not applicable

Abstract citation ID: dead093.819

P-475 Effect of transdermal estradiol on endometrial preparation in frozen-thawed embryo transfer: A randomized control study.

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Study question: Is patient usage of transdermal estradiol more effective than oral estradiol on endometrial preparation in frozen-thawed embryo transfer cycles?

Summary answer: This study showed no significant differences in clinical pregnancy rates between the 2 groups. Transdermal gel have advantage of better patient comfort, lesser side effects.

What is known already: Estrogen and progesterone are crucial factors for endometrial preparation in frozen embryo transfer (FET) cycles. Studies assessing different forms of estradiol in FET have already been published. However, literature and studies in Vietnam are still limited to evaluating, research evaluating transdermal estrogen in ART. This study aimed to evaluate the use of transdermal estradiol gel and compare it with oral estradiol for the endometrium preparation in frozen-thawed embryo transfer (FET) cycles.

Study design, size, duration: This randomized clinical trial (RCT) included 380 frozen embryo transfer (FET) cycles from February 2020 to August 2021 at the IVF Department, Hung Vuong Hospital.

Participants/materials, setting, methods: They were randomized into one of the two groups. Group A (n = 190) received oral estradiol and group B (n = 190) received estradiol gel. In both groups, medication was started on day 2 of the menstrual, and endometrial thickness was monitored by ultrasound. Main outcomes were the following: serum estradiol level , endometrial thickness,.... Secondary outcomes were CPR, MR, and side effects in the 2 groups. Statistical testing was performed with Stata 14.

Main results and the role of chance: There was a significant difference in estradiol level on the day of progesterone administration and embryo transfer between the two groups (270.5 pg/ml versus 186.5 pg/ml, p, p. <0.001). According to the comparison, the two groups were not significantly different in the endometrial thickness on the day of progesterone administration (p = 0.85). as the data suggest, the total dose of estradiol used and the total number of days of dosing were similar in the two groups. The biochemical and clinical pregnancy rates were similar between the two groups and did not a significantly differ (56.2% vs. 52.2%, p = 0.474). The rate of miscarriage was higher than in the study group, but it was not statistically significant. Almost 20.3% of patients (n = 37) in group A had mild adverse effects when compared with only 10.1% (n = 18) in group B, and this was clinically significant (P = 0.007).

Limitations, reasons for caution: The limitation of our study was not asses ongoing pregnancy rate and live-birth rate.

Wider implications of the findings: Patients who estradiol valerate is contraindicated such as, high risk for deep vein thrombosis, hyperlipidemia, and clotting disturbance we can use oestrogel to be safe. It is suggested that estradiol transdermal patches be used instead of oral estradiol in FET cycles

Trial registration number: ISRCTN15301227

Abstract citation ID: dead093.820

P-476 Metformin ameliorated testosterone-induced ferroptosis via inhibiting ferritinophagy in trophoblasts of polycystic ovary syndrome (PCOS) placentas

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Study question: Does trophoblasts of PCOS patients with aberrant iron metabolism, and how does metformin ameliorate androgen-induced ferroptosis in trophoblasts *in vivo* and *in vitro*?

Summary answer: Metformin ameliorates placental development and attenuates spontaneous miscarriage rate in early gestation by inhibiting ferritinophagy in trophoblasts of PCOS patients with hyperandrogenism.

What is known already: PCOS is a common reproductive endocrine disease in women of reproductive age, and a large proportion of patients present with hyperandrogenism. Studies have found that the incidences of spontaneous miscarriage and adverse pregnancy outcomes in PCOS patients are higher than those in healthy controls, which placental dysfunction is an important factor. It is related to mitochondrial dysfunction, oxidative stress and metabolic disorders. Ferroptosis is an iron-dependent and phospholipid peroxidation-mediated cell death. Studies have shown that iron ions participate in a variety of biochemical reactions in humans, and its abnormal metabolism may lead to some diseases especially in the reproductive system.

Study design, size, duration: Villi at gestational weeks of 6-8 were collected from 18 healthy controls and 18 women with PCOS. Human trophoblast stem cells (hTSCs) were isolated from fresh villi (6-8 weeks, n = 3) and cultured with testosterone, ferroptosis or autophagy inhibitors, metformin, or *NCOA4* small interfering RNA. A dihydrotestosterone (DHT)-induced PCOS C57BL/6 mouse model was treated with or without metformin (3 months before mating) (n = 20 per group). Mouse placentas were obtained at embryonic days 11.5 (n = 50 per group).

Participants/materials, setting, methods: The levels of iron ions and lipid peroxidation were measured by PGSK staining, BODIPY^{C11} staining and MDA assay kit. The expressions of ferroptosis-related genes and proteins were assessed by qPCR, western blot and immunofluorescence. The binding of androgen receptor (AR) and NCOA4 to the putative androgen response element (ARE) was examined by ChIP, EMSA and luciferase assay. The influence of metformin on ferritinophagy in placentas of PCOS mice was confirmed by western blot and immunofluorescence.

Main results and the role of chance: The levels of iron ions and lipid peroxidation in villi at gestational weeks of 6-8 were higher in PCOS patients than in healthy controls. *In vitro* experiments, testosterone-induced ferroptosis was ameliorated by ferroptosis inhibitors, and ferritinophagy was mitigated by autophagy inhibitors or *NCOA4* knockdown in primary cultured hTSCs. Testosterone induced ARs into the nucleus, where the recruitment of AR homodimers along with NCOA4 to the positive ARE on the *SQSTM1* promoter, leading to autophagy induction and degradation of ferritin heavy chain 1 (FTH1) in autophagosomes, resulting in the release of ferritin bound irons as free ferrous irons. Metformin ameliorated testosterone-induced ferroptosis in hTSCs to a certain extent by inhibiting ferritinophagy. Compared to those of control mice, the placentas of DHT-treated PCOS mice showed increased levels of iron ions and lipid peroxidation, and altered expressions of ferroptosis-related proteins. Hyperandrogenism also induced ferritinophagy in labyrinthine zone of placentas in PCOS mice. Metformin ameliorated placental development was associated with decreased iron ions accumulation by inhibiting ferritinophagy in PCOS mouse placentas, which observably attenuated embryo absorption rate and spontaneous miscarriage incidence in the early gestation of PCOS mice.

Limitations, reasons for caution: The numbers of PCOS patients and mouse placentas in this study were small. The placental labyrinthine zone was not separated from the endocrine junctional zone and maternal decidua for molecular analyses. In addition, it would be better to isolated and cultured mouse TSCs to explore mechanisms on ferritinophagy in further.

Wider implications of the findings: This study provides further evidence for metformin as a PCOS treatment, which ameliorates placental development and attenuates spontaneous miscarriage rate in early gestation.

Trial registration number: not applicable

Abstract citation ID: dead093.821

P-477 Bidirectional association between ovarian reserve and spontaneous miscarriage: findings from the clinical IVF data to epidemiological evidence and genetic links

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Study question: To determine whether the association between ovarian reserve and spontaneous miscarriage risk is bidirectional and caused by shared genetic risk loci.

Summary answer: Bidirectional association between ovarian reserve and spontaneous miscarriage was observed. This may be attributed to five shared risk genes (SYCP2L, TP63, GSPT1, RIF1, and PRRC2A).

What is known already: Previous studies have shown that women with lower serum anti-Müllerian hormone (AMH) had an increased risk of embryo aneuploidy or spontaneous miscarriage risk, compared to women with higher AMH concentration, although not all studies agree. The most recent meta-analysis postulated that the association between diminished ovarian reserve (DOR) and miscarriage may be not causal, but that the two occurrences merely share a common cause, such as other systematic clinical conditions or past exposure. Inspired by this hypothesis, we conducted the present analyses to clarify this bidirectional association between DOR and miscarriage and further elucidate plausible mechanisms by using genetic data.

Study design, size, duration: There were four data sources. 1) The Clinical IVF data were retrospectively collected from an academically affiliated Reproductive Medicine Center (17,786 cycles included). 2) We further analyzed data from the UK Biobank (UKB) which is a large-scale, population-based, prospective cohort study (35,316 white women included). 3) The GWAS summary statistics for age at natural menopause (ANM) and spontaneous miscarriage were obtained from published GWAS studies. 4) The individual-level genotype data were also extracted from UKB.

Participants/materials, setting, methods: There were four analysis modules, 1) IVF data to test the association of ovarian reserve and miscarriage risk by using logistic regression models, 2) UKB data to test the association of spontaneous miscarriage history and ANM by using linear regression models, 3) GWAS summary data to test the overall genetic enrichment between miscarriage and ANM by plotting Q-Q plot, and 4) individual-level genotype data to identify specific shared genes by using 'pleio' R package.

Main results and the role of chance: In the analysis of clinical data, the risk of miscarriage was 1.5 (95% CI: 1.22-1.85, $p < 0.001$) times in the group with AMH<1.1 ng/ml, compared to the group with AMH >=1.1 ng/ml after adjustment. In the analysis of UK Biobank data, one more spontaneous miscarriage history would lead to 0.02 (95% CI: 0.00-0.03, $p = 0.032$) years earlier in the standardized ANM after adjustment. Participants with >=2 spontaneous miscarriage history would have 0.05 (95% CI:0.01-0.08, $p = 0.011$) years earlier in standardized ANM, compared with participants with <=1 spontaneous miscarriage. In the analysis of GWAS summary statistics, we found that there were 11,736,532 single nucleotide polymorphisms (SNPs) tested by both GWAS studies of ANM and miscarriage. In the stratified conditional Q-Q plot, successive leftward deflections did not show which suggested that an overall genetic overlap between miscarriage and ANM was not observed. In the analysis of the individual-level genotype data, we identified five shared genetic risk loci that affect both miscarriage and menopause. They were all novel risk genes for spontaneous miscarriage. Of these five genes, SYCP2L, TP63, GSPT1, and RIF1 were associated with congenital malformations and PRRC2A was associated with autoimmune disease, which are factors that possibly play a role in the occurrence of miscarriage.

Limitations, reasons for caution: The UKB epidemiology data and genetic data were restricted to participants of European ancestry. Further studies are needed in non-white populations. Second, the predictive value of GWAS summary data for miscarriage may be limited which results in a null finding in the conditional Q-Q plot.

Wider implications of the findings: Our findings could be of interest to IVF clinicians in patient counseling regarding the prognosis of IVF treatment and genetic counseling for miscarriage. Our findings encourage further research focus on the shared genetic architecture and common pathophysiological basis of diminished ovarian reserve and miscarriage which may bring new therapeutic opportunities.

Trial registration number: not applicable

Abstract citation ID: dead093.822

P-478 The decreases in serum soluble programmed cell death ligand −1 level are associated with missed miscarriage in humans

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Study question: The perturbations in serum levels of soluble forms of programmed cell death-1 (sPD-1) and its ligand (sPD-L1) in missed miscarriage (MM) are unknown.

Summary answer: Dysfunction in PD-L1 signal has been demonstrated in MM as evidenced by a lower circulating sPD-L1 level and declined expressions of PD-L1 in placenta.

What is known already: Programmed cell death-1 (PD-1) and its ligand (PD-L1) comprise important immune inhibitory checkpoint signaling to maintain pregnancy. During pregnancy, PD-L1 is highly expressed in the trophoblasts of the placenta. The reduced placental PD-L1 expression is associated with the risk of miscarriage. Therefore, disturbance of PD-1/PD-L1 axis in the fetomaternal interface is susceptible to be an important pathogenesis for pregnancy loss. Interestingly, the soluble form of these immune checkpoint molecules is detectable and associated with immunosuppression, it raises the diagnostic potential to monitor their level to monitor pregnancy health.

Study design, size, duration: In the discovery cohort, we enrolled 108 patients with MM and 115 body mass index (BMI)-matched, healthy women with a full-term pregnancy at 6 to 13 weeks of gestation. Another cohort of 25 MM patients and 25 subjects with induced abortion was recruited for validation.

Participants/materials, setting, methods: Blood samples were collected at the first prenatal visit for HP women or on the day of dilatation and curettage surgery (D&C) for subjects with MM or IA to measure serum sPD-1 and sPD-L1 levels. Placenta samples were harvested during the D&C within cohort 2 to examine related gene and protein expression.

Main results and the role of chance: Our results showed that circulating sPD-L1 levels were reduced by 50% in patients with MM (55.71 ± 1.54 pg/mL) compared to HP controls (107.27 ± 5.48 pg/mL, $P<0.001$) and remained significant after adjusting for maternal age and gestational age, whereas no significant differences in sPD-1 were observed between the two groups. Applying serum sPD-L1 levels achieved an area under the receiver operating characteristics curve (AUROC) of 0.83 (95% CI: 0.77 to 0.88, $P<0.001$) with the optimal cut-off value of 81.52 pg/ml to detect MM. Our data in the validation cohort further demonstrated that sPD-L1 was lower in MM patients relative to IA subjects. Likewise, placental PD-L1-related gene expressions were downregulated at both mRNA and protein levels in miscarriage samples relative to samples in IA groups and were positively associated with sPD-L1 levels in maternal circulation

Limitations, reasons for caution: This study was conducted in East Asian women, further studies are required for the evaluation of the serum PD-1 and sPD-L1 levels in different ethnic groups. Secondly, due to the cross-sectional nature of the study design, whether the sPD-L1 levels could utilize as a predictive biomarker is questioned.

Wider implications of the findings: The maternal serum sPD-L1 level has the potential to be a diagnostic aid for pregnancy maintenance. Moreover, insufficient PD-L1 expression in the feto-maternal interface may act as one of the potential pathogenic events to trigger early pregnancy loss.

Trial registration number: not applicable

Abstract citation ID: dead093.823
P-479 Prognosis for ongoing pregnancy after recurrent implantation failure (RIF) following IVF/ICSI treatment

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Study question: What is the cumulative ongoing pregnancy rate within one year after visiting the RIF outpatient clinic in patients with RIF after IVF/ICSI treatment?

Summary answer: The cumulative ongoing pregnancy rate was 42.9% with a mean time to pregnancy of 8.8 months (95% CI 7.5-10.1 months).

What is known already: An estimated portion of 10-15% of IVF patients experience RIF. Multiple add-on treatments have been proposed, however, evidence for effective clinical therapeutic options still remain scarce. Although it is described that RIF patients might eventually benefit most from a 'keep calm and carry on' approach, in practice patients often seek clearer answers on their pregnancy chance and request further investigations/interventions. It would be helpful to give patients insight in their chances of achieving pregnancy after RIF, but at this moment little is known about their prognosis. This is further complicated by the lack of consistency in the definition of RIF.

Study design, size, duration: A prospective cohort study on 42 RIF patients after one-year follow-up was performed, as part of the MURIM (Multidisciplinary Research on Repeated Implantation Failure and Recurrent Miscarriages) study. Baseline characteristics including an endometrial assessment and ReceptIVFity (vaginal microbiome) test were collected at the RIF outpatient clinic. After a follow-up duration of minimal one year participants were asked to complete a questionnaire regarding fertility treatment and pregnancy outcome. Missing information was received via medical files.

Participants/materials, setting, methods: RIF patients aged 18 to 38 years old visiting the RIF outpatient clinic at Maastricht University Medical Centre+ between April 2019 until September 2021 were included. RIF was defined as consecutive implantation failure of three high quality embryos or ten embryos without a quality criterion. Clinical characteristics, pregnancy outcome and time to pregnancy was analyzed by survival analysis and cox hazard regression analysis via SPSS version 20.0. A p-value of < 0.05 was considered statistically significant.

Main results and the role of chance: Forty-two out of 44 contacted patients responded to the questionnaire (response rate = 95.5%). The ongoing pregnancy rate was 42.9% (Standard Deviation (SD): 0.50%) during the first year after visiting the RIF outpatient clinic. Mean time to ongoing pregnancy was 8.8 months (95%-CI: 7.5-10.1 months). The mean amount of embryo transfers (ETs) during this year was 2 (SD: 1.78). The ongoing pregnancy rate per ET (by survival analysis) for the first, third and sixth ET was 33.1%, 49.3% and 63.8%, respectively. To correct for a possible overestimation of the cumulative ongoing pregnancy rate per ET by normal survival analysis, pregnancy rates were calculated again using the number at risk of the total group during the first ET (pessimistic cumulative pregnancy rate). The pessimistic cumulative ongoing pregnancy rates were 33.1%, 42.9% and 48.6%, respectively.

When comparing baseline characteristics by univariable cox hazard regression between women with and without ongoing pregnancy, no significant differences were found. Nineteen pregnancies were reported during the one-year follow-up, one conceived spontaneously (5.3%); four via fresh ET (IVF=15.8% and ICSI=5.3%); and 14 by frozen ET (IVF=26.4% and ICSI=47.4%). The pregnancies resulted in 14 livebirths (73.7%); one miscarriage (5.3%); one stillbirth (5.3%); and three ongoing pregnancies (15.8%).

Limitations, reasons for caution: Although this is the first study that prospectively describes pregnancy prognosis after RIF, it is limited by the short follow-up period and relatively small sample size. The RIF outpatient clinic consultation might have contributed to the obtained pregnancy results but the proportionality is unclear as there is no control group.

Wider implications of the findings: The obtained ongoing pregnancy prognosis after RIF is encouraging and justifies a conservative approach after three failed ETs. Furthermore, the high pregnancy rate indicates the need of well-defined, individualized diagnostic criteria to define RIF and to be able to deviate between couples with 'bad luck' and an underlying (treatable) cause.

Trial registration number: NL66835.068.18/METC18-040

Abstract citation ID: dead093.824
P-481 Additional sub-cutaneous progesterone: a putative strategy to counteract poor clinical outcomes after vitrified-warmed euploid blastocyst transfer?

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Study question: Is there an association between additional subcutaneous progesterone (a-sP) during luteal phase support for vitrified-warmed euploid blastocyst transfers and the clinical outcomes?

Summary answer: a-sP was mainly administered to worse-prognosis patients:positive-pregnancy-test (PPT), but also biochemical-pregnancy-loss (BPL) rates were lower than control, thereby involvingsimilar live-birth-rate (LBR) per transfer.

What is known already: Frozen-embryo-transfer (FET) implementation is increasing worldwide, and luteal-phase-support is a hot-topic. Serum/uterine progesterone levels are key in eliciting immunological tolerance and uterine quiescence, while supporting the processes underlying embryo implantation. Yet, a consensus is missing on progesterone administration (formulation, route, dosage, and duration). Endometrial biopsy studies showed increased progesteroneconcentration after vaginal administration, while sP has excellent pharmacokinetics with faster peak serum concentration.Combining both administration routes might compensate cases ofpoor vaginal absorption and contribute to reaching adequate systemic/uterine levels. This synergy might be beneficial especially in RIF-patients, subject to lower LBRs also in the context of euploid ET.

Study design, size, duration: Retrospective analysis of 775 vitrified-warmed euploid blastocysttransfers conducted January2020-March2021. Three of 12 gynecologists administrated a-sP as luteal phase support mostly to patients with a worse reproductive history (i.e., longer duration of infertility and/or higher number of previous failed IVF). We assessed the clinical outcomes (PPT, BPL, miscarriage and LBR) in the group of patients who used a-SP (N = 128) versus the control(N = 647), adjusting for confounders in logistic regression analyses.

Participants/materials, setting, methods: Only euploid non-mosaic blastocysts were transferred. All patients had normal uterine cavities and thyroid function. Endometrial preparation was performed with either hormone-replacement-therapy (estradiol valerate 6mg/die plus vaginal micronized progesterone 800mg/die) or modified-natural-cycle (hCG administration plus vaginal micronized progesterone 400mg/die). In the a-sP group, the supplementation was started 3 days before FET. In case of pregnancy, the therapy was continued until the 8thweek.

Main results and the role of chance: The patients in the control and a-sP groups were similar for oocyte age (37.6 ± 2.7yr versus 37.5 ± 2.7yr), age at

ET (37.7 ± 3.2yr versus 37.7 ± 3.4yr), and body-mass-index (22.2 ± 2.4 versus 22.0 ± 2.8yr). Conversely, the patients in the a-SP group experienced longer duration of infertility (3.5 ± 1.9yr in the control versus 4.1 ± 2.6yr, p = 0.05), and had already undergone ≥1 previous IVF cycle (37%) and ≥2 failed ETs (22%) more frequently than the control (27% and 12%, respectively;p=0.05). The endometrial preparation protocol was similar in the two groups (76% hormone-replacement-therapy and 24% modified-natural-cycle). Also, blastocyst quality and day of transfer were similar. In the study period, the prevalence of a-sP administration increased from first to second-third ETs (13% to 29%). The confounders identified on the clinical outcomes were blastocyst quality, day of transfer and consecutive number of ET. Therefore, these features were included in multivariate logistic regressions. PPT rates were 61.1% (N = 395/647) and 49.2% (N = 63/128, p = 0.01) in the control and a-SP groups, respectively (multivariate-OR:0.65, 95%CI 0.44-0.97;adjusted-p=0.04). BPL rates were 9.1% (N = 36/395) and 1.6% (N = 1/63, p = 0.04), respectively (multivariate-OR:0.14, 95%CI 0.02-0.99;adjusted-p=0.05). Miscarriage rates were 14.2% (N = 51/359) and 6.5% (N = 4/62;p=0.1), respectively (multivariate-OR:0.4, 95%CI 0.14-1.16;adjusted-p=0.1). Lastly, LBRs were 47.6% (N = 308/647) and 45.3% (N = 58/128), respectively (multivariate-OR:1.04, 95% 0.7-1.55;adjusted-p=0.8).

Limitations, reasons for caution: Retrospective analysis. Progesterone levels are not routinely assessed in our clinical practice. A larger prospective study only in poor prognosis patients is required to assess the putative benefit of a-sP.

Wider implications of the findings: About 30% of patients undergoing FET suffer from inadequate progesterone levels, possibly impacting the clinical outcomes, yetscarcely detectable through its serum levels. In these women, vaginal progesterone could be insufficient, and a-sP can act as a rescue strategy against reduced endometrial receptivity via lower BPL.

Trial registration number: Not applicable

Abstract citation ID: dead093.825

P-482 Bacterial vaginosis in a subfertile population undergoing fertility treatments: a prospective cohort study

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Study question: Does bacterial vaginosis (BV) influences pregnancy rates during fertility treatments?

Summary answer: BV does not significantly impact ongoing pregnancy or live birth rates but could affect miscarriage rates.

What is known already: BV is known to influence several obstetric outcomes, such as preterm delivery and endometritis. Only few studies investigated the effect of BV in subfertile women, and studies found a negative effect of BV on fecundity especially in the in vitro fertilisation (IVF) population.

Study design, size, duration: Observational prospective study, 76 couples attending a fertility clinic in the Netherlands between July 2019 and June 2022, undergoing a total of 133 attempts of intra uterine insemination (IUI) or IVF.

Participants/materials, setting, methods: Participants undergoing IUI, IVF or intra cytoplasmatic sperm injection (ICSI). Vaginal samples taken at oocyte retrieval or insemination were analysed on qPCR BV and 16S rRNA gene microbiome analysis of V1-V2 region. Logistic regression with a Generalized Estimated Equations (GEE) analysis was used to account for multiple observations per couples. Main study outcome was defined as ongoing pregnancy at 12 weeks. Secondary outcomes were miscarriage rate and live birth rate.

Main results and the role of chance: A total of 26% of the 133 samples tested positive for BV. No significant differences were observed in ongoing pregnancy or live birth rates based on BV-status (OR 0.50 (0.16-1.59), aOR 0.32 (0.09-1.23)) or microbiome community state type (CST). There was a tendency of more miscarriages based on positive BV status (OR 4.22 (1.10-16.21), aOR 4.28 (0.65-28.11)) or microbiome CST group III/IV. Sample outcomes of qPCR BV were in accordance with their microbiome analyses. On baseline groups significantly differed on smoking status and body mass index (BMI). Odds ratios were adjusted for smoking status, BMI and socioeconomic status.

Limitations, reasons for caution: Numbers of the initial sample size calculation were not met. Therefore, numbers may be too small to draw firm conclusions.

Wider implications of the findings: This study describes the influence of BV or an abnormal microbiome in a subfertile population undergoing fertility treatments. A possible effect of lifestyle factors on BV-status is shown. Combining qPCR and microbiome results and analysing both IUI and IVF/ICSI treatments can contribute to better generalisability to the consulting room.

Trial registration number: Z21.031

Abstract citation ID: dead093.826

P-483 Application of RNA-sequencing based predictive model for endometrial WOI in patients with recurrent implantation failure: a prospective cohort study

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Study question: Is intrauterine pregnancy rate following a FET timed by the rsERT with hour precision higher than the conventional FET among patients with recurrent implantation failure?

Summary answer: Personalized embryo transfer (pET) guided by this newly optimized rsERT significantly improved pregnancy outcomes of patients with recurrent implantation failure (RIF).

What is known already: Asynchrony between blastocyst and endometrium is responsible for implantation failures. Hence, accurate prediction for endometrial window of implantation (WOI) would maximize the effectiveness of assisted reproductive technology (ART). Previously, we have established a predictive model for endometrial WOI (rsERT) by three-time points sampling from the same patient at 48-hour intervals during one menstrual cycle. To avoid multiple sampling and build a more efficient WOI prediction tool, estimation method with hour precision by single time point sampling is urgently needed.

Study design, size, duration: This was a two-phase strategy involving model optimization and benefit evaluation with a prospective controlled trial. The study was performed in a tertiary hospital setting between September 2018 and December 2021. In the first phase (September 2018-June 2020), 91 participants were recruited to optimize the rsERT. In the second phase (June 2020-December 2021), 176 patients with RIF were enrolled to valida the clinical efficacy of this newly optimized rsERT.

Participants/materials, setting, methods: First phase: endometrium samples were obtained from sampling on LH + 5, +7, +9 or P + 3, +5, +7 respectively. Samples were predicted by the rsERT. Only those in agreement with theoretical WOI timing and from whom successfully obtained intrauterine clinical pregnancy after subsequent FET were eligible. Second phase: 88 patients in the experimental group underwent pET timed by rsERT after sampling at LH + 7 or P + 5. 88 patients in the control group performed standard embryo transfer without sampling.

Main results and the role of chance: This newly optimized predictive model could provide hour-based results of endometrial WOI and have an average accuracy of 94.51% by using 10-fold cross validation. In the second phase, a total of 88 NGS were constructed for RNA-sequencing by using endometrial biopsy samples from RIF patients in the experimental group (n = 88). The results indicated WOI displacement in 40 of 88 (45.45%). Among them, advanced WOI occurred in two patients (2/40, 5.00%), and

delayed WOI occurred in 38 patients (38/40, 95.00%). The baseline clinical parameters, including age, BMI, infertility duration, types of infertility, number of previous failed cycles, main aetiology of infertility, the prevalence of pre-implantation genetic screening/diagnosis (PGS/PGD), endometrial thickness, endometrial types, the percentage of transferred blastocysts, good-quality embryo transferred rate and number of transferred embryos were all comparable between the two groups ($P > 0.05$). The intrauterine pregnancy rate (IPR) and implantation rate (IR) of the experimental group were significantly improved compared to those of the control group (for IPR: 61.36% vs. 31.82%, RR 3.630, 95%CI 1.958-6.732, $P = 0.000$; for IR: 42.86% vs. 24.66%, RR 2.292, 95%CI 1.392-3.772, $P = 0.001$). Moreover, in the experimental group, pregnancy outcomes did not exhibit remarkable differences between the natural cycle and hormone replacement (HRT) cycle.

Limitations, reasons for caution: Caution is warranted due to the non-randomized design and limited sample size of the study. Besides, embryonic factors should be taken into consideration as well. Hence, a multi-center randomized controlled trial of rsERT combined with PGT is needed to further validate its clinical efficacy.

Wider implications of the findings: These data add a new alternative of predictive model for endometrial WOI among Chinese women.

Trial registration number: the National Key Research and Development Program of China

Abstract citation ID: dead093.827
P-484 PGT-A and euploid transfer is more efficient than untested transfer in patients obtaining 1 or 2 blastocysts: a propensity score matching-based study

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Study question: Does PGT-A and euploid transfer involve any clinical advantage with respect to untested transfer inpatients obtaining 1 or 2 blastocysts?

Summary answer: PGT-A involved less transfers, less miscarriages, and a shorter time from oocyte retrieval to cycletermination without affecting the cumulative-live-birth-rate per patient.

What is known already: Meiotic chromosomal aneuploidies are the main cause of implantation failures and miscarriages andshow a U-shaped association with women fertility. Their prevalence increases from 25-30% in women <35 years to >90% around menopause. In IVF, PGT-A via comprehensive-chromosome-testing techniques allows identifying euploid blastocysts, whose live-birth-rate (LBR) per transferand miscarriage-rate (MR) per clinical pregnancy are respectively higher and lower than untestedblastocysts. From an intention-to-treat perspective, PGT-A cannot improve the cumulative-live-birth-rate (CLBR), but no report exists either of an impact on this key clinical outcome.Nevertheless, more data are needed about PGT-A performance in couples producing 1 or 2 blastocysts.

Study design, size, duration: We gathered a dataset of 369 non-PGT and 1461 PGT-A cycles with 1-2 blastocysts obtained bynaïve patients (April-2013 to July-2022). Patients were matched 1:1 for maternal age (36 ± 3.1 years)and number of MII-oocytes inseminated (5.7 ± 3.3) through propensity-score-matching, thereby producing a database of 242 cycles per arm. The primary outcome was LBR per transfer. Secondary outcomes were MR per clinical pregnancy, CLBR per concluded cycles and the time to cycle termination.

Participants/materials, setting, methods: ICSI of all own metaphase-II oocytes retrieved and blastocyst culture were systematically conducted. In the PGT-A group, trophectoderm biopsy without day3 zona-pellucida drilling andcomprehensive-chromosome-testing to assess full-chromosome non-mosaic aneuploidies wereperformed. Blastocyst cryopreservation was conducted via vitrification. Only euploid/untestedsingle transfers were conducted. The groups were comparable also for BMI, FSH, AMH, sperm factor, cumulus-oocyte-complexes, 2PN-zygotes, maturation, fertilization and blastulation rates. 99 and 143 patients obtained 1 and 2 blastocysts, respectively, in both groups.

Main results and the role of chance: The 242 non-PGT patients transferred 1.45 ± 0.5 blastocysts. 57% and 43% performed one ($N = 139$) and two ($N = 103$) transfers, respectively. 165 patients (68%) did not undergo a fresh transfer because of OHSS risk (25%), premature progesterone elevation (20%), inadequate endometrium(23%), fever/COVID-19 (5%), couple's will (27%).In the PGT-A group, the euploidy rate was $48.8 \pm 43.3\%$, 0.8 ± 0.7 euploid blastocysts were obtained, and 0.6 ± 0.6 transferred ($N = 151$) ($p = 0.01$ versus non-PGT). 45% of the PGT-A patients did not have euploid blastocysts ($N = 110/242$), 47% and 8% underwent one ($N = 113$) and two ($N = 19$) transfers, respectively. In the non-PGT and PGT-A group, the LBR per transfer was 17% ($N = 61/351$) versus 41%($N = 62/151$; Multivariate-OR:3.9, 95%CI 2.4-6.3, adjusted-p=0.01; post-hoc power=99%). The MR was 30% ($N = 26/87$) versus 16% ($N = 12/74$; Multivariate-OR adjusted for blastocysts' quality and day: 0.4,95%CI 0.2-0.9, adjusted-p=0.03), respectively. To date, 94% ($N = 228/242$) and 92%($N = 223/242$) of the cycles were concluded ($p = 0.5$) and the CLBR was 25% ($N = 57/228$) versus 27%($N = 60/223$; Multivariate-OR adjusted for maternal age:1.1, 95%CI 0.7-1.7, adjusted-p=0.6). The days between oocyte retrieval and cycle termination (PGT-A report without euploid blastocysts, last transfer without LB or first transfer with LB) were 125.1 ± 144.4 and 74.5 ± 95.6 ($p = 0.01$; confirmed adjusting for maternal age and number of blastocysts obtained in a linear regression).

Limitations, reasons for caution: Retrospective design. The mean maternal age at our private IVF center is 39 years with ≈70% requesting PGT-A. Nevertheless, all women are counseled about their age-specific prevalence ofaneuploidies.

Wider implications of the findings: PGT-A to assess full-chromosome non-mosaic aneuploidies and non-PGT involve similar CLBR incouples obtaining 1 or 2 blastocysts, but the former approach is faster (shorter time to cycletermination), safer (less miscarriages) and more efficient (less transfers). A cost-effectivenessanalysis is in our pipeline.

Trial registration number: not applicable

Abstract citation ID: dead093.828
P-485 Performance of ERA test after PGT-A in RIF patients defined according to ESHRE 2023 good practice recommendations: a retrospective case-control study

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Study question: Is Endometrial-Receptivity-Array (ERA) test clinically-useful among Repeated-Implantation-Failure (RIF) women defined according to ESHRE 2023 good-practice-recommendations (GPR) and undergoing single euploid blastocyst transfer?

Summary answer: ERA-test showed a higher "non-receptive endometrium" rate among RIF. Nevertheless, the live-birth-rate (LBR) per first euploid transfer was independent from progesterone timing according to ERA-test.

What is known already: ERA-test evaluates the expression of 238 genes from an endometrial biopsy. It aims at predicting the window of implantation and adjust progesterone timing accordingly. The data are controversial due to poor study design and/or limited sample size. Few studies investigated ERA-test in a setting with euploid transfers and/or among RIF patients. Moreover, RIF definition is largely inconsistent leading to a significant bias in the interpretation of the existing literature and treatment. ESHRE 2023 GPR revised RIF definition. Here, we leveraged this updated definition and PGT-A to assess ERA-test diagnostic/therapeutic performance in these women.

Study design, size, duration: Retrospective case-control study during 2013-2021 including 2211 patients with at least one euploid blastocyst after PGT-A. Hysteroscopy was conducted in case of suspect uterine pathologies, biopsy in case of suspect inflammation, and therapy in case of endometritis. 265 patients (12%) were classified RIF according to the ESHRE GPR (= no successful implantation despite an estimated cumulative chance ≥60%). 48 RIF patients requested ERA-test before undergoing vitrified-warmed euploid single blastocyst transfer, while 217 did not.

Participants/materials, setting, methods: qPCR/NGS-based PGT-A was conducted to assess non-mosaic aneuploidies. Normal thyroid function, vitamin-D levels, uterine cavity, and absence of sactosalpynx were confirmed. Whenever the ERA test suggested altered receptivity, progesterone timing was modified accordingly. The primary outcome was LBR per first euploid transfer in the non-ERA versus ERA groups, with a sub-analysis in ERA receptive versus non-receptive. Outcomes were adjusted for confounders assessed via logistic regressions.

Main results and the role of chance: RIF patients requesting ERA-test were older (39.1 ± 4.5 versus 36.7 ± 3.5 years, $p < 0.01$), and had already undergone 3.7 ± 2.0 transfers resulted in implantation failures (1.5 ± 1.3 of euploid blastocysts) versus 2.6 ± 1.7 transfers (1.0 ± 0.8 of which of euploid blastocysts) in the control ($p < 0.01$). Nevertheless, all these variables were not associated with the primary outcome under investigation. 44 non-RIF patients requested ERA-test in the same study period. Notably, the rate of pre-/post-receptive endometria was 39.6% (N = 19/48) among RIF and 13.6% (N = 6/44) among non-RIF women ($p < 0.01$). In fact, maternal age was not associated with "non-receptive" responses after ERA-test, the number of previous implantation failures showed an Odds-Ratio 1.29, 95%CI 1.03-1.62, $p = 0.03$. Blastocyst quality and day of full-blastulation were similar in ERA and non-ERA patients, as well as in ERA receptive and non-receptive patients. Positive pregnancy test, biochemical-pregnancy-loss, and miscarriage rates were all similar among non-ERA and ERA, and among ERA receptive and non-receptive patients. Indeed, also the LBR was similar among non-ERA and ERA (N = 98/220, 44.5% versus N = 17/48, 35.4%, $p = 0.26$; Odds-Ratio adjusted for blastocyst quality and day of full-blastulation: 0.76, 95%CI:0.39.1.48, $p = 0.42$) and among ERA receptive and non-receptive patients (N = 10/29, 34.5% versus N = 7/19, 36.8%, $p = 0.99$; Odds-Ratio adjusted for blastocyst quality and day of full-blastulation: 0.99, 95%CI:0.3-3.4, $p = 0.99$).

Limitations, reasons for caution: Our standard clinical workflow does not entail ERA-test, but the couple may request it. The study is retrospective with limited sample size. Patients requesting ERA-test were older with more previous failures. Non-ERA and ERA receptive patients underwent either Hormone-Replacement-Therapy or Modified-Natural-Cycle. Cost-effectiveness analyses are missing.

Wider implications of the findings: A promising association was reported between the novel definition of RIF and "non-receptive" responses, suggesting that ERA-test might unveil endometrial receptivity issues. Nevertheless, positive, and negative predictive values upon implantation are largely missing and its clinical utility as a therapeutic tool in RIF women remains questionable.

Trial registration number: none

Abstract citation ID: dead093.829

P-486 Alterations in endometrial programming lead to recurrent in vitro fertilization failures

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Study question: Could patients with recurrent implantation failures (RIF) display alterations on the decidualization program conditioning decidual cells' functions and the inflammatory response during peri-implantation period?

Summary answer: Endometrium from RIF-patients displays alterations into the decidualization program and into the induction of a physiological inflammation preventing the attachment and cell-migration for embryo implantation

What is known already: The decidualization program starts on each menstrual cycle and implies not only phenotypical changes on the endometrial stromal cells, but also in their secretory profile. Moreover, this process induces a physiological and sterile inflammatory response associated with inflammatory mediators production, such as IL-1β. At the same time, several molecules associated with receptivity are modulated; increasing the expression of adhesion molecules and metalloproteinases, while decreasing MUC-1 to allow embryo attachment and implantation. Even nowadays with different techniques that can identify a competent embryo and the implantation window, RIF-patients cannot reach implantation and the mechanisms involved have not been elucidated yet.

Study design, size, duration: First, a bioinformatic analysis was performed using standardized pathways involved in angiogenesis, placentation, decidualization, inflammation and immune regulation from public databases (Reactome, Gene Ontology Biological Process, WikiPathways, KEGG). Based on arrays that compare gene expression in endometrial biopsies from RIF or fertile women, we selected those that connect two or more processes and we validated their modulation in endometrial samples. Functional processes, such as migration and inflammatory production were also evaluated in primary cultures.

Participants/materials, setting, methods: Endometrial samples were obtained from fertile and RIF patients during the secretory phase. The Investigation and Ethics Committee from San Isidro, Argentina approved this study. RT-qPCR was performed to test decidualization markers: IGFBP1, PRL, PGR; inflammation: IL-1β, NLRP3, PTGS1; angiogenesis balance : CXCL14, ARG2, VEGFA; adhesion molecules: ITGA8 and MUC1. IL-1β production was quantified by Flow Cytometry. Wound healing assay was performed in human endometrial stromal cells derived from cell line and RIF patient's biopsy

Main results and the role of chance: First, we performed bioinformatic analysis based on standardized pathways in public databases. We focused on genes that connected and were modulated in processes involved in implantation, angiogenesis, placentation, decidualization, inflammation and immune regulation. Then, we validated the expression of 15 genes with the highest score in biopsies from RIF or fertile women taken on LH + 7. We found

decreased IGFBP1 expression, an early decidualization marker, and the progesterone receptor ($p < 0.0001$), suggesting alterations in the decidualization program. Since inflammation is associated with decidualization, we evaluated the IL-1β pathway. RIF biopsies had reduced expression of NLRP3, associated with inflammasome assembly, accompanied by a reduction in PTGS1 and IL-1β production in comparison with endometrial samples from fertile women. Moreover, when we evaluated genes involved in processes regulated by inflammation, we observed increased expression of MUC1, avoiding blastocyst adhesion, and decreased ITGA8 preventing its attachment accompanied by decreased MMP9 levels ($p < 0.005$). Also, we observed an imbalance between pro and anti-angiogenic factors (VEGFA vs CXCL14). Finally, we isolated and cultivated stromal cells from RIF samples to analyze migration ability, a process that mediates blastocyst inclusion into the decidua, by wound healing assay. These primary cultures showed a differential migration pattern compared with decidualized stromal cells.

Limitations, reasons for caution: The present results were obtained using endometrial samples from RIF patients and fertile women obtained during the secretory phase of the menstrual cycle. Even though the samples represent the endometrium after in vivo decidualization, further studies are necessary to elucidate whether these mechanisms operate similarly in vivo.

Wider implications of the findings: Decidualization process induces a physiological and sterile inflammatory response associated with the modulation of several adhesion molecules and MMP to allow the attachment and embryo implantation. However, this initial inflammatory response is differentially induced in RIF patients in comparison with fertile women, suggesting its relevance as a potential pharmacological target.

Trial registration number: This work was funded by the National Agency of Sciences and Technology ANPCyT (PICT 2018-4715 and PICT 2028-2461 to RR) and University of Buenos Aires (UBACyT UBACyT 20020090200034 to RR).

Abstract citation ID: dead093.830
P-487 Preimplantation genetic testing for aneuploidy for recurrent pregnancy loss: a systematic review and meta-analysis

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Study question: Can preimplantation genetic testing for aneuploidy (PGT-A) improve the live rate of recurrent pregnancy loss(RPL)?

Summary answer: PGT-A can improve the live birth rate and reduce the rate of miscarriages in women with RPL following in vitro fertilization-embryo transfer (IVF-ET).

What is known already: PGT-A has been widely used in women with RPL over the past decade. However, there is no consensus supporting this approach to the management of RPL.

Study design, size, duration: Systematic review and meta-analysis according to PRISMA guidelines. The research population was focused on RPL women. The intervention was PGT-A. The comparator was IVF/ICSI without PGT-A or expectant management. The primary outcome was the live birth rate per woman, and the secondary outcomes were the miscarriage rate and clinical pregnancy rate.

Participants/materials, setting, methods: PubMed, EMBASE, The Cochrane Library, and trial registers were searched for studies on PGT-A and RPL up to October 2022. There were no limitations regarding the year of publication or duration of follow-up but to the English language. The primary outcome was the live birth rate per woman, and the secondary outcomes were the miscarriage rate and clinical pregnancy rate.

Main results and the role of chance: Out of 1466 search results, 9 studies (including one RCT, one prospective pilot study, and seven retrospective cohort studies) were included in the review. Seven of these studies compared the live birth rate of RPL with PGT-A versus without PGT-A following in vitro fertilization-embryo transfer (IVF-ET). PGT-A significantly increased live birth rate (odds ratio(OR): 2.07; 95% confidence interval: 1.88 to 2.27) and lowered miscarriage rate (OR: 0.21; 95% confidence interval: 0.13 to 0.34). However, PGT-A did not improve the clinical pregnancy rate. In two studies comparing the live birth rate/ongoing pregnancy of RPL with PGT-A versus expectant management, the result shows that PGT-A did not improve the live birth rate (OR: 1.11; 95% confidence interval: 0.74 to 1.67).

Limitations, reasons for caution: Limitations include the retrospective design and heterogeneity of studies included, limiting comparison and pooling of data. Some studies have limited sample sizes. The data collected in the database could not exclude anatomic or chromosomal abnormalities in women with RPL.

Wider implications of the findings: PGT-A can improve the live birth rate and reduce the rate of miscarriages in women with RPL following IVF-ET. However, there is no evidence of a beneficial effect of PGT-A if compared with expectant management. It is important to evaluate carefully the application of PGT-A in clinical practice.

Trial registration number: not applicable

Abstract citation ID: dead093.831
P-488 Pregnancy loss rate in recurrent pregnancy loss patients according to gestational week: life tables for clinical guidance of patients during supportive care before week 24

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Study question: What is the hazard for a new pregnancy loss (PL) each gestational week (GW) reached in RPL patients and does it depend on baseline characteristics?

Summary answer: The PL rate declines for every GW passed during pregnancy and the hazard rate depends on age, BMI, conception method, and history of late PL.

What is known already: A few studies in healthy women have provided information about the risk of PL for each GW reached but were highly biased due to variable entry time. No similar study has been performed in recurrent pregnancy loss (RPL) patients who often ask their clinician about their pregnancy prognosis for each GW passed. No tool based on baseline characteristics have been provided to guide patients on the risk of a new PL at a specific GW

Study design, size, duration: In a prospective cohort study from January 2016 to January 2023, 517 RPL patients with ≥2 previous, consecutive PLs, no uterine or chromosomal abnormalities, and who during follow-up had ≥1 pregnancies after admission to the specialized RPL Clinic of Western Denmark were included. All patients were guided to contact the clinic immediately after a positive urine-hCG test to have an early ultrasound scan from GW 5. All registered pregnancies after admission were included (N = 639).

Participants/materials, setting, methods: Life table analyses on the risk of a PL according to GW from GW 4-24 were performed on the first and on all subsequent pregnancies after admission, respectively, and hazard ratios (HR) for age (</≥35 years), BMI (</≥30), smoking (no/yes), previous livebirth (no/yes), conception method (natural/IUI versus IVF/ICSI/FET), number of previous PLs (≥/<5), and previous late PL (no/yes) were calculated by univariate log-rank test for equality and a multivariate Cox proportional hazard model.

Main results and the role of chance: The median GW of PL after admission (n = 169) was 6 (IQR 4-7). In total, 32.1% of patients had ≥1 PL after admission with a cumulative hazard of 0.37 (95%CI 0.32-0.43). The cumulated hazard at GW 7 was 0.27 (0.23-0.32) and 0.34 (0.30-0.41) at GW 12 at which times 76.5% and 94.0% of PLs, respectively, had occurred. The life tables for first pregnancy outcome stratified by baseline characteristics showed that the risk of PL per GW was independent of smoking (HR = 1.14 [0.76-1.73], p = 0.514), prior livebirth (0.77 [0.59-1.01] p = 0.056) and number of previous PL (1.21 [0.84-1.75] p = 0.302), while significantly differing curves and hazard rates were found for maternal age >35 (1.36 [1.00-1.85], p = 0.043), BMI >30 (1.39 [1.00-1.97], p = 0.050), previous late PLs (1.80 [1.20-2.70], p = 0.005), and conception by ART (1.75 [1.39-2.56], $p < 0.001$), respectively, in comparison to their counterparts (BMI<35, no

late PL, and natural conception). The results can be interpreted such as for each week the pregnancy has progressed intact, the risk of PL decreased significantly faster in recurrent PL patients with lower age and BMI, no prior late PL, and pregnancy after natural conceptioncompared with patients in the opposite groups[OBC1] . Comparable results were found when all pregnancies after admission were included.

Limitations, reasons for caution: For the stratified analyses, the size of each subgroup is small and therefore these life table analyses should be interpreted with caution. The risk of missing registration of early PLs is expectedly small, as patients are encouraged to take contact and have all PL registered to receive best supportive care.

Wider implications of the findings: The results illustrate that advice to RPL patients about expected pregnancy success at different GW of progressing pregnancy should be modified according to certain baseline characteristics. The time analysis graphs can help clinician inform their patients on the pregnancy prognosis and potentially alleviate the patient's stress.

Trial registration number: no applicable as no human specimens were used. Database registered locally by: 2018-5

Abstract citation ID: dead093.832
P-489 Uterine fluid-immune cell analysis at the time of the embryo transfer

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Study question: What are the subtypes of immune cells present in the upper and lower parts of the uterus at the time of frozen embryo transfer (FET)?

Summary answer: Our study is the first study confirming different immune cell subsets in the freshly collected uterine fluid at the time of the embryo transfer.

What is known already: Although endometrial receptivity is a key factor in influencing implantation in both naturally conceived and assisted reproductive technology (ART) cycles, very little is known about the endometrium milieu around the time of implantation. Previous studies have demonstrated the presence of several cytokines in the endometrium affect implantation. However, there is lacking data about the presence of immune cell subtypes within the endometrium and in the uterine cavity at the time of implantation.

Study design, size, duration: This study was approved by the Institutional Review Board (# 49174). The study was designed as a prospective observational cohort study between May 2021 and December 2022 at a single academic-based fertility center. All patients underwent at least one In Vitro Fertilization cycle (IVF) and have frozen embryos. Twenty-four participants were recruited for this study which was conducted during the frozen embryo transfer cycle (FET) regardless of the outcome of previous cycles.

Participants/materials, setting, methods: Trial transfer catheter was introduced under ultrasound guidance into lower uterine segment. Upon removal, the tip was rinsed in IMDN medium containing 10% FBS (lower). Embryo was then placed in upper uterus under ultrasound guidance. The tip of transfer catheter was rinsed in separate aliquot of the above media (upper). After centrifugation, pelleted cells were stained for the following surface markers: CD45, CD3, CD19, CD4, CD8, gamma delta TCR, CD25, CD127, CD66b, CD14, CD16, CD56.

Main results and the role of chance: Upon staining the pelleted cells, we were able to identify viable leukocytes from samples obtained from both, upper and lower uterus (0.1257×10^6 cells +/- SD 0.3167), (0.1230×10^6 cells +/- SD 0.1171), respectively. Among total viable cells, there was no significant difference in both the percent and number of CD45+ cells between the upper and lower uterus (9.880 +/- 6.983, 13.67 +/- 9.792, $p = 0.1980$) respectively. However, there was significantly higher expression of CD3+ ($p = 0.006$), CD19+ ($p = 0.0319$) and CD14+ ($p = 0.0189$) cells in samples collected from upper compared to lower uterus. Within all CD3+ cells, we found that gamma delta T cells (GDT) were the major population of T cells in both upper and lower uterus. In contrast, CD8+ T cells were significantly higher in the lower uterus when compared to the upper uterus ($p = 0.009$). There was no statistically significant difference in the expression of CD4+ T cells, T regulatory cells, NK cells (CD56+), neutrophils (CD66b+) and monocytes (CD14+) between upper and lower uterus.

Limitations, reasons for caution: Limitations of our study include number of participants as well as the number of parameters analyzed by flow cytometry. To maximize information from each sample, multicolor flow cytometry can give critical insight into immune cell composition of the uterine milieu and the difference in expression between upper and lower uterus.

Wider implications of the findings: We believe the immune milieu at the time of embryo transfer will affect implantation. Understanding the composition of immune cell will guide further research in identifying optimal immune milieus that favor implantation. Comprehensive analysis of endometrium is expected to lead to new diagnostic and therapeutic approaches to improve IVF outcomes.

Trial registration number: not applicable

Abstract citation ID: dead093.833
P-490 Impact of follicular phase characteristics on mid-luteal progesterone and ongoing pregnancy rates: Lessons learned from true natural cycle frozen embryo transfer

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Study question: Is there an association between follicular phase endocrine and ultrasonographic characteristics and ongoing pregnancy rates (OPRs) in true-natural cycle (t-NC) frozen embryo transfers (FETs)?

Summary answer: Endometrial thickness one day before ovulation is the only independent predictor of ongoing pregnancy (OP) in t-NC FET cycles.

What is known already: In ovulatory women, NC FET is currently being proposed instead of the hormonal replacement treatment (HRT) protocol since HRT is associated with increased maternal and obstetric risk factors, most probably due to the absence of a corpus luteum. However, the presence of ovulation in regularly cycling women does not guarantee a receptive endometrium. Currently, there is a lack of data regarding the impact of follicular phase endocrine and ultrasonographic parameters on mid-luteal progesterone (P_4) levels and OPRs in t-NC FET cycles.

Study design, size, duration: A cohort study of 229 women who underwent warmed blastocyst transfer employing t-NC. Three strategies were adopted. During January 2017-June 2017, serum P_4 was not monitored. During July 2017-June 2020, serum P_4 was routinely monitored on the FET-1 day. During July 2020-August 2022, a rescue strategy with daily 25-mg subcutaneous progesterone was adopted for patients with P_4 levels between 7-10 ng/mL. In patients with serum $P_4 < 7$ ng/mL FET was cancelled.

Participants/materials, setting, methods: Staying in-town and having regular menstrual cycles, permitting intensive endocrine and ultrasonographic monitoring (daily serum estradiol [E_2], luteinizing hormone [LH], P_4, and transvaginal ultrasonography), starting mid-cycle until confirmation of ovulation was the only inclusion criteria. Only the first FET cycle per patient was included. The primary outcome measure was OPR. The impact of follicular phase endocrine and ultrasonographic parameters on serum P_4 levels on the FET-1 day and OPRs were evaluated using regression analysis.

Main results and the role of chance: Follicular phase parameters, including follicular phase length, late follicular phase E_2, LH, P_4 levels, and maximal follicle diameter before ovulation, were not significant predictors of ongoing

pregnancy (OP) in t-NC FET. In contrast, the endometrial thickness one day prior to ovulation was a significant independent predictor of OP (adjusted OR = 1.29, 95% CI:1.06-1.56). When endometrial thickness before ovulation was assessed in quartiles, univariate OPRs in the 25th (<9.1 mm), 25-50th (9.1-10.1mm), 51-75th (10.2-11.6 mm) and >75th (>11.6 mm) percentiles were 43.3%, 47.3%, 71.7%, and 54.7%, respectively (p=0.016). Following logistic regression analysis, the adjusted ORs for OPR of the 51-75th (3.69, 95% CI:1.42-9.56) and >75th (2.86, 95% CI: 1.06-7.73) percentiles were significantly higher when compared with that of < 25th. The three strategies for monitoring/acting (rescue if needed) based on serum P_4 levels on the FET-1 day were associated with similar adjusted ORs for OP. Notably, cancellation of cycles with serum P_4 <7 ng/mL and adopting a rescue policy for those with 7-10 ng/mL did not increase OPRs. After multivariate linear regression analysis, late follicular E_2 levels (ß-coefficient: 0.399 ng/mL, 95% CI:0.007;0.021) and BMI (ß-coefficient: −0.292 ng/mL, 95% CI: −0.730;-0.222) were the only significant predictors of P_4 levels on the FET-1 day.

Limitations, reasons for caution: Limitations include the cohort study design evaluating three different policies, the single point serum P_4 assessment, and adopting an arbitrary lower cut-off of < 7 ng/mL for cycle cancellation and 7-10 ng/mL for a rescue strategy.

Wider implications of the findings: Endometrial thickness one day before ovulation was the only significant predictor of OP in t-NC FET cycles. Apart from endometrial thickness, none of the follicular phase parameters were significant predictors of OP. Single-point assessment of serum P_4 may not be reliable when assessing reproductive outcomes in t-NC FET.

Trial registration number: Not applicable

Abstract citation ID: dead093.834

P-491 The efficacy of subcutaneous versus vaginal progesterone for luteal phase support - a comparison in 723 blastocyst hormone replacement therapy frozen embryo transfer cycles

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Study question: Are ongoing pregnancy rates (OPRs) similar between subcutaneous (sc) progesterone (P) and vaginal P administration in hormone replacement therapy (HRT) frozen embryo transfer (FET) cycles?

Summary answer: The OPRs were similar between sc and vaginal P administration; however, 15.8% of patients in the vaginal group needed additional P as rescue therapy.

What is known already: There is a paucity of data comparing sc and vaginal P administration in HRT-FET cycles. In HRT-FET cycles, there seem to be marked interpersonal differences in serum progesterone (P_4) levels following the same dose and route of P administration. Impaired reproductive outcomes have been reported when serum P_4 levels are below a certain threshold (10 ng/ml) on the FET/FET-1 day; rescue with supplemental sc P in these cases might rectify reproductive outcomes. With sc P, only one study reported comparable OPRs in four quartiles of serum P_4 on the day of FET in HRT cycles.

Study design, size, duration: A cohort study was conducted during December 2019-November 2022, including a total of 723 consecutive warmed blastocyst transfer cycles, employing HRT. Each patient was included only once. The inclusion criteria were female age ≤44 years old, BMI ≤40 kg/m^2, and having available day-5/6 vitrified blastocyst(s) after warming. Pre-implantation genetic testing cycles for aneuploidy (PGT-A) were included, whereas PGT for monogenic disorders/structural re-arrangements and the presence of uterine anomalies were excluded from the study.

Participants/materials, setting, methods: Following estrogen priming, sc P 25 mg twice-daily (n = 249) or vaginal P gel 90 mg twice-daily (n=474) were administered. The serum P_4 level was measured on the FET-1 day, i.e., day 5 of P supplementation. In patients with serum P_4 levels <8.75 ng/ml on the FET-1-day, additional exogenous sc P, 25 mg/day (rescue), was provided. The primary outcome measure was OPR. A generalized additive model was performed to study functional relationships between P_4 and OPR.

Main results and the role of chance: The mean circulating P_4 levels on the FET-1 day were 26.1 ± 8.1 ng/ml and 13.8 ± 5.5 ng/ml in the sc P and vaginal P gel groups, respectively ($p < 0.001$). In the vaginal P group, 15.8 % of patients needed sc P rescue, whereas no patient required rescue in the sc P group. All unadjusted reproductive outcome measures, including positive hCG per embryo transfer, implantation, clinical pregnancy, pre-clinical loss, miscarriage rates, and OPRs, were comparable between the sc P and vaginal P gel groups. After P rescue, when logistic regression analysis was performed to delineate the independent predictor(s) of ongoing pregnancy, only female age, number of blastocysts transferred, blastocyst morphology, and PGT-A were found to be the significant predictors. Importantly, sc P administration, necessitating no rescue, provided similar OPRs (adjusted OR = 1.22 95% CI:0.87-1.80) compared to vaginal P, in which rescue was needed in 15.8% of the patients. The impact of serum P_4 levels on adjusted reproductive outcome measures was evaluated by percentiles (<10th, 10–49th, 50–90th, and >90th percentiles). All serum P_4 percentiles had similar OPRs, and no significant association was observed between serum P_4 levels and OPRs in both sc and vaginal P (with rescue if needed) groups.

Limitations, reasons for caution: Cohort study, lack of cost and side-effect analyses.

Wider implications of the findings: Subcutaneous P 25 mg twice-daily secures a serum P_4 above 8.75 ng/ml, whereas additional exogenous P (rescue) was needed in 15.8% of patients receiving vaginal P administration only. The OPR was similar between the sc P alone and vaginal P groups after sc P rescue of the vaginal group.

Trial registration number: Not applicable

Abstract citation ID: dead093.835

P-492 The relationship between dominant follicle development and clinical outcomes of hormone replacement therapy-frozen embryo transfer: a retrospective clinical study

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Study question: Dose the dominant follicle development occurred in the hormone replacement therapy-frozen embryo transfer cycles affect the clinical pregnancy outcomes?

Summary answer: The development of dominant follicles in HRT-FET cycles does not affect the clinical pregnancy rate, early miscarriage rate and live birth rate.

What is known already: The hormone replacement therapy is one of the most commonly used endometrial preparation protocols for frozen embryo transfer due to the convenience of administration and stability of pregnancy outcomes. Howere, here are 4-10% cycles accompanied by the development of dominant follicles.In order to avoid uncontrollable follicular development in HRT cycles, clinicians tried to increase the dosage of exogenous oestrogen or take oestrogen in advance, which still could not totally inhibit follicular growth. In addition, gonadotropin releasing hormone agonist is also used. However, excessive GnRHa use will increase the treatment cost and prolong the treatment time.

Study design, size, duration: This is a noninterventional, retrospective, observational, single-centre cohort study. From 2012 to 2019, 13251 cycles were included in this study.

Participants/materials, setting, methods: This study involved 13251 HRT-FET cycles. In 13107 cycles , there was no dominant follicle development in HRT cycles, while in 144 cycles, there was dominant follicle development. We used a multivariable logistic regression model and adopted

propensity-score matching, in order to study the impact of dominant follicle development on the clinical pregnancy outcomes of HRT-FET cycles, and aim to clarify the appropriate criteria for canceling HRT-FET cycles with dominant follicle development.

Main results and the role of chance: Considering the differences in the number of cycles and baseline characteristics between the two groups, we adopted propensity-score matching to identify the cycle cohort with similar baseline characteristics. After propensity-score matching, the baseline characteristics of patients in the two groups were similar. There was no difference in the number of transferred embryos and the proportion of transferred blastocysts between the two groups. There was no significant difference in clinical pregnancy rate, early miscarriage rate and live birth rate between the two groups. In addition, univariate analysis was also used to identify confounding factors that may affect clinical pregnancy outcomes). The baseline factors (female age, male age, AFC, baseline FSH level, infertility duration, number of previous embryo transfer cycles) and treatment factors (endometrium thickness, number of transferred embryos and type of transferred embryos) were selected as the adjustment variables of multivariate regression analysis. There was no significant correlation between dominant follicular development in HRT-FET cycles and clinical pregnancy rate (adjusted OR = 1.162, 95% CI: 0.737-1.832, P = 0.52).

Limitations, reasons for caution: The main limitation of this study is the retrospective design. In addition, there is a significant difference between two groups. In a real life setting, these results are cautiously applicable.Further prospective investigations are needed to elucidate the impact of dominant follicle development on clinical outcomes.

Wider implications of the findings: Our retrospective study suggests that the development of dominant follicles in HRT-FET cycles does not affect the clinical pregnancy rate, early miscarriage rate and live birth rate. Therefore, it is not necessary to cancel the FET cycle immediately when the dominant follicle development is monitored in the HRT-FET cycle.

Trial registration number: not applicable

Abstract citation ID: dead093.836

P-493 Bleeding during early pregnancy after Hormone Replacement Therapy Frozen Embryo Transfer (HRT-FET) – is it more common than we think?

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Study question: How common is bleeding in early pregnancy after HRT-FET? And does bleeding affect the reproductive outcome in this population?

Summary answer: A total of 47% of HRT-FET patients experience bleeding before the 8th week of gestation, however, bleeding does not seem to affect the reproductive outcome.

What is known already: Bleeding occurs in 20% of spontaneously conceived pregnancies. Most pregnancies will proceed to term although episodes of heavy bleeding are associated with an increased risk of miscarriage. The prevalence of bleeding after assisted reproductive technology including in vitro fertilization seems to be higher and, in some reports, up to 36%. However, the knowledge is scanty and in particular the knowledge regarding bleeding during HRT-FET is sparse. As HRT-FET in clinical practice increases, knowledge on the occurrence of early bleeding during pregnancy and the impact on the reproductive outcome in this population is needed.

Study design, size, duration: We performed a cohort study including 489 patients undergoing HRT-FET. The trial was conducted from January 2020 to November 2022 in a public fertility clinic.

Participants/materials, setting, methods: Patients with autologous vitrified blastocyst were treated in a standard HRT-FET protocol. In the event of a positive HCG-test an early pregnancy scan was performed around 8 weeks of gestation. During this visit the women answered a questionnaire covering pregnancy related symptoms, wellbeing, and if bleeding occurred, the type and duration of the bleeding. The information was verified through medical files.

Main results and the role of chance: In this HRT-FET cohort we found that a total of 47% (150/322) of patients with a positive pregnancy test experienced bleeding before 8 weeks of gestation. Mostly the bleeding was described as spotting with a median of 2 days (range 0.5—16 days). Out of 150 patients with one or several bleeding episodes, a total of 107 patients (71 %) had an ongoing pregnancy at 12 weeks of gestation. In comparison, 172 patients reported no bleeding episodes and a total of 115 (67%) of these patients had an ongoing pregnancy at 12 weeks of gestation. This difference was found to be non-significant (p = 0,39).

Limitations, reasons for caution: The patients in our study were treated in a standard HRT-FET protocol. The results may not be transferable to other treatment protocols. Also the data is based on patients self-reported symptoms. Therefore some degree of recall bias may be present.

Wider implications of the findings: Episodes of early bleeding during pregnancy are associated with distress for the pregnant woman – especially in previously infertile patients. Knowledge about the frequent occurrence of bleeding during early pregnancy after HRT-FET and that this does not affect the reproductive outcome will help the clinician reassure and guide the patient.

Trial registration number: 2019-001539-29

Abstract citation ID: dead093.837

P-494 Relevance of intravenous immunoglobulins in patients with unexplained recurrent miscarriages that occurred at the same gestational weeks every time

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Study question: Are intravenous immunoglobulins (IVIG) effective for the selected patients who experienced unexplained recurrent miscarriages (RM) that occurred at the same gestational weeks every time?

Summary answer: IVIG before and after the gestational week-limit (GWL), where miscarriage occurred at the same gestational weeks every time,may improve pregnancy outcomes in RM patients.

What is known already: Treatment for patients with unexplained RM are challenging. Since the efficacy of immunotherapy with paternal mononuclear cells has been denied in 1999, some treatment options (e.g., intralipid, G-CSF) have been published with conflicting results. In terms of IVIG, the same story has been described. Because previous studies for IVIG treatment included very heterogeneous group of patients, different dose, different intervals of IVIG, and different starting time of infusions. Recently, a double-blind, randomized, placebo-controlled study for unexplained RM women has been published in 2022, which revealed that IVIG (0.4g/kg) for five consecutive days at 4-5 weeks of gestation improved pregnancy outcomes.

Study design, size, duration: We performed a retrospective study between September 2013 and May 2021 in two fertility centers. We included 106 patients in the study who experienced 2 or more miscarriages between 5 and 21 weeks of gestation that occurred at the same gestational weeks every time, who had all negative results for our routine RM work up and still unsuccessful result after treating these conditions, and whose products of conceptus (POC) revealed at least one normal karyotype.

Participants/materials, setting, methods: IVIG (Venoglobulin IH 5%, Japan Blood Products Organization, 0.4g/kg) was injected twice before and after 1 week of GWL. If the GWL was 8 weeks of gestation, IVIG should be injected 7 and 9 weeks. We defined successful IVIG treatment as live birth. When the pregnancy ended in miscarriage, POC was performed as much as possible. All participants provided written informed consent, and Institutional Review Board approval was obtained.

Main results and the role of chance: Of 106 patients, average age was 37.5 years (28-47 years) and the mean number of previous miscarriages was 3.1. Total 128 cycles of IVIG attempt resulted in 90 (70.3%) successful

pregnancies, 36 miscarriages and 2 biochemical pregnancies. After excluding abnormal karyotype of POC (N = 7), 68.6% (83/121) was successful. Per patients, 81.1% (86/106) had at least one live birth. Of 32 patients who experienced miscarriages at the first IVIG attempt, 16 patients got pregnant. Following second IVIG attempt resulted in 12 live births (75.0%). The average weight of live birth babies was 3038g and the birth week was 38.8 weeks of gestation (34-41 wk) for singleton pregnancy (N = 83), whereas 2529g with 35.9 weeks of gestation (33-37 wk) for twin pregnancy (N = 7). Four (4/83, 4.8%) and five (5/7, 71.4%) preterm deliveries were observed for singleton and twin pregnancy, respectively. One baby showed pulmonary hemorrhage resulting in hospitalization in the Neonatal Intensive Care Unit, other live birth babies were without any abnormal findings. Hypertensive disorders of pregnancy were noted in two women resulting in cesarian section delivery.

Limitations, reasons for caution: This study was retrospective study without any control group and was conducted at only two fertility centers. In future clinical research, it is necessary to conduct randomized controlled trials using placebo group to demonstrate the effectiveness of IVIG before and after the GWL using euploid blastocysts.

Wider implications of the findings: IVIG before and after the GWL adjusted for each patient may improve pregnancy outcomes in RM patients. Although IVIG is expensive, the current method requires only two IVIG injections, that may be acceptable for much more patients who want to avoid miscarriages and who didn't use IVIG for economic reasons.

Trial registration number: not applicable

Abstract citation ID: dead093.838

P-495 What is the optimal cut off value of the number of egg donations from the same egg donor lifetime?

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Study question: Do repeated ovarian stimulation cycles in the same donor have a negative impact on clinical outcome?

Summary answer: There is no negative impact of the consecutive ovarian stimulation cycles on embryological parameters and outcomes from the same donor.

What is known already: Different country regulations required from single stimulation to maximum 6 stimulations for egg donors in lifetime. Modern assisted reproductive technologies (ART) clinics should consider this issue described above from the point of view of the number of oocytes retrieved during controlled ovarian stimulation cycles - this is a balance between efficacy of the ART in infertility treatment programs and donor safety. Despite some studies have underscored the likely negative impact of repeated cycles on outcomes it remains up for debate. The age groups of 20-30 years old egg donors may give similar clinical outcomes for 1st-6th attempt of stimulation cycles.

Study design, size, duration: In this single center, 4 years of retrospective study included oocyte donors (n = 96) with repeating cycles of ovarian stimulation (COS). Cycles took place between January 2018 and December 2022 in an IVF clinic with respect to at least 6 months interim between each cycle.

We evaluated the concordance between the follicle-to-oocyte index (FOI), number of oocytes retrieved, maturation rate (MR), utilization rate (UR) and the numbers of attempts from 1st to 6th.

Participants/materials, setting, methods: The mean age of the patients in this study was represented in dynamics (SD) from 1st COS program 23,2 (3,2) to 27,8 (3,7) years during the final 6th cycle, all of them passed through 6 consequence COS programs.. The association between each COS cycle' parameters and the differences in COS output was assessed by T-Student (parametric) or U-Mann-Whitney (non-parametric).

Main results and the role of chance: We observed that mean number (SD) of mature oocytes retrieved was non significantly different and raised from the 1st attempt –18,46 (8,89), to 5th - 22,03 (10,16), with decreasing during 6th COS – 21,55 (12,06). MR and UR indicators had a fluctuating picture of the dynamics of values. They either increased or decreased from one cycle to another. Mean values of MR was the lowest after 3rd COS (81%) and the highest – 4th COS (84%). UR dynamic was similar with lowest value after 1st cycle (12,0%) and the maximum 17,9% after 6th COS program. FOI parameter was measured within values of 73,82 – 81,02: min value 73,82 (16,40) was observed after the second stimulation cycle and max value – after fifth (mean = 81,02 ± 18,26). These changes were found to be not significantly different ($p > 0.05$). Regarding egg donation cycle outcome for recipients (age average 34,57) attempt 1st –3rd gave 96,14% fertilization rate, 66,89% used blastocysts rate and 60,77% clinical pregnancy rate. Recipients (average age 33,19) attempting 4th- 6th gave 94.17% fertilization rate, 64,09% used blastocysts rate and 63,19% clinical pregnancy rate.

Limitations, reasons for caution: This study is limited regarding retrospective nature and by the small samples size. Expanding the number of participants is the possible next step, in order to challenge or rather confirm our preliminary findings.

Wider implications of the findings: The controversial dynamic of the changes of the embryological parameters and COS outcomes in the same donor's pool after repeated stimulation cycles with no statistically observed differences can be grounds for making a positive decision both personally by the donor and the IVF specialist.

Trial registration number: NA

Abstract citation ID: dead093.839

P-496 The effect of adding letrozole to the combined treatment of caesarean scar pregnancy with methotrexate followed by hysteroscopic evacuation of products of conception

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Study question: Does adding letrozole to combined cesarean scar pregnancy (CSP) treatment cause a greater decrease in B-human chorionic gonadotropin (B-hCG) concentrations and less blood loss?

Summary answer: The addition of letrozole to the combined CSP treatment does not result in a greater decrease in B-hCG concentrations or less blood loss.

What is known already: There is no reference treatment for CSP as the limited number of cases precludes the extrapolation of results. In our center we successfully use two-step treatment with methotrexate followed by hysteroscopic removal of products of conception (POC). The time in between is needed to achieve a decrease in the trophoblast's vital potential (B-hCG fall) and its vascularization. The suppression of estradiol production by letrozole may interfere with the physiological effect of progesterone, which is essential for the maintenance of pregnancy. Additional administration of letrozole could further reduce the vital potential of the pregnancy, resulting in a lower rate of complications.

Study design, size, duration: A prospective cohort tertiary single-center study (consent no. 1072.6120.321.2020) was conducted among women with CSP from January 2021 to December 2022. Only women with increasing concentrations of B-hCG and consent of the Bioethics Committee for termination of pregnancy were included. Women with decreasing B-hCG concentrations were excluded. All enrolled women gave informed written consent to participate in the study.

Participants/materials, setting, methods: All women received a single dose of 100 mg MTX intravenously and 50 mg MTX in intra-amniotic injection (day 0), along with 30 mg potassium chloride in case of positive fetal heartbeat (FH). Women who consented were additionally given letrozole 5 mg orally (day 0) for 10 days. B-hCG concentration was measured on day 0,4,7.

After obtaining satisfactory decrease in B-hCG and POC vascularization, women underwent hysteroscopic evacuation of POC. Blood loss parameters were measured.

Main results and the role of chance: The study included 28 women aged 27-41, with a gestational age of 6-12 weeks. FH was present in 17/28 (60.7%) women. Of the 28 women, 16 (57.1%) received additional letrozole (arm 1). There was no significant difference between arm 1 vs. arm 2 (without letrozole) in terms of gestational age (7.69 ± 1.54 vs. 8.00 ± 2.09 weeks, p = 0.85), FH positivity (10/16, 62.5% vs. 7/12, 58.33%; p = 1), BMI (25.39 ± 5.08 vs. 26.24 ± 6.51; p = 0.82), day 0 B-hCG (47656.89 ± 37747.87 vs. 51870.83 ± 45838.87 mIU/ml; p = 0.89) and day 0 hemoglobin (Hb) (12.98 ± 0.74 vs. 12.74 ± 1.26 g/dl; p = 0.57). Timing of hysteroscopy was not significantly different between arm 1 vs. arm 2 (day 22.19 ± 14.44 vs. 31.42 ± 25.83; p = 0.42). The dynamics of B-hCG decline in arm 1 vs. arm 2 between days 4-0 (-3295.67 ± 15133.13 vs. −6883.00 ± 20029.88 mIU/ml; p = 0.71) and 7-4 (-13137.82 ± 13765.14 vs. −19145.75 ± 23526.70 mIU/ml; p = 0.68) was not significantly different. There were no differences between arm 1 vs. arm 2, in decrease in Hb concentration on day 1 post-hysteroscopy (-1.79 ± 1.67 vs. −2.19 ± 1.68 g/dl, p = 0.53), blood loss volume (318.75 ± 437.37 vs. 591.67 ± 744.02 ml; p = 0.29) and rate of hemorrhage (4/16, 25% vs. 3/12, 25% cases, p = 1).

Limitations, reasons for caution: The limitations of the study are the small sample size, the single-center nature of the study, and the arbitrarily set dosing regimen of letrozole.

Wider implications of the findings: The results do not support the use of letrozole in the above regimen as an adjunct to combined CSP treatment. Trials with a larger sample size and with a different letrozole dosing regimen are needed to confront the results.

Trial registration number: 072.6120.321.2020

Abstract citation ID: dead093.840
P-497 High-concentration of E2 post-controlled ovarian hyperstimulation promotes cervical extracellular matrix degradation by up-regulating UBA52

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Study question: To explore the effect of high concentration of E2 after controlled ovarian hyperstimulation (COH) on the extracellular matrix of cervical tissue in the second trimester.

Summary answer: The high concentration of E2 after COH promotes cervical ECM degradation leading to cervical insufficiency of pregnancy in ART by up-regulating UBA52.

What is known already: Assisted reproductive technology (ART) is a double-edged sword. While effectively solving infertility, ART also associated with many obstetric complications like preterm birth, although, the reason is currently unclear. Cervical insufficiency (CI) caused by degradation of extracellular matrix (ECM) is one of the most important causes of preterm birth. All patients undergoing ART need to receive controlled ovarian hyperstimulation (COH), leading to a sharp rise of the serum level of E_2, which has a significant impact on the blood system, liver and kidney, but the effect on the cervix is unclear.

Study design, size, duration: 1.Retrospectively analyzed the clinical data of our center to compare the incidence of cervical insufficiency between ART singleton pregnancy and natural singleton pregnancy. 2. Establishing a post-COH pregnancy mice model, to analyze how the concentration of E2 affect the cervical ECM.

Participants/materials, setting, methods: The incidence of CI and the serum level of E_2 on the day of human chorionic gonadotropin (HCG) in ART singleton pregnancies and natural singleton pregnancies was compared. The pregnant mice models after COH were established to detect the serum level of E_2 and to investigate the structure of the cervical ECM. We detected the overall expression level of collagen and selected the mark gene using RNA-seq.

Relevant biological experiments were used for verification 14/5000

Main results and the role of chance: The incidence of CI in ART singleton pregnancies was significantly higher than that in natural singleton pregnancies (7.80% *vs.* 3.54%, $p<0.0001$) and so was the serum level of E_2 in patients with CI during pregnancy than those without (3333 ± 1116.61 pg/ml, n = 40 *vs.* 2437 ± 1072.2 pg/ml, n = 9, $p=0.034$). Furthermore, the serum level of E_2 was markedly higher in the COH pregnant mice models (175.68 ± 38.72 pg/ml, n = 10 *vs.* 235.43 ± 67.24 pg/ml, n = 11, $p=0.024$) and the expression of collagen was reduced significantly, and DEGs related to ECM organization pathway was significantly enriched. Our results indicated that matrix metalloproteinases (MMPs) should not be responsible for the collagen degradation in ECM of cervix. Interestingly, the ubiquitination level of type I collagen increased compared with the control group both *in vitro* and *in vivo* experiments. *UBA52*, a component of the ubiquitin-encoding gene, has been shown to strongly upregulated among these ubiquitination related genes (URGs), and its expression was demonstrated to be E_2 concentration dependent. Also, an estrogen response element (ERE) was detected in *UBA52* promoter region, and direct binding of ER to ERE was validated, implying that *UBA52* might be a key regulator for collagen degradation.

Limitations, reasons for caution: Firstly, the findings of clinical data were still inevitably subject to retrospective study limitations. Secondly, we found that *UBA52* may be the target gene that causes the increased ubiquitination of type I collagen in mouse cervical tissue after COH, but the deeper related mechanism was still unclear.

Wider implications of the findings: Our research provides evidence that a high concentration of E_2 after COH is harmful to cervical function leading to cervical insufficiency, which further underlines the importance of providing special care to women who use ART for pregnancy.

Trial registration number: not applicable

Abstract citation ID: dead093.841
P-498 Towards precision medicine: maternal genetic profiles as determinant of adequate decidualization and reproductive success; a prospective study in oocyte donation cycles.

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Study question: To determine the impact of genes related to decidualization and immune cell activation as predictors for reproductive success.

Summary answer: Women with unfavorable genetic profiles for Human Leukocyte Antigen (HLA)-F and Killer Immunoglobulin-like Receptor (KIR) genes showed decreased pregnancy rates.

What is known already: KIR genes encode receptors present on uterine Natural Killer (uNK) cells that are essential during placentation and early gestation. It has been established that women of KIR AA genotype show lower reproductive success than KIR BX, due to the lack of activation of these immune cells. Additionally, another gene, HLA-F, expressed in endometrial cells, also seems to be involved in the recruitment and supplementary activation of uNKs. Certain alleles of this gene appear to decrease its expression in the uterus and hinder the processes in which it is involved during the decidualization process, decreasing implantation rates and increasing time-to-pregnancy.

Study design, size, duration: We conducted a prospective cohort study with 75 patients, with no history of recurrent miscarriage or implantation failure, who attended our institution to undergo assisted reproduction treatments (ART) with donated oocytes, between January 2021 and December 2022. By using the egg donation model, we were able to investigate the endometrial cell population avoiding the oocyte factor bias

Participants/materials, setting, methods: All patients included were under 45 years of age (40.5 ± 2.9 years), had a normal BMI (21.6 ± 1.9 kg/m^2) and had not been diagnosed with uterine pathologies or immunological conditions. All recipients were genotyped for the KIR and HLA-F genes and pregnancy, miscarriage (MR) and live birth rates (LBR) obtained in their egg donation cycles were recorded. Fisher's test was used to determine statistically significant differences ($p < 0.05$) between groups

Main results and the role of chance: Patients were divided into two groups according to their KIR genotype: 29 KIR AA (inhibitor) and 46 KIR BX (activator). In each group, allelic load was determined for the three HLA-F regions studied (RS1362126, RS2523405 and RS2523393). For all these regions, patients with favorable HLA-F alleles and KIR BX genotype had higher pregnancy rates than KIR AA patients with unfavorable HLA-F alleles (85.42 % vs 54.17% for RS1362126 region, OR = 4.956, p = 0.008; 83.33% vs 58.62% for RS2523405 region, OR = 3.529, p = 0.0295; 82.5% vs 57.69% for RS2523393 region, OR = 3.457, p = 0.0462). No significant differences were obtained for MR or LBR. This demonstrates the involvement of KIR and HLA-F in embryo implantation and that the coincidence of unfavorable alleles for both decreases reproductive success considerably, probably due to impaired decidualization and lack of uNK recruitment and activation.

No significant differences were obtained for the rest of the genetic profiles, except when comparing pregnancy rate of KIR AA women with favorable HLA-F alleles to KIR AA women with unfavorable HLA-F alleles for the RS1362126 region (79.41% vs 54.17%, OR = 3.264, p = 0.04). The latter finding suggests that the lack of uNK cell activation, so evident in KIR AA patients, could be compensated by expressing favorable HLA-F alleles.

Limitations, reasons for caution: The low sample size of this study does not allow us to compare groups taking into account other genes, such as embryonic HLA-C, which also, and in combination with KIR genotypes, has been shown to play a role in proper implantation and placentation.

Wider implications of the findings: uNKs activation is required to promote embryo implantation. Combination of unfavorable genetic profiles involved in these immune mechanism leads to lower pregnancy rate. The study of these genetic profiles can help to predict reproductive success, thus identifying patients susceptible to receive immunological treatment before embryo transfer, possibly decreasing their time-to-pregnancy.

Trial registration number: Not applicable

Abstract citation ID: dead093.842

P-499 Individualization of the mean luteal phase after frozen embryo transfer with hormone replacement therapy

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Study question: Objective: To determine in embryo cryotransfer cycles with hormone replacement therapy whether progesterone levels five days after transfer < 15.7 ng/mL correlate with reproductive outcomes.

Summary answer: Conclusion: We cannot affirm that low levels of progesterone in the mid luteal phase after cryotransfer are an independent factor of evolutionary gestation.

What is known already: n the transfer of frozen embryos with hormone replacement therapy after a period of endometrial preparation with estrogens for 10-12 days, luteal phase support with vaginal progesterone is administered as many days as the embryo to be transferred, as reported by Van de Vijver et al 2001. After reporting that low progesterone levels on the day of transfer were associated with worse outcomes, our purpose is to evaluate progesterone levels after transfer with the first prospective study monitoring post-transfer progesterone levels to see if its supplementation correlates with better reproductive outcomes.

Study design, size, duration: Study desing: Analytical, observational, longitudinal, prospective study conducted at the Assisted Reproduction Unit of Hospital Quironsalud Barcelona between December 2021 and May 2022. 159 patients aged 18-42 years who have undergone a cycle of frozen embryo transfer (CT) of their own or donated embryos with hormone replacement therapy.

Participants/materials, setting, methods: Material and methods: Determine in embryo cryotransfer cycles with hormone replacement therapy with estradiol, luteal phase support of micronized natural progesterone five full days prior to the transfer of a blastocyst.Determination of progesterone levels five days after transfer, if levels >= 15.7ng (group C) we followed the same guideline, if < 15.7 ng one group was supplemented with 25 mg Psc (group A), and another group of patients (group B) was not supplemented.

Main results and the role of chance: Results: Total of 159 patients with mean age of 37.86 years, mean antimuleriana value 2.95 ng/ml, antral follicle count of 11.54 and mean endometrial thickness of 8.93mm. 55.97%(n = 89) TC had P4D+10 <15.7ng. Pregnancy rate (BHCG>5mIUI/mL) was higher in the group with progesterone 5 days after transfer (P4D+10) >=15.7ng with 65.71% CI95%[53.31,76.38] p = 0.65, and in the group of P4D+10 < 15.7 ng became pregnant 53.57% CI95%[34. 21,71.99] p = 0.53 of group A with respect to 45.90% IC95%[33.26,59.06] p = 0.45 group B. Clinical evolutionary pregnancy rate was higher in group A 80% vs 67.86% of group B, p-value=0.06. Abortion rate lower in group A 20% vs 32.14% group B, p = 0.01.

Limitations, reasons for caution: Limitations. There are few previous studies that allow us to support our arguments, the variability of progesterone levels in blood throughout the day can alter the results,nevertheless, and the sample size is small and it is difficult to find significant differences.

Wider implications of the findings: We did observe that >50% (55.97%) of the patients presented low values of progesterone after the embryo frozen transfer, with an improvement in pregnancy rate 53.57% vs 45.90% when these were rescued with subcutaneous progesterone. About 7% of pregnancies would be lost without this monitoring and supplementation.

Trial registration number: not applicable

Abstract citation ID: dead093.843

P-500 Relationship of midluteal serum progesterone levels and pregnancy outcomes after day-3 embryo-transfer(ET) in hormonal replacement therapy (HRT) cycles with intramuscular progesterone: a prospective cohort study

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Study question: Does midluteal(day-9 of progesterone) serum progesterone level in HRT cycles with intramuscular progesterone affect the clinical pregnancy rate (CPR)/early miscarriage rate(MR) and live birth rate(LBR)?

Summary answer: Midluteal serum progesterone between 32.2-43.7ng/ml results in significantly greater CPR versus that with lower/higher levels and significantly greater LBR compared to that with lower levels.

What is known already: Evidence points to low serum progesterone levels at the time of FET being associated with lower ongoing pregnancy rates. Recently, the relation of midluteal serum progesterone levels in HRT cycles with vaginal progesterone as luteal phase support (LPS) has been elaborated indicating better pregnancy outcomes with an optimum range of serum progesterone/higher levels. There is no data establishing the association of pregnancy outcomes with midluteal serum progesterone levels when intramuscular progesterone is used as LPS in HRT cycles.

Study design, size, duration: A prospective cohort study conducted between December 2019-March 2022 at a tertiary centre with 196 patients(204 cycles), both-autologus(AO)-56.3% and donor oocytes(DO)- 43.6%: fresh ET-16.6% and frozen ET(FET)-83.3% with similar ICSI, vitrification and ET protocols(2/3 Grade A day-3 embryos). CPR, early MR (≤13 weeks miscarriage/clinical pregnancy) and LBR (≥28 weeks, LBs/ETs) were compared. Serum progesterone was measured on the morning of day-5 post-ET(day 9th from the start of progesterone). Most patients delivered at the same institute.

Participants/materials, setting, methods: Patients were non-smokers with normal hysteroscopy. Oral estradiol 6-8mg was initiated from Day 1-2 (for 10-17 days). When ET ≥ 7mm and serum progesterone <1ng/ml, intramuscular progesterone 100mg/day was started (daily till day-4 post-ET, then every 3rd day till week 8). Vaginal progesterone gel was added from day 5 post-ET till week 10.

Serum progesterone levels were categorized in tertiles. Chi-square, t-tests and multiple regression analysis was used for statistical analysis. Two-tailed *P*<.05 indicated statistical significance.

Main results and the role of chance: Mean age was 34.76 ± 5.36 years with a mean BMI 26.4 ± 4.38kg/m^2 Overall, CPR was 106/204(51.9%), LBR 78/204(38.2%) and early MR 28/106(26.4%) Midluteal serum progesterone levels ranged from 11.1-261ng/ml and were divided into tertiles(T) T1<32.0ng/ml(n = 68),T2 32.2-43.7ng/ml(n = 68),T3 >43.7ng/ml (n = 68) Median progesterone levels of T1,T2,T3 were 27.7ng/ml(Interquartile range [IQR] 7.0), 38.4ng/ml (IQR 5.0) and 51.2ng/ml (IQR 12.6) respectively. Mean age and mean BMI was not significantly different across the tertiles *P*=.27, *P*=.20 respectively CPR was significantly different across T1 32/68(47%),T2 45/68(66.1%)and T3 29/68(42.6%) *P*=.014. CPR was significantly more in T2 versus T1 and T3, (OR:2.2, 95%CI:1.1-4.4 *P* =.02) and (OR:2.6,95%CI:1.3-5.3, *P*=.006), respectively. T3 versus T1, *P* =.60 LBR was also significantly different across the tertiles [T1 20/68(29.4%), T2 34/68(50%),T3 24/68(35.3%) *P*=.03] LBR in T2 was significantly more than in T1(OR: 2.4,95% CI:1.2-4.9, *P*=.02). There was no significant difference betweenT3 versus T1 and T2 versus T3, *P*=.46, *P*=.08, respectively. Early MR wasn't significantly different across T1 12/32(37.5%), T2 11/45(24.4%) and T3 5/29(17.2%), *P*=.18 Serum progesterone levels were not significantly affected by age r = 0.0257, *P*=.71 or BMI r = 0.0097, *P*=.89 Multivariate logistic regression showed that age affected CPR after adjusting for BMI, midluteal serum progesterone levels, origin of oocytes, fresh ET/FET(AOR 0.92: 95% CI:0.86-0.98, *P*=.*01*).

Limitations, reasons for caution: This is a single centre study with limited sample size. Including the measurement of serum progesterone at the time of ET would have given a better perspective of the patient specific behavior of progesterone as LPS. Larger studies with different LPS protocols are needed to validate the results.

Wider implications of the findings: In HRT cycles with intramuscular progesterone, an optimum midluteal serum progesterone level is related to better pregnancy outcomes. Factors causing the wide variation of progesterone levels need to be evaluated so as to individualize the dose. Identifying the appropriate time frame for optimising the midluteal progesterone levels should be explored.

Trial registration number: not applicable

Abstract citation ID: dead093.844

P-501 Live birth rates (LBR) are not influenced by a change in embryo quality imposed by embryo cryopreservation and thawing in single euploid frozen embryo transfers.

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Study question: Is a change of embryo quality through vitrification and thawing associated with a reduced LBR when a single euploid frozen embryo transfer is performed?

Summary answer: Live birth rates are not influenced by a change in embryo quality through embryo cryopreservation and thawing in single euploid frozen embryo transfer.

What is known already: Embryo aneuploidy is the most significant single factor and accounts for about 50% of implantation failure and miscarriage in human reproduction. Preimplantation genetic test for aneuploidy (PGT-A) presents a mean to identify euploid embryos, thereby improving pregnancy rates and reducing early pregnancy losses. However, after PGT-A on trophectoderm cells at blastocyst stage, embryos have to be cryopreserved and warmed later for the embryo transfer procedure. Embryo quality is known to impact implantation and ongoing pregnancy rates, therefore, the aim of this analysis was to evaluate the impact of a change in embryo quality between pre-vitrification and post-thaw on the outcome.

Study design, size, duration: This retrospective observational study included a total of 611 pregnant patients after frozen-thawed euploid single embryo transfer (FET) at a tertiary IVF centre between April 2017 and December 2020. All embryos transferred were cryopreserved after trophectoderm biopsy for PGT-A, performed either on day 5 or day 6. Each patient was only included once into the analysis. Endometrial preparation for FET was performed either in a natural cycle (NC) or as a hormonal replacement cycle (HRT).

Participants/materials, setting, methods: Patients with infertility, undergoing ovarian stimulation and FET in either a HRT or a NC as endometrial preparation approach, and who achieved a pregnancy, were included. Embryos were morphologically graded pre-vitrification and post-thaw based on inner cell mass and trophectoderm morphology on the day of cryopreservation. Four groups were initially established (top, good, fair, poor), hence for the analysis, fair and poor qualities were combined.

Main results and the role of chance: A total of 591 patient were analysed after exclusion of 20 patients due to the occurrence of late miscarriage and ectopic pregnancy. Patients' characteristics are as follows (mean ± SD (min-max)): age 33.87 ± 5.55(20-47) years; AMH 3.20 ± 3.28ng/mL(0.01-35.72); BMI(body mass index) 26.61 ± 4.86kg/m^2(14.84-40.31) and AFC 14.00 ± 7.33(1-35).

The changes in the embryo quality pre-vitrification and post-thaw were categorized in "no change", "improved" or "degraded". All groups were compared to top-quality-no change group. A binary logistic analysis evaluated the following groups regarding their combined impact on LB: top quality-degraded (odds ratio (OR)0.702; 95% confidence interval (CI)0.302-1.635, p = 0.413); good quality-no change (OR 1.022; CI:0.457-2.287; p = 0.958); good quality-degraded (OR 0.787; CI:0.330-1.880; P = 0.590); good quality-improved (OR 0.301; 0.056-1.628; P = 0.163); fair+poor quality-no change(OR 0.521; 0.203-1.340; P = 0.176); fair+poor quality-degraded (OR 0.283; 0.056-1.438; P = 0.128) and fair+poor quality-improved (OR 1.917; 0.431-8.534; P = 0.393). No impact on the probability of LB was seen in any combined group of change in embryo quality with its pre-grades. Furthermore, no association was found for age, BMI, AMH, pre-vitrification and post-thaw quality. Embryos biopsied on day 6 instead day 5 had a decreased chance of a LB (OR 0.516; 0.305-0.875, p = 0.014). Further on, NC was associated with significantly higher chance of LB compared to HRT cycle (OR 2.629; 1.606-4.305, p = <0.001).

Limitations, reasons for caution: Limitation is the retrospective design of the study and the grading of the embryos, which might be subjective and bearing interobserver variability.

Wider implications of the findings: Whereas a change in the embryo quality does not impact the chance of LB after single euploid FET, a NC approach increases the odds for LB. This fact should be considered for a personalized treatment approach and in the choice of the endometrial preparation protocol.

Trial registration number: not applicable

Abstract citation ID: dead093.845

P-502 Higher progesterone levels on the day of frozen embryo transfer is associated to better reproductive outcomes and higher obstetric complications: a retrospective cohort study

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Study question: Can serum progesterone (P) levels on the day of frozen embryo transfer (ET) compromise reproductive outcomes and/or obstetric complications?

Summary answer: Serum P levels was related to higher clinical pregnancy rate on frozen ET cycles. However, women with P level≥8.8 ng/ml presented higher obstetric complications.

What is known already: P levels <9.2 ng/ml on the day of ET significantly decreased ongoing pregnancy rate (OPR) in 211 donated oocyte cycles. In another study of the same group, serum P levels <8.8 ng/ml on the day of ET presented lower OPR in both own or donated oocyte cycles. Another study shown that low serum P4 the day prior to euploid FET can benefit from association of injectable P and achieve similar reproductive outcomes compared to those with initial higher P levels.

Study design, size, duration: This is a retrospective cohort study that included 509 women who have undergone frozen embryo transfer. In this study was included only first cycle of each patient. The women were divided into two groups according P levels: 1) P level <8.8 ng/ml (n = 388) and 2) P level≥8.8 ng/ml (n = 121). The analysis included the period between 01 January 2020 to 31 December 2021.

Participants/materials, setting, methods: All participants of study who underwent frozen ET after an artificial endometrial preparation cycle with estradiol valerate or 17βestradiol and vaginal micronized progesterone(600 mg/day). Age, body mass index(BMI), cause of infertility, and reproductive outcomes(clinical pregnancy, miscarriage, twin pregnancy, live birth rates) and obstetric complications (prematurity, ectopic pregnancy, acretism, hypertensive syndrome in pregnancy, gestational diabetes, intrauterine growth restriction rates) were analyzed. Only women without uterine abnormalities and with endometrium≥6.5 mm were included in this study.

Main results and the role of chance: Clinical pregnancy rate did not significant statistical between the 1 and 2 groups. Live birth rate (p = 0.01) was significantly higher in group 2. Other reproductive outcomes were similar between the groups. In relation to obstetric complications, miscarriage rate was significantly higher in group 1 (p < 0.01) and prematurity (>0.01), acretism (> 0.01), and gestational diabetes (p = 0.01) rates were higher in group 2. The P level did not present relation to cause of infertility. The logistic regression analysis shown that only good embryo quality was predictor to clinical pregnancy rates.

Limitations, reasons for caution: These outcomes were shown only in women without uterus abnormality and that used vaginal progesterone. It is necessary caution to considerer these results to general population. Furthermore, serum P level was analyzed in single moment without definition of interval between last vaginal P dose administration and blood sample collected.

Wider implications of the findings: Monitoring P levels on FET day should be performed routinely on artificial cycles because it can be related to higher live birth rate. However, new studies can confirm if higher P levels on FET day or adjust of P level according P value will be related to better reproductive outcomes.

Trial registration number: not applicable

Abstract citation ID: dead093.846

P-503 Immunological therapies in women undergoing assisted conception: a systematic review and meta-analysis

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Study question: What is the effectiveness and safety of immunological therapies in women undergoing assisted conception?

Summary answer: The evidence on the overall effectiveness and safety of immunomodulators was scarce and uncertain, although some drugs may lead to improved outcomes in selected women.

What is known already: Embryo implantation is a pre-requisite for pregnancy success. It requires a state of immune tolerance within the maternal endometrium permitting the attachment and subsequent invasion into the decidua of the semi-allogenic blastocyst. Mechanistic data suggesting that heightened immune responses may impair implantation have led to the widespread use of immunomodulatory therapies in clinical practice. These include drugs such as aspirin, heparin, corticosteroids, intralipid and intravenous immunoglobulin (IVIG). However, despite the common use of immunomodulatory drugs in women having in vitro fertilisation (IVF), there is no consensus on their effectiveness and safety, owing to a paucity of well-designed interventional studies.

Study design, size, duration: We searched MEDLINE, EMBASE, PubMed and CENTRAL until September 2022. We selected randomised controlled trials (RCTs) investigating immunological therapies in women undergoing IVF, including aspirin, heparin, corticosteroids, granulocyte-colony stimulating factor (G-CSF), intralipid, intravenous immunoglobulin (IVIG), tumour necrosis factor-alpha inhibitors and peripheral blood mononuclear cells (PBMCs), evaluated alone or in combination, versus no intervention, placebo or any other immunomodulator(s). The primary outcomes were the rates of live birth or ongoing pregnancy (LBR/OPR) and miscarriage per participant.

Participants/materials, setting, methods: Two reviewers independently selected studies and extracted data. We conducted pairwise meta-analyses according to participants' phenotypes as reported by the trialists, including: good prognosis, previous implantation failure, autoimmunity, high inflammation, thin endometrium, and low ovarian reserve. Using the Cochrane-RoB-1 tool, we restricted our primary analyses to RCTs at low risk of selection and other biases. We presented effect estimates as risk ratio (RR) with 95% confidence interval (CI) and considered I^2>50% as representing substantial heterogeneity.

Main results and the role of chance: Our searches identified 14,893 records. We included a total of 84 studies in the qualitative synthesis, of which 43 contributed to the meta-analyses, evaluating in total 7,301 participants and 12 treatment comparisons. The primary analysis showed that aspirin resulted in little to no difference in LBR (RR 0.97, 95% CI 0.94-1.23; n = 688 women; moderate-certainty evidence) and MR (RR 1.20, 95% CI 0.73-1.99; n = 988 women; low-certainty evidence) compared with placebo. The data did not identify a difference in LBR/OPR in women using subcutaneous heparin (RR 1.30, 95% CI 0.80-2.12; n = 150 women; very low-certainty evidence) or IVIG (RR 1.28, 95% CI 0.32-5.16; n = 51 women; very low-certainty evidence) versus placebo. For the remaining interventions, the primary analysis was uncertain of an effect or insufficient for quantitative synthesis. However, the sensitivity analysis including all studies, irrespective of risk of bias, showed that corticosteroids may improve LBR in women with thyroid autoimmunity (RR 2.33, 95% CI 1.04-5.25; n = 60 women; very low-certainty evidence); intrauterine G-CSF may improve LBR in women with a thin endometrium (RR 2.57, 95% CI 1.24-5.29; n = 304 women; very low-certainty evidence); and intrauterine PBMCs may improve LBR in women with previous implantation failure (RR 2.03, 95% CI 1.33-3.10; n = 312 women; very low-certainty evidence).

Limitations, reasons for caution: For most of the interventions under study, the evidence was of low or very-low certainty, owing to limitations in study design, imprecision, indirectness and inconsistency. Further, very few studies reported on adverse events, highlighting a worrying lack of data on the safety of immunological therapies for women undergoing assisted conception.

Wider implications of the findings: A scarcity of well-designed RCTs limits the evidence on immunomodulators in women undergoing IVF. However,

we identified positive signals for some therapies in specific conditions (e.g. corticosteroids for thyroid autoimmunity, intrauterine G-CSF for thin endometrium, and PBMCs in women with previous implantation failure). This merits further investigation in high-quality trials.

Trial registration number: PROSPERO registration number CRD42021294031

Abstract citation ID: dead093.847
P-504 Pregnancy outcome of intravenous immunoglobulin in women with recurrent pregnancy loss

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Study question: Does intravenous immunoglobulin(IVIG) increase live births in recurrent miscarriage(RM) women with cellular immune abnormality such as high NK cell level and cytotoxicity and TNF-α/IL-10 ratio?

Summary answer: IVIG treatment increased the live birth rate of RM women with abnormal cellular immunity.

What is known already: Various causes of RM have been identified but even with a detailed evaluation, almost half of the cases have unidentified etiologies. Immune imbalance is one of the proposed potential etiologies of these idiopathic RM. To regulate abnormal cellular immunity, treatment such as IVIG is proposed to improve pregnancy outcomes. However, the use of IVIG in RM is still controversial.

In addition, one of the widely used indicators of immune imbalance in pregnancy is the NK cell level, but there is a disagreement in setting the cut-off value. The percentage varies from 12 to 18%.

Study design, size, duration: RM was defined as women with two or more spontaneous abortions and in total, 987 RM women visited Department of Obstetrics and Gynaecology, Konyang University Hospital(KYUH) from January 2007 to December 2020. Only those with a full evaluation and known treatment outcome were included. A total 204 idiopathic RM and 209 RM women with known aetiology were enrolled.

Participants/materials, setting, methods: We investigated the pregnancy outcome by sorting the patients into seven subgroups depending on abnormal cellular immunity including NK cell level, NK cell cytotoxicity and TNF-α/IL-10 ratio. Then, to verify the cut-off value(16.1%) of NK cell level which we set in our previous study, patients were classified into three groups according to their NK cell level: 1) <12%(low), 2) 12-16%(moderate), 3) >16%(high).

Main results and the role of chance: Among all RM women with at least one abnormal cellular immunity were treated with IVIG and the overall live birth rate (LBR) was 80.2%. The group which did not have IVIG treatment showed an overall LBR of 78.0%. Within the 7 groups with abnormal cellular immunity, the group with both high NK cell toxicity and TNF-α/IL-10 ratio showed the highest LBR, 100% of LBR and the group with both high NK cell level and TNF-α/IL-10 ratio showed the lowest treatment outcome, 71.4%. To verify the adequacy of our cut-off value, after excluding those with high NK cell cytotoxicity and TNF-α/IL-10 ratio, 152 patients were classified into low, moderate, and high level of NK cells. LBR were 81.0%, 72.5%, and 76.9%.

Limitations, reasons for caution: The study was designed retrospectively, resulting in numerous follow-up losses. It is a single-centered study that may not be enough to generalize the diagnosis method and treatments from these findings. Moreover, IVIG is an expensive drug that may lead to certain patient preferences.

Wider implications of the findings: The study may provide evidence in selecting RM patients with immune abnormalities to be treated with IVIG and raise their pregnancy outcomes. We can also apply these results to the reproductive failure group. A detailed evaluation and an evidence-based treatment are important.

Trial registration number: not applicable

Abstract citation ID: dead093.848
P-505 Role of intramuscular immunoglobulin in improving the live birth rate in ANA positive women with recurrent pregnancy loss

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Study question: In ANA positive women with Recurrent Pregnancy Loss, could addition of intramuscular Immunoglobulin along with appropriate adjuvant therapy improve the live birth rate ?

Summary answer: In ANA positive RPL patients, addition of Intramuscular immunoglobulin along with glucocorticoids and low dose aspirin improves the live birth rate

What is known already: RPL is a frustrating condition which affects 1-3% of women trying to conceive. Presence of ANA is associated with autoimmunity, which affects trophoblast development and vascular remodeling process thereby resulting in pregnancy loss. Latest ESHRE guidelines also suggest ANA testing for explanatory purpose. Aspirin has anti-platelet and anti-inflammatory effects, and glucocorticoids exhibit a beneficial effect in autoimmune diseases. Therefore, they are considered conventional adjuvant therapies in ANA-positive women with RPL. Addition of Immunoglobulin for RPL patients benefits by the down-regulation of primary antibody production, the modulation of complement activation and Suppresses Nk cell cytotoxicity

Study design, size, duration: 72 ANA positive patients with Unexplained RPL were enrolled in the study. Anatomical, metabolic ,genetic and other immunological causes for RPL were excluded. Out of 72 patients 36 patients were allotted to the treatment group, who received intramuscular immunoglobulins along with Low dose aspirin and glucocorticoids, remaining 36 patients were allotted to the control group, who received only Aspirin and glucocorticoids. Duration of study extended from jan2021 till Dec 2022

Participants/materials, setting, methods: 72 patients with unexplained RPL underwent ANA screening using Indirect Immunofluorescence technique in the pre conception period. Patients with a titre ≥1:80 were considered to be significant and enrolled in the study. Patients were allotted to the Treatment and Control group based on personal choice of the patient after informed consent. Live birth rate was the primary outcome analyzed.

Main results and the role of chance: There were no significant differences in socio-demographic characteristics between group. Patient characteristics – Age, BMI, Ovarian reserve, sperm factor and number of previous Pregnancy losses were matched.Treatment group who received Intramuscular immunoglobulin once in 3 weeks right from the time of positive pregnancy test till 28 weeks along with low dose aspirin and glucocorticoids had a live birth rate of (23/36) 63.8 % whereas Control group who received only Low dose Aspirin (75 mg) and Glucocorticoids had a live birth rate of 41.6 % (15/36). Other obstetric outcomes which were analysed were Pre Eclampsia and GDM which were comparable between both the groups.Preterm labour was marginally higher in the control group.

Limitations, reasons for caution: Because of limited sample size results need to be interpreted with caution. Screening for ANA antibodies in patients were done only preconceptionally, and we were not aware about their ANA titres in pregnancy. This study involves only patients who conceived with IVF treatment and doesn't include spontaneous natural conception

Wider implications of the findings: ANA screening in RPL cases is suggested to prognosticate the outcome. Addition of Immunoglobulins significantly increases the live birth rate in these cases. Role of Immunoglobulin in patients with altered cellular immunity has to be further studied which is likely to benefit other cases of unexplained RPL.

Trial registration number: not applicable

Abstract citation ID: dead093.849

P-506 Administration of low molecular weight heparin alleviate immune physiology in recurrent implantation failure modulating CCL2-CCR2 axis during frozen embryo transfer: effort from clinic to bench

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Study question: Does low molecular-weight heparin (LMWH) administration modulate CCL2-CCR2 axis in recurrent implantation failure (RIF) patients undergoing frozen embryo transfer (FET) to improve pregnancy salvage?

Summary answer: LMWH treatment attests beneficial immunological tolerance in vivo and in vitro through modulation of CCL2-CCR2 axis suggesting possible restoration of weak pro-inflammatory response in RIF.

What is known already: Immune imbalance in proportion of activated macrophages (M1) and alternatively activated macrophages (M2) impede endometrial receptivity and contribute to RIF. CCL2 (M2-asscociated) - CCR2 (t-helper-2 associated) axis orders macrophage polarization by influencing expression of functionally relevant and polarization associated genes and down-modulate pro-inflammatory cytokine production. Immunological effects of LMWH regulate pregnancy-promoting processes at foetal-maternal interface to provide an appropriate implantation process; outcomes being controversial. We aim to investigate the potential immunoregulatory properties of LMWH in patients with RIF undergoing FET, with particular regard to effects on macrophages and T-helper (Th) cells; two key players in promoting immune tolerance during pregnancy.

Study design, size, duration: This prospective cohort study constituted 121 women with RIF (≥3 failed embryo transfer) undergoing FET at Institute of Reproductive Medicine between January to October 2022. An incremental dose (4-12mg/day) of estradiol with (Group A; n = 62) or without (Group B; n = 59) LMWH (40 mg/day) from d2 to d12 with sequential ultrasound following intra-muscular progesterone (100mg/day) for 4 days was administered in all participants. Blood samples were collected to isolate plasma and peripheral-blood-mononuclear-cells (PBMCs).

Participants/materials, setting, methods: Monocytes were purified from PBMCs and cultured with GM-CSF or M-CSF to analyze factor/s which control CCL2-CCR2 expression along macrophage polarization by flow cytometry. Influence of CCL2 on GM-CSF-cultured macrophages (GM-MØ) and M-CSF-cultured macrophages (M-MØ) specific gene markers was analysed using quantitative RT-PCR (qRT-PCR). To assess immunological effects of LMWH in-vivo, Th-specific cytokine profile/s and respective chemokines were measured from plasma by Luminex xMAP technology. Statistical significance was set at $p < 0.05$.

Main results and the role of chance: The clinical pregnancy rate was higher in group A. (A vs B: 37.09% vs. 27.11%, p = 0.24). LMWH exposure significantly decreased the secretion of CCL2 ($p < 0.01$) and CCL22 ($p < 0.001$) in GM-CSF-cultured macrophages, while it increased CCL2, CCL20 production ($p < 0.001$) in both GM-MØ and M-MØ. However, expression of CCL7 was significantly lower ($p < 0.01$) in M-MØ and GM-MØ generated from CD14+ CD16+ monocytes and CD14++ CD16- monocytes respectively under LMWH exposure. qPCR analysis revealed a significant increase ($p < 0.01$) in the expression of various GM-MØ–specific genes including EGLN3, and SERPINE1 in macrophage supernatant post LMWH treatment. Expression of various M-MØ-specific marker/s such as IGF1, FOLR2, and HTR2B, is significantly up-regulated ($p < 0.01$) in Group B. Group A, although documented a decreased concentration/s (pg/ml) of Th17-type cytokine/s (IL-6 (1.88 ± 0.65 vs. 0.91 ± 0.43; $p < 0.04$ and IL-23 (17.94 ± 9.76 vs. 7.78 ± 3.43; $p < 0.01$)), at end of treatment, concentration of TGF-β was significantly higher (3909.05 ± 1248.35 vs. 2469.83 ± 1058.71; $p < 0.01$) which cue to probable maintenance in balance of inflammatory response at the time of implantation. No differences were observed in Th1-type cytokine level/s (IFN-γ and TNF-α). CXCL10, CXCL11, CXCL1, CXCL8, CCL17, CCL22 do not differ in vivo.

Limitations, reasons for caution: Beneficial effects of LMWH administration with a good sample size are clearly needed to confirm this hypothesis; hence result/s should be dealt with caution. Further to this, inclusion of other arm/s (corticosteroids, intralipid and/or intravenous immunoglobulin) will make the study much robust and conclusive.

Wider implications of the findings: The identification of atypical Th1-associated pro-inflammatory effects of LMWH drives to evaluate modulation of CCL2 expression to limit or potentiate macrophage activation in RIF; especially where anticoagulant effect is not the primary goal. Further, modification of cytokine expression/s might potentiate novel strategy derived from deregulated macrophage polarization.

Trial registration number: Not Applicable

Abstract citation ID: dead093.850

P-507 Oxidative/Nitrative Stress Biomarkers Increased the Risk of Recurrent Pregnancy Loss

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Study question: Whether oxidative/nitrative stress biomarkers increased the risk of recurrent pregnancy loss (RPL)?

Summary answer: Patients with higher oxidative/nitrative stress biomarkers of 8-NO_2Gua and HNE-MA had an increased risk of recurrent pregnancy loss compared with controls.

What is known already: In reproductive medicine, recurrent pregnancy loss (RPL) is a major problem including the most unknown and lack of evidence-based diagnostics and therapies. Certain oxidative stress markers play an important role in the progress of some diseases. In one study, subsequent increases in 8-OHdG levels in the RPL group indicated extensive DNA damage and the lipid peroxidation products dramatically increased before an abortion occurred. Large unknowns of other indicators of oxidative stress, such as markers of lipid peroxidation (8-isoPF 2α, HNE-MA, and MDA), and DNA damage (8-NO2Gua), are studied for their impacts on RPL.

Study design, size, duration: We used an established case-control study, the Taiwan Recurrent Pregnancy Loss and Environmental Study (TRIPLES), which included 514 reproductive age (20-50) women (including 397 cases and 117 controls) from obstetric clinics at National Cheng Kung University Hospital in southern Taiwan from 2013 to 2022.

Participants/materials, setting, methods: The physicians diagnosed two or more consecutive miscarriages before 20 weeks of pregnancy as RPL cases. They excluded uterine anomalies, maternal diseases, chromosomal abnormalities, polycystic ovarian syndrome (PCOS), premature ovarian failure, and endometriosis. As a control group, we included married women, who delivered at least one child without artificial reproduction, underwent laparotomy for other clinical reasons, and did not suffer from the above-mentioned gynecological diseases.

Main results and the role of chance: Recurrent pregnancy loss (RPL) groups were older (mean=35.13) and had more time to prepare for pregnancy (mean=10.66 months). Their marital status mainly was married, their household income exceeded around 33,000 USD, and they consumed coffee habits. The median oxidative/nitrative stress biomarkers of 8-NO2Gua and HNE-MA in the case group were significantly higher than those in the control group (6.15 and 30.12 vs. 3.77 and 21.54 ug/L, $p<0.001$), indicating that the oxidative stress biomarkers of 8-NO2Gua and HNE-MA could be an essential effect factor in RPL. By categorizing the data by tertile of oxidative/nitrative stress biomarkers, we found that the RPL risk was 3.19 times higher in the third tertile of log 8-NO2Gua than in the first tertile (OR: 3.19, 95% CI: 1.66–6.10) and that the RPL risk in log HNE-MA was higher in the third tertile (OR: 2.05, 95% CI: 1.12–3.74). After adjustment for age, time to pregnancy (months), and coffee drinking habits, the RPL risk in the third tertile of log 8-NO2Gua was 2.01 times significantly higher than that in the first tertile (AOR: 2.01, 95% CI: 1.00–4.04).

Limitations, reasons for caution: Limitation of a case-control study. The oxidative stress biomarkers 8-NO2Gua and HNE-MA were significantly higher in the RPL groups, however, the causes of oxidative stress (like multiple environmental exposures, etc.) remain more studies to elucidate.

Wider implications of the findings: We provided evidence for oxidative/nitrative stress biomarkers in RPL patients, which implies the reduction of oxidative/nitrative stress may improve the progress of RPL. As important information for preventive medicine, more studies are needed to understand the adverse outcome pathway of oxidative/nitrative stress in RPL.

Trial registration number: MOST 109-2314-B-400 −022 -MY3 and EM-112-PP-11

Abstract citation ID: dead093.851

P-508 The adverse effect of a previous late miscarriage on the subsequent pregnancy outcomes: a retrospective cohort study across more than ten years

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Study question: To explore whether a previous late miscarriage (LM) has a prognostic impact on the subsequent pregnancy outcomes in in-vitro fertilization (IVF) women.

Summary answer: Women who had a LM for unexplained factor or cervical factor following first embryo transfer had a significantly poor pregnancy prognosis after the subsequent transfer.

What is known already: It has been reported that a history of early miscarriages is significantly associated with poor pregnancy outcomes. However, whether and how previous LM might influence future pregnancy outcomes still remain unclear.

Study design, size, duration: A retrospective cohort study was performed among 1072 infertile women who had a LM following the first embryo transfer from January 2008 to December 2020 at Center for Reproductive Medicine, Shandong University.

Participants/materials, setting, methods: 1072 infertile women who had a late miscarriage following first embryo transfer were grouped by the causes, 458 women with unexplained factor (unLM), 146 women with fetal factor (feLM), 412 women with cervical factor (ceLM), 56 women with trauma factor (trLM). Subgroup analysis and binary logistic regression were performed to evaluate the associations between late miscarriages with different causes and subsequent pregnancy outcomes.

Main results and the role of chance: Compared with general IVF population, the early miscarriage rate was significantly higher in the unLM group [8.28% vs 13.47%, adjusted OR (95%CI): 1.596(1.119-2.276), P=0.01]. Further, women with a unLM or ceLM had a dramatically elevated risk of recurrent late miscarriage [for unLM:4.24% vs 9.43%, adjusted OR (95%CI): 1.907(1.237-2.939), P=0.003; for ceLM: 4.24% vs 15.53%, adjusted OR (95%CI): 2.682(1.820-3.952), P=0.000] and a consequently reduced frequency of live birth [for unLM: 49.96% vs 43.01%, adjusted OR (95%CI): 0.746(0.612-0.908), P=0.004; for ceLM: 49.96% vs 38.59%, adjusted OR (95%CI): 0.611(0.494-0.756), P=0.000].

Limitations, reasons for caution: This study was limited by the retrospective design from a single center and the sample size in feLM and trLM group is small. Further studies are needed to verify our results and ascertain the mechanisms underlying the reported associations.

Wider implications of the findings: Only one previous late miscarriage resulted from unexplained factor or cervical factor was significantly associated with a higher risk of miscarriage and a lower live birth rate after subsequent embryo transfer. Women with only one late miscarriage should be informed the great risk and given intense surveillance during subsequent pregnancy.

Trial registration number: not applicable

Abstract citation ID: dead093.852

P-509 Genomic characterization of couples and their embryos/fetuses with idiopathic recurrent pregnancy loss

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Study question: To what extent do genomic variants contribute to idiopathic recurrent pregnancy loss (RPL)?

Summary answer: Pathogenic uniparental disomy, copy number variations, and single nucleotide variants were identified in 18 of 83 couples and their embryos/fetuses with idiopathic RPL.

What is known already: RPL is defined as the loss of two or more pregnancies before 24 weeks gestation. The etiologies of RPL include antiphospholipid antibody syndrome, and anatomic, endocrinological and chromosomal abnormalities. About 50% of RPL is unexplained and is termed as idiopathic RPL. Chromosomal aberrations and genetic variants have been identified to be associated with idiopathic RPL, demonstrating that genomic factors underlie idiopathic RPL.

Study design, size, duration: We applied genome sequencing to study genomic variations that causing idiopathic RPL. Couples and their embryos/fetuses were collected and sequenced. Genomic variations from women and men experiencing RPL and their embryos/fetuses were analyzed to fully characterize the genomic contribution to idiopathic RPL.

Participants/materials, setting, methods: We recruited 83 Chinese couples with idiopathic RPL and collected 249 samples from their embryos/fetuses and peripheral blood. Genomic DNA of those samples were sequenced, and pathogenic chromosomal abnormalities and genetic variants were identified.

Main results and the role of chance: Generally, pathogenic variants were identified in RPL females, males and embryos/fetuses. One uniparental disomy, 9 copy number variations, and 1 homozygous and 1 *de novo* single nucleotide variants (SNVs) were identified pathogenic in 12 RPL embryos/fetuses. Five and three pathogenic SNVs were identified in 4 RPL females and 2 RPL males respectively. Many SNVs affect the genes involved in sexual development. In total, 18 of 83 (21.69%) couples with idiopathic RPL could be explained by genomic variation, providing information for clinical treatments.

Limitations, reasons for caution: The sample size is relatively small, and they are from one medical center.

Wider implications of the findings: Genomic variations in females, males and their embryos/fetuses can cause RPL, and identification of genetic causes will facilitate further precision intervention.

Trial registration number: not applicable

Abstract citation ID: dead093.853

P-510 Low neighbourhood socioeconomic status is associated with lower cumulative ongoing pregnancy rate after in vitro fertilization treatment

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Study question: Is there an association between neighbourhood socioeconomic status (SES) and cumulative ongoing pregnancy after 2.5 years of in vitro fertilization (IVF) treatment?

Summary answer: Low and middle neighbourhood SES is associated with lower odds of an ongoing pregnancy within 2.5 years of IVF treatment than high neighbourhood SES.

What is known already: Low SES is known to have a negative impact on general health and a variety of medical conditions, including perinatal health. However, not much data is available on the impact of SES on IVF treatment outcome.

Study design, size, duration: This is a retrospective observational study of 3720 couples undergoing IVF or IVF-ICSI treatment between 2006 and 2020.

Participants/materials, setting, methods: Neighbourhood SES was assigned to each couple based on the postal code of residence. Subsequently, SES was categorized into low (<p20), medium (p20-p80), and high (>p80). Multivariable logistic regression analyses were performed with cumulative ongoing pregnancy within 2.5 years as outcome variable, SES category, female age, BMI, smoking status (yes/no), and interaction terms for age*SES and BMI*SES were used as covariates.

Main results and the role of chance: There was no difference in ongoing pregnancy rates between SES groups after the first fresh embryo transfer or in the average number of IVF treatment cycles performed. However, the cumulative ongoing pregnancy rates differ significantly between SES groups (Low; 43.6%, medium; 50.9%, high; 54.1%). Low SES had significantly lower odds for achieving an ongoing pregnancy within 2.5 years (OR = 0.06 (95%CI 0.02-0.22)). The interaction terms age*SES and BMI*SES showed attenuation of this association with increasing age and BMI (OR = 1.07 (95%CI 1.022 – 1.12) and OR = 1.61 (95%CI 1.25 – 2.09), respectively). The associations with medium SES were similar, but less pronounced (OR = 0.16 (95%CI 0.05 – 0.50) with OR = 1.04 (95%CI 1.00 – 1.09) and OR = 1.41 (1.12 – 1.77) for the interaction terms with female age and BMI respectively.

Limitations, reasons for caution: We were not able to perform additional analysis on individual characteristics like educational level, ethnicity or language proficiency due to lack of data.

Wider implications of the findings: In the Netherlands, health insurance is mandatory. Our study showed that even with equal access to fertility care, patients living in a low SES neighbourhood are disadvantaged. This underlines the importance of taking the whole wellbeing of the patient into account, before starting an IVF treatment.

Trial registration number: not applicable

Abstract citation ID: dead093.854
P-511 Prolonging the time of vaginal progesterone supplementation does not improve pregnancy outcomes after day 6 blastocyst transfer

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Study question: Does the administration of vaginal progesterone for 6 days improve pregnancy outcomes when transferring day 6 blastocysts, as opposed to 5 days of progesterone treatment?

Summary answer: We observed similar pregnancy outcomes after 5 and 6 days of vaginal progesterone supplementation following day 6 blastocyst transfer in oocyte recipient cycles.

What is known already: Progesterone administration promotes the final phase of endometrial preparation for embryo transfer. However, the optimal duration of progesterone transformation still remains unknown. The implantation potential of blastocysts formed at different timepoints is also contentious, as blastocysts on day 6 have been linked to poorer clinical outcomes compared to their day 5 counterparts. It is unclear whether this effect stems from an intrinsic impaired implantation capacity of slow-developing blastocysts, or a displaced window of implantation (WOI). It has been hypothesized that 6 days of progesterone administration may improve clinical outcomes after frozen day 6 blastocyst transfer.

Study design, size, duration: Retrospective cohort study of oocyte recipients undergoing single day 6 blastocyst transfer, between January 2020 and December 2022. This study compared two groups: day 6 blastocysts transferred after 5 days of progesterone administration (n = 284) and day 6 embryo transfers performed after 6 days of progesterone administration (n = 69).

Participants/materials, setting, methods: Our study included oocyte recipients who underwent a single embryo transfer of a day 6 blastocyst and hormonal replacement treatment for endometrial preparation with vaginal external progesterone administration (800mg/24h) for either 5 or 6 days prior to embryo transfer. Outcomes were analyzed using unpaired t-test or Fisher's test and logistic regression. Multivariate models were adjusted for oral or transdermal estrogen administration and endometrial thickness on the day of embryo transfer. P-values <0.05 were considered significant.

Main results and the role of chance: Mean maternal age was 43.5 years, while patients had a mean BMI of 25.1. Mean days of estrogen therapy prior to embryo transfer were 22.4, with 75.3% of patients using transdermal estrogen administration, and achieving a mean endometrial thickness of 9.6 mm on the day of transfer. Mean progesterone levels on the day of transfer were 11.9 ng/ml. Thirty-seven percent of the day 6 blastocysts were of high quality. None of the demographic or cycle characteristics were significantly different between the study groups. When considering outcomes of day 6 blastocyst transfers, we found no significant differences in biochemical (26.33% vs 20.13%, p = 0.343), clinical (22.06% vs 17.46%, p = 0.49) and ongoing (14.64% vs 9.52%, p = 0.41) pregnancy rates between 5 and 6 days of progesterone administration. Our adjusted analysis further corroborated these results.

Limitations, reasons for caution: The retrospective nature of the study and low number of transfers performed after 6 days of progesterone treatment warrant careful interpretation. This study only evaluated endometrial preparation by vaginal progesterone administration in oocyte recipients and cannot be extrapolated to other routes of progesterone administration or other patient populations.

Wider implications of the findings: Poorer reproductive outcomes associated with the transfer of day 6 blastocysts appear to be related to a reduced implantation capacity of slow-developing embryos rather than a displaced WOI due to rising progesterone. WOI appears wider than assumed, as prolonged progesterone transformation times had no effect on pregnancy outcomes.

Trial registration number: Not applicable

Abstract citation ID: dead093.855
P-512 Cell-mediated immunity as a biomarker of predisposition in recurrent miscarriage.

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Study question: Are alterations in the peripheral cellular immune system a potential risk factor for recurrent miscarriage?

Summary answer: Percentage alteration of some circulating immune cell subpopulations could be used as biomarkers or potential therapeutic targets in recurrent miscarriage.

What is known already: About 50% of patients with recurrent miscarriage (RM) have no known cause of the disease. The maternal immune system plays an important role in maternal-fetal tolerance, so an imbalance in the maternal immune system could trigger reproductive failure in women. Antiphospholipid syndrome is the only immunological alteration with sufficient evidence of association with RM, however, there are many other immunological parameters under investigation that appear to mediate the maternal-fetal relationship such as KIR receptors and HLA-C. Other authors are focusing their studies on reproductive immunology at the cellular level, looking at the role of different lymphocyte subpopulations in RM.

Study design, size, duration: Prospective observational analytical study (cases and controls) conducted in the Immunology Department of the Hospital General Universitario Gregorio Marañón (Madrid, Spain) from 2019 to 2022. It included a group of 49 non-pregnant women with a clinical history of recurrent miscarriages of unknown cause, and two control groups, one with 40 women of childbearing age who had at least one live newborn and another with 60 women of childbearing age who had no history of pregnancy.

Participants/materials, setting, methods: The inclusion criteria for the study were: two or more gestational losses (< 22 weeks of pregnancy). Patients with severe uterine pathologies, genetic disorders, thrombophilias, obesity, infections, coeliac disease without gluten restriction, uncontrolled endocrine disorders, immunomodulatory treatment, autoimmune disorders and documented RM were excluded from the study.

An immunophenotypic study of T, B, NK and NKT lymphocyte subpopulations and monocytic series was performed. Cell characterization was carried out by 6-colour flow cytometry in peripheral blood.

Main results and the role of chance: When comparing the cellular profile of RM patients with the profile of both control groups, we observed alterations in the percentage levels of CD3+ T cells, CD8+ T cells, NKT cells and non-switch and class switch memory B cells in the patient group, suggesting the presence of a predominantly pro-inflammatory peripheral cellular immune profile in RM patients. In contrast to previous studies by our research group, we did not observe significant differences between the percentage levels of NK cells and activated CD8+ T cells (HLA DR+) in the patient and control groups, which may be explained by the high and exhaustive patient selection in this study.

After association study of these alterations with RM, we identified levels of NKT cells > 5%, CD8+ T cells > 26.50%, non-switch memory B cells > 22% and class switch memory B cells > 32% as new biomarkers of RM risk. In addition, the combination of at least two of these new biomarkers was found to significantly increase the likelihood of developing the disease, suggesting that RM is a complex pathology in which multiple factors may influence the pathogenesis of the disease.

Limitations, reasons for caution: Among the limitations of our study, we can highlight the collection of control group variables from databases developed in previous studies, in addition to a small sample size.

Wider implications of the findings: The determination of these immunological biomarkers in RM allows us to evaluate the role of immunomodulatory therapies on these biomarkers in order to evaluate possible new treatments to help prevent immune-mediated reproductive failure, thus contributing to a more personalized medicine.

Trial registration number: Not applicable

Abstract citation ID: dead093.856
P-513 Effect of embryo migration after embryo transfer with fresh oocyte donation cycles on pregnancy outcomes

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Study question: Is there any effect of embryo migration on clinical pregnancy rate and live birth rate in fresh oocyte donation cycles?

Summary answer: Clinical pregnancy rate and live birth rate were not affected from embryo migration in any direction.

What is known already: Embryo transfer is the final crucial step to affect success rates and up to date there has been vast amount of studies to optimize embryo transfer for better implantation and increasing clinical pregnancy rate and live birth rate. Embryos are loaded on the catheter mostly in the order of air-embryo-air-medium. By doing so, we indirectly visualize embryo(s) via air bubbles and we rely on bubble position as a reflector of embryo position. In literature, there are no studies investigating whether there is any embryo migration after 60 minutes of embryo transfer and the effect of this migration on pregnancy outcomes.

Study design, size, duration: The study included fresh oocyte donation (OD) cycles of recipient women. In vitro fertilization (IVF) cycles, frozen-thawed embryo transfer cycles, cryopreserved-thawed OD cycles were excluded.

Participants/materials, setting, methods: The migration distance was assessed by ultrasound twice, one of them immediately after embryo transfer (ET) and the second one at 60th minutes of ET. All embryos were expulsed to 10-20 mm from the fundus. The first group consisted of patients whose embryos migrated towards fundus, second group with embryos remained between 10 and 20 mm from fundus and the third group including embryos migrated towards cervix. Three groups were compared for pregnancy outcomes.

Main results and the role of chance: A total of 611 fresh OD cycles were recruited in this study. At 60 minutes after ET; in 123 patients (20.1%) embryos were located nearer than 10 mm to the fundus (Group 1), in 476 patients (77.9%) embryos were located at same initial expulsed zone (Group 2) and in 12 patients (2%) embryos moved more away from fundal endometrium towards cervix (Group 3). In group1, there were 96 clinical pregnancies (CPR:78.0%) and 78 live births (LBR: 63.4%). In group 2, CPR and LBR were 72.1% (n = 343) and 66% (n = 314), respectively. In group 3, there were 8 clinical pregnancies (CPR:66.7%) and all had live birth (LBR:66.7%). There was no significant difference in terms of CPR and LBR between the groups.

Limitations, reasons for caution: Our study is the first to search the impact of embryo migration on live birth rate in fresh OD cycles with high number of patients. Although our population had sufficient number of participants with analyzing LBR; we believe prospective designed studies are required.

Wider implications of the findings: Our study reveals that the concept of embryo migration is a fact and almost 20% of embryos migrate, whether towards fundus or cervix. On the other hand, whether embryo stayed static or migrated, CPR and LBRs are high in freshOD cycles, independent from any possible migration.

Trial registration number: NCT044855669

Abstract citation ID: dead093.857
P-514 Uterine Contractility during Follicle Aspiration and Embryo Transfer and IVF/ICSI Outcomes: the WAVES study

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Study question: What is the influence of uterine contractility of in-vitro fertilisation (IVF) patients at follicle aspiration (FA) and embryo transfer (ET) on clinical pregnancy outcomes?

Summary answer: At time of embryo transfer, a lower contraction frequency, and increased contraction coordination is associated with more favourable chances of ongoing pregnancy after IVF/ICSI treatment.

What is known already: Uterine peristalsis is the rhythmic, wave-like motion of the subendometrial layer of the uterus. Various subjective methods using visual interpretation suggest that uterine peristalsis features are different in the various stages of the menstrual cycle, and they are thought to be important for fertility and early embryo implantation. Recently, a new automated quantitative method to measure uterine contractility was validated in a small number of IVF patients to analyse uterine peristalsis on transvaginal ultrasound (TVUS) recordings with speckle-tracking. With this method a new contraction feature – coordination – can be assessed alongside frequency, direction, amplitude.

Study design, size, duration: This study is part of an ongoing multi-centre prospective observational cohort study investigating uterine contractility on

TVUS. Our study included patients undergoing IVF/ICSI treatment with good quality TVUS recordings from 2017 to 2023. Patients received fresh ET on Day 3 or Day 5.

Participants/materials, setting, methods: 128 IVF/ICSI patients undergoing fresh ET were included from participating centres. Patients underwent TVUS within 1 hour prior to FA(n = 61/128), and/or within one hour before ET (n = 67/128). Uterine contraction frequency (CF), amplitude, velocity and coordination were measured by applying dedicated speckle tracking and strain analysis. The primary outcome was ongoing pregnancy (OP, viable pregnancy >10 weeks gestational age). The independent T-test and Mann-Whitney U test were applied to compare features between groups.

Main results and the role of chance: 39.1% of the IVF/ICSI patients (50/128) achieved ongoing pregnancy. Most patients underwent IVF/ICSI treatment due to a male factor (29.0%) or idiopathic subfertility (31.9%). Age, BMI and embryo quality were comparable for the pregnant vs. non-pregnant groups ($p > 0.05$ for all). CF was significantly higher during FA vs. ET (1.70 ± 0.26 vs. 1.54 ± 0.23,[RC(1] $p < 0.001$), as well as contraction velocity (0.79 ± 0.22 vs 0.63 ± 0.16, $p < 0.001$). Contraction amplitude was also lower during ET vs. FA (0.06 IQR 0.03 vs. 0.08 IQR 0.04, $p < 0.001$[RC(2]). During FA, no significant differences were seen in contraction features for pregnant vs. non-pregnant groups (all $p > 0.05$). At ET, a lower mean CF was significantly associated with OP(1.46 ± 0.18 vs. 1.57 ± 0.19 [RC(3] contraction/min, $p = 0.016$), as well as presence of more coordinated uterine contractions (0.22 ± 0.09 vs. 0.33 ± 0.14, $p < 0.001$[RC(4]). No significant differences were found for the features of amplitude and velocity.

Limitations, reasons for caution: Further validation of these results is ongoing, including expansion of the sample size. No sub-analysis has yet been done to assess the effect of additional IVF/ICSI treatment characteristics (i.e. stimulation protocol, type of subfertility) on uterine contractility and chance of pregnancy.

Wider implications of the findings: Uterine contractility changes its character depending on the timing of IVF treatment, with different characteristics seen at FA vs. ET. The most favourable contraction profile for ongoing pregnancy after ET seems to be uterine contractions with relatively low frequency and good coordination. These findings may support decision-making at ET.

Trial registration number: not applicable

Abstract citation ID: dead093.858
P-515 EHD1 impaired endometrial decidualization linked to recurrent implantation failure through SENP1-mediated progesterone resistance

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Study question: Recurrent implantation failure (RIF) patients exhibit poor endometrial receptivity and abnormal decidualization with reduced effectiveness to progesterone, an intractable clinical problem with elusive mechanisms.

Summary answer: EHD1 overexpression promoted the SUMOylation and ubiquitinated degradation of the PRB protein leading to decreased transcriptional activity and responsiveness of endometrial stromal cells to progesterone.

What is known already: Abnormal decidualization is a significant cause of infertility in RIF patients. A previous study has demonstrated that EH homeodomain 1 (EHD1) is significantly elevated in the endometrium of RIF patients and plays an essential role in the decidualization of human endometrial stromal cells (HESCs). However, far less is known about the function of EHD1 in the endometrium during embryo implantation.

Study design, size, duration: After approval from the Ethics Committee of Nanjing Drum Tower Hospital (2013-081-01), endometrial specimens were collected from women who received treatment at the Reproductive Center of Nanjing Drum Tower Hospital from January 2020 to December 2021. Twelve RIF women and Twelve fertile normal control women were enrolled in the present study. Endometrium in the middle and late stages of secretion was obtained by aspiration and curettage 5-7 days after ovulation.

Participants/materials, setting, methods: The expression and location of EH homeodomain 1 (EHD1) in endometrium were evaluated by IHC, qRT-PCR and Western blotting. Transcriptomic analysis was performed to predict the mechanism by which EHD1 is mediated in human endometrial stromal cells (HESCs) decidualization. Luciferase reporter assays and Western blotting were used to detect the protein activity and stability of progesterone receptor B (PRB) regulated by EHD1. The potential mechanisms were further confirmed through immunoprecipitation, nucleocytoplasmic separation and immunofluorescence experiments.

Main results and the role of chance: EHD1 was abnormally elevated in RIF patients and linked to aberrant endometrial decidualization. EHD1 overexpression inhibited progesterone receptor (PGR) transcriptional activity and the responsiveness of HESCs to progesterone. Indeed, EHD1 overexpression promoted the SUMOylation of PRB, leading to ubiquitinated degradation of the PRB protein. Supplementation with the de-SUMOylated protease SENP1 ameliorated EHD1-repressed PRB transcriptional activity. In addition, the expression of PRL and IGFBP1 was rescued by interfering with the expression of EHD1 in HESCs from RIF patients.

Limitations, reasons for caution: In our study, we provided novel insight into the molecular scaffold of EHD1 to mediate the ubiquitination of PRB for degradation. However, our data do not account for the specific mechanisms by which EHD1 promotes the ubiquitination of PRB, and The specific enzymes involved remain to be elucidated.

Wider implications of the findings: We demonstrated that abnormally high expression of EHD1 in endometrial stromal cells attenuated the activity of PRB associated with progesterone resistance in a subset of women with RIF.

Trial registration number: not applicable

Abstract citation ID: dead093.859
P-516 Defective decidualization secretome in severe preeclampsia

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Study question: How defective decidualization (DD) induces a differential secretome compromising embryo invasion in severe preeclampsia (sPE).

Summary answer: DD endometrial stromal cells isolated from sPE patients secrete a differential secretome in response to hormonal stimuli.

What is known already: Endometrial decidualization is a primary driver of embryo invasion and placentation. This process is highly coordinated by estradiol and progesterone, which transform the endometrial stromal cells (ESCs) into decidualized cells specialized in the secretion of specific molecules. The secretome of ESCs regulates the endometrial differentiation into the decidua, including immune system and endothelial function regulation. Previously, we have demonstrated the existence of *in vitro* and *in vivo* defective decidualization (DD) in women who suffered sPE due to an impaired response to hormonal stimuli. However, the molecular mechanism that connects DD with a hostile maternal environment for embryo invasion remains unexplored.

Study design, size, duration: Human ESCs from women who suffered sPE (n = 3) and women with normal pregnancies as controls (n = 3) were isolated from endometrial biopsies collected at the time of late-secretory phase. ESCs were cultured using an established *in vitro* decidualization model, including three technical replicates per sample and condition. Culture media (n = 36) were analysed by mass spectrometry to characterize their global secretome, unveiling the differential secreted factors in DD underlaying sPE. The study duration was 12 months.

Participants/materials, setting, methods: For *in vitro* decidualization, isolated human ESCs were cultured for three days in the absence or presence of hormonal stimuli (0.5mM cAMP + 1μM MPA) in serum-free media. Culture

media from decidualized and non-decidualized conditions were collected to analyse the secreted proteins by high-resolution mass spectrometry-based proteomics. Protein quantification was performed in the MaxQuant and Perseus software. This approach enables the characterization of the global secretory program as well as the discovery of proteins.

Main results and the role of chance: We identified and quantified a total of 1.117 proteins secreted by ESCs. In the transition from non-decidualized to decidualized status, the secretome of the control group showed significant differences in the abundance of 83 proteins (p-value<0.05), including two classical markers of decidualization (IGFBP1 and PRL), as well as other hormonally regulated proteins (e.g. VEGFA, WNT4 and MFAP2), supporting the validity of our experimental method. Then, we compared the secretome of decidualized ESCs from controls versus sPE patients, unveiling the altered differential secretome linked to DD. We detected 160 proteins significantly deregulated in sPE compared controls (p-value<0.05) during DD, including proteins reported as risk factors of sPE (e.g. LPA and TF), decidualization modulators (e.g. MFAP2 and TRAP1), mediators of maternal-fetal communication (NPTX1) and immune response regulators (e.g. C3, C5, C7, and SERPIN). Downregulated proteins were associated with cytoskeleton organization and small GTPase mediated signal transduction, consistent with defective decidualization. In contrast, upregulated proteins were implicated in complement activation, regulation of humoral and inflammatory response. A heatmap based on the 160 deregulated proteins successfully classified samples into sPE and controls, supporting the existence of a differential secretome in DD linked to sPE.

Limitations, reasons for caution: Sample size for *in vitro* decidualization was based on six patients, analysing two conditions and three technical replicates per patient (N total samples=36) to ensure robustness and reproducibility. Samples clustering in the heatmap support the biological variance was higher than the technical variance, supporting the effectiveness of the hallmark identified.

Wider implications of the findings: ESCs isolated from women who suffered sPE show an aberrant differential secretome linked to DD. These secreted factors into the decidual microenvironment might impact in embryo invasion, supporting the maternal contribution to sPE. Understanding the mechanisms underlying DD might provide new biomarkers for sPE risk prediction.

Trial registration number: Not applicable

Abstract citation ID: dead093.860
P-517 Downregulated INHBB in endometrial tissue of recurrent implantation failure patients impeded decidualization through the ADCY1/cAMP signalling pathway

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Study question: To identify the mechanism of Inhibin Subunit Beta B (INHBB) in the regulation of human endometrial stromal cells (HESCs) decidualization in recurrent implantation failure (RIF).

Summary answer: INHBB was decreased in endometrial stromal cells of women with RIF, which attenuated decidualization in vitro by suppressing ADCY1-induced cAMP production and cAMP-mediated signalling.

What is known already: INHBB encodes a preprotein subunit of inhibin/activin, functional cytokines belonging to the transforming growth factor-β (TGF-β) family. INHBB mRNAs were expressed in human decidualized endometrium from the first trimester of pregnancy and increased as the gestation progressed. Furthermore, INHBB expression increases in the mouse uterus in areas undergoing decidualization. These studies indicated that INHBB might play an essential role in endometrial decidualization.

Study design, size, duration: The Medical Ethics Committee of Nanjing Drum Tower Hospital approved this study (No. 2013-081-01). Written informed consent was obtained from each participant. Between January 2020 and November 2021, 28 infertile and 18 fertile control women aged 20-40 years with normal and regular menstrual cycles (25-35 days) and no history of steroid hormone medication in the last 3 months were included in the study.

Participants/materials, setting, methods: RNA-seq was conducted to identify the differentially expressed genes in the endometria from control and RIF patients. RT-qPCR, WB, and immunohistochemistry were performed to analyse the expression levels of INHBB in decidualised HESCs. RT-qPCR and immunofluorescence were used to detect changes in decidualization after knockdown INHBB. Then, RNA-seq was used to dig out the mechanism of INHBB regulating decidualisation.

Main results and the role of chance: Our results showed significantly reduced expression of INHBB in endometrial stromal cells of women with RIF. In addition, INHBB was increased in the endometrium of the secretory phase and significantly induced in in-vitro decidualization of HESCs. Notably, with RNA-seq and siRNA-mediated knockdown approaches, we demonstrated that the INHBB-ADCY1-mediated cAMP signalling pathway regulates the reduction of decidualization. We found a positive association between the expression of INHBB and ADCY1 in endometria with RIF (R2= 0.3785, P = 0.0005).

Limitations, reasons for caution: Our results indicated that INHBB regulates cAMP level by inducing the expression of ADCY1, while the regulation mechanism between INHBB and ADCY1 in endometrial decidualization requires further exploration.

Wider implications of the findings: The decline of INHBB in HESCs suppressed ADCY1-induced cAMP production and cAMP-mediated signalling, which attenuated decidualization in RIF patients, indicating that INHBB is an essential component in the decidualization process.

Trial registration number: not applicable

Abstract citation ID: dead093.861
P-518 The effect of inactivated SARS-CoV-2 vaccination on the outcome of the first frozen-thawed embryo transfer cycle

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Study question: Is there an effect of inactivated severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) vaccination on the outcome of the first frozen-thawed embryo transfer(FET) cycle.

Summary answer: Inactivated SARS-CoV-2 vaccination did not affect the outcome of the first FET cycle, including clinical pregnancy rate, early miscarriage rate and newborns' birth weight.

What is known already: SARS-CoV-2 vaccination can significantly reduce the risk of severe disease and death after SARS-CoV-2 infection. However, due to the lack of vaccine safety data, the infertile population who plan to undergo ART treatment have high concerns about the safety of vaccine vaccination. To date, there are merely few researches aimed to determine the impact of inactivated SARS-CoV-2 vaccination on reproductive and neonatal outcomes of women attempting FET.

Study design, size, duration: This was a retrospective cohort study of patients who underwent the first FET cycle in the Reproductive Center of Ningbo Women and Children Hospital from November 1st 2021 to December 19th 2022. All patients were followed up until January 19th 2023. Patients whose age was >40 years old were excluded. The patients were organized into groups based on age, anti-Müllerian hormone (AMH) levels, antral follicle count (AFC), body mass index (BMI), and vaccination or not.

Participants/materials, setting, methods: Patients fully vaccinated with two doses of Sinopharm or Sinovac inactivated vaccines(Group A, n = 452) were compared with unvaccinated patients(Group B, n = 452) who were matched by age, AMH, AFC and BMI. The baseline characteristics and clinical outcomes of each group were compared.SPSS version 26.0 (SPSS Inc., USA) was used for all data analyses.

Main results and the role of chance: There were no statistically significant differences in age, AMH, AFC, BMI, infertility duration, infertility type, infertility causes, fertilization type, ovarian stimulation protocol, number of oocytes

retrieved(9.24 vs 9.92, p = 0.102), endometrial preparation protocol, endometrial thickness(9.40 vs 9.29mm, p = 0.344), embryo stage, number of embryos for transfer(1.4 vs 1.42, p = 0.589) between two groups. No difference was found between vaccinated and unvaccinated patients in clinical pregnancy rate(55.31% vs 57.52%, p = 0.502), early miscarriage rate(10.4% vs 11.54%, p = 0.681). The newborns' birth weight were comparable between two groups(2971.44 vs 3156.83g, p = 0.66).

Limitations, reasons for caution: This is a single-center retrospective study and further accumulated data is warranted to validate the findings. Because of the short follow-up time, some patients are still pregnant, hence we await live birth outcomes.

Wider implications of the findings: These findings contribute to the evidence regarding the reproductive safety of inactivated SARS-CoV-2 vaccination for fertility-seeking women, and it can provide further theoretical reassurance for doctors and patients to vaccinate before pregnancy.

Trial registration number: not applicable

Abstract citation ID: dead093.862
P-519 Is oral dydrogesterone a good alternative to vaginal micronized progesterone for luteal phase support in women receiving oocyte donation?

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Study question: Does oral dydrogesterone (OD) in luteal phase support (LPS) provide equivalent clinical pregnancy and miscarriage rates as micronized vaginal progesterone (MVP) in oocyte donation recipients?

Summary answer: No significant difference in clinical pregnancy and miscarriage rates was observed when using OD instead of MVP for LPS in oocyte donation.

What is known already: Dydrogesterone has a higher bioavailability than MVP due to its high specificity for progesterone receptors allowing the use of lower doses limiting side effects. In addition, because of its route of administration, its tolerance is better. The use of OD for LPS in fresh IVF cycles is now well recognized. However, few data are available on its use in frozen-thawed embryo transfer especially in artificial cycles. In this situation, the lack of corpus luteum leads to a total dependence on exogenous progestogens for implantation and maintenance of the pregnancy.

Study design, size, duration: A retrospective observational study was performed from prospectively collected data in the ART Department of Lille University Hospital from July 2018 to July 2022.

Participants/materials, setting, methods: Our study analysed 372 oocyte donation cycles with embryo transfer. Recipients underwent endometrial preparation by hormone replacement treatment (artificial cycles). LPS was provided by weekly intramuscular progesterone (500 mg/2ml) and either OD 40 mg per day or MVP 800 mg per day for 12 weeks if the pregnancy test was positive. The primary endpoint was clinical pregnancy rate.

Main results and the role of chance: This study compared the outcome of 162 transfers with dydrogesterone + IM progesterone to the outcome of 210 transfers with the MVP + IM progesterone. No significant difference was found between the two groups except for the number of embryos transferred, embryo quality, donor BMI and fresh or frozen status of the embryo. After adjustment for these criteria, our two groups were comparable in clinical pregnancy rates with 36.67% in the MVP group versus 30.25% in the OD group (OR 0.868 [0.529 ; 1.423], p = 0.57), ongoing pregnancy rates (29.05% versus 25.31%, OR 0.993 [0.591; 1.669], p = 0.97), miscarriage rates (7.62% versus 4.94%, OR 0.640 [0.248; 1.650], p = 0.35) and live birth rates (26.67% versus 17.69%, OR 0.727 [0.412; 1.284], p = 0.27).

Limitations, reasons for caution: The main limitation of this study is the non-randomisation of data between the two treatment groups.

Wider implications of the findings: Our study suggests that dydrogesterone has a place in our LPS strategies in artificial cycle for frozen embryo transfers or in oocyte donation, especially as this route is often preferred by patients and very well tolerated. Further prospective cohorts or randomized trials could continue to inform clinical practice on LPS.

Trial registration number: DEC 16-25

Abstract citation ID: dead093.863
P-520 Risk factors for early pregnancy loss in women undergoing in vitro fertilization-embryo transfer

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Study question: What are the risk factors for early pregnancy loss (EPL) in infertility patients undergoing in vitro fertilization-embryo transfer (IVF-ET) cycles?

Summary answer: The risk factors for EPL were maternal age, 14-day β-human chorionic gonadotrophin(β-hcg) levels and cycle type.

What is known already: Pregnancy obtained through assisted reproductive technology still has a high risk of spontaneous early pregnancy abortion, and the cause of which is unclear. It not only affects the rate of ongoing pregnancy and live birth, but also increases the psychological and economic burden of patients. At present, there have been many studies on the risk factors of early pregnancy loss in IVF/ICSI-ET at domestic and overseas, but the results are different, which may be related to the selection of different research objects, sample size and statistical methods.

Study design, size, duration: A retrospective study of 6107 embryo transfer cycles collected from January 2018 to December 2018 was performed. The cycles were divided into the EPL group (n = 987) and the live birth group (n = 5120) according to the cycle outcomes.

Participants/materials, setting, methods: Baseline data and clinically relevant indicators were compared between the two groups, including maternal age, infertility years, infertility type, body mass index (BMI), tubal factor, ovulatory dysfunction, endometriosis, male factor, antral follicle count (AFC), single gene related genetic disease, follicle-stimulating hormone (FSH), estradiol (E2), luteinizing hormone (LH), 14-day β-hcg levels, prolactin (PRL), progesterone (P), testosterone (T), anti-Müllerian hormone (AMH), cycle type and the number of transplanted embryos.

Main results and the role of chance: The EPL rate of the infertility patients undergoing fresh/frozen-thaw embryo transfer cycle was 16.16%. Univariate analysis showed that there were statistically significant differences between the two groups in maternal age, AFC, 14-day β-hcg levels, T, AMH, infertility years, cycle type, infertility types, chromosome disease and male factors ($P < 0.05$). The results of binary logistics regression analysis showed that maternal age, 14-day β-hcg levels and cycle type were related factors to EPL ($P < 0.05$).

Limitations, reasons for caution: This study lacked some baseline data, such as adverse pregnancy history, abortion history, and previous transplant failure history, and male factors such as BMI, age, semen results, smoking history. The information about fetal chromosomes was also missing.

Wider implications of the findings: Clinical prevention and treatment of risk factors can be targeted to improve pregnancy outcomes after transplantation.

Trial registration number: none

Abstract citation ID: dead093.864

P-521 A gene expression risk signature of endometrial failure for prognosis in In Vitro Fertilization (IVF) patients

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Study question: It is possible to identify a characteristic pattern of endometrial gene expression indicative of implantation failure, which is independent of implantation window displacements?

Summary answer: An endometrial transcriptomic signature was able to identify patients with a > 3-fold increased risk of implantation/pregnancy failure with 95% accuracy.

What is known already: Implantation failure of endometrial origin is a complex and multifactorial symptom with diverse causes, diagnosed in IVF patients after repeated failures with good quality embryos. A generation of gene expression tools have assumed that Window of implantation (WOI) displacement is the principal cause of this condition, but strategies seeking to counteract this problem by adjusting the day of embryo transfer have not yielded convincing improvements in outcomes. However, it is conceivable that other forms of endometrial disruptions, relevant to embryo implantation, could exist. New endometrial diagnostic strategies are needed to understand, diagnose and potentially treat patients affected with such problems.

Study design, size, duration: A prospective multicenter study between January 2018 and December 2021 recruited 281 Caucasian IVF patients (mean age of 39.4 ± 4.8 years and BMI of 22.9 ± 3.5 kg/m^2) undergoing hormone replacement therapy and encompassing 114.5 ± 7.2 h of progesterone administration at time of endometrial biopsy. Following experimental quality controls and clinical follow up, 186 patients who had a good quality embryo transferred in the cycle after endometrial biopsy collection were included for gene discovery and prediction performance.

Participants/materials, setting, methods: The expression of 404 genes selected for their potential to distinguish endometrial timing and/or endometrial disruption was measured. Transcriptomic variation related to progression of the menstrual cycle was removed using transcriptomic endometrial dating (TED) and linear models. Study groups were established according to clinical and gene expression parameters through a semi-supervised artificial intelligence procedure. Gene signature discovery and a cross-validation processes were undertaken to define predictive expression patterns. Reproductive outcomes were compared between prognosis profiles.

Main results and the role of chance: We developed a procedure called Clinically Acute Transcriptomic Stratification (CATranS), combining clinical parameters and deep transcriptomic molecular characterisation to stratify patients according to endometrial prognosis: 'poor' (n = 137) or 'good' (n = 49). These two transcriptomic profiles were associated with differing reproductive outcomes in the single embryo transfer following biopsy: pregnancy rate (45.1% vs 79.6%, poor vs good prognosis, respectively, p = 3.8E-5); live birth rate (56.4% vs 97.5%, p = 3.0E-06); clinical miscarriage rate (27.9% vs 2.6%, p = 0.0020); biochemical pregnancy rate (20.4% vs 0%, p = 0.0023). Patients with a poor prognosis profile had 3.3-times higher relative risk of a transferred embryo failing to implant or a pregnancy not being sustained to term, compared with good prognosis patients. Initially, a reference dataset was used to build a prototype for diagnosing endometrial failure, revealing that a gene expression signature consisting of 135 genes was the most predictive. Prediction performance was estimated using a 5-fold 100-times cross-validation process, resulting in a median accuracy of 0.92, median sensitivity of 0.96, and median specificity of 0.84. From these 135 predictive genes, 122 were differentially expressed (FDR<0.05) in endometrial poor prognosis, 59 up- and 63 down-regulated, most involved in functional processes such as regulation (17%), metabolism (8.4%), immune response and inflammation (7.8%).

Limitations, reasons for caution: We describe a potential new strategy for evaluating endometrial competence. However, to confirm predictive value, validation using additional samples from patients independent of signature discovery set is required. Further research to identify potential treatments for patients classified as poor prognosis is needed, providing a tailored clinical pathway for these patients.

Wider implications of the findings: The prototype described is a novel concept, potentially leading to development of a new generation of tools for diagnosis of fertility problems related to endometrial factors. The 'poor' prognosis profile is not caused by asynchronies in menstrual cycle progression, opening the possibility of finding new treatment pathways for these patients.

Trial registration number: NA

Abstract citation ID: dead093.865

P-522 Embryo transfer procedural parameters do not predict IVF cycle outcome

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Study question: Do embryo transfer (ET) procedural parameters affect the clinical outcome, in terms of positive human chorionic gonadotropin (hCG), clinical pregnancy and live-birth?

Summary answer: Observation of ET procedural parameters, either as single parameters or combined in a model, does not seem to have a predictive value on cycle outcome.

What is known already: It has been reported that several ET procedural parameters of technical nature may influence IVF outcome. Observational studies on variables during and post ET from mucus presence to catheter reload, have been published and data hitherto is conflicting leaving practitioners confounded. It is essential to evaluate the effect of the reported ET performance parameters, clarifying the degree of effect on a singular or combinatorial level. This will assist practitioners in recording and analysing strictly factors that hold high-significance predictive value, instead of redundantly recording moot parameters that may be unfit to serve as key performance indicators post ET.

Study design, size, duration: A prospective observational study was performed between 3/2018 and 9/2022 in a single IVF center. ET procedures for a total of 1417 women were assessed. The performance parameters studied and associated with clinical outcomes were the following: time of ET, presence of cervical mucus in the catheter, presence of blood, catheter reload, employment of tenaculum and stylet, resistance during transfer, ease of transfer according to the practitioner and discomfort as experienced by the patient.

Participants/materials, setting, methods: Only women of normal endometrial and uterine anatomy and function were included. Genetic and endocrine abnormalities, azoospermia and PGT cycles were excluded. Embryo grading was performed according to Veeck's and Gardner's system depending on developmental stage. All ETs were performed employing the same type of soft catheter. The clinical outcomes evaluated were positive hCG, clinical pregnancy, and live-birth. Associations were evaluated on a singular or combinatorial level between the ET performance parameters and clinical outcomes.

Main results and the role of chance: A univariate logistic regression was employed to evaluate the possible effect of each of the ET procedural parameters. None of the ET parameters presented with statistical significance. The employment of ultrasound, the number, and the quality of transferred embryos, maternal age as well as the physician and embryologist performing the transfer were evaluated as covariates in a univariate regression model. Number (p = 0.01) and quality of transferred embryos (p = 0.003), as well as maternal age (p = 0.004) presented with statistical significance. The multivariate approach yielded similar results. To evaluate the possibility that interactions between the ET procedural parameters could affect cycle outcome a

model was created evaluating all possible interactions and combination of the ET parameters. To adjust for possible multiple comparison bias, the Bonferroni correction was employed. Only the presence of mucus along with significant resistance seemed to present with statistical significance ($p < 0.0001$). However, when adjusting for the covariates the combination of cervical mucus and significant resistance did not provide a statistically significant effect on clinical outcomes and thus cannot be considered as an independent predictor. When subgrouping only for women below the age of 35 with at least one top quality embryo, no statistically significant association was observed.

Limitations, reasons for caution: The sample size could be a reason for caution when interpreting the results of the present study as a larger number of women presenting with each combination may be required to reach the threshold of statistical significance. The single-center nature of the study may present as another limitation.

Wider implications of the findings: Data indicates that ET procedural parameters hold no predictive value singularly or combinatorially. The only robust predictive factors are embryo quality, number, and maternal age. While further studies are required to cement this finding, it may be the case that the time-consuming recording and analysis of such parameters is redundant.

Trial registration number: Not applicable

Abstract citation ID: dead093.866
P-523 3D Sw71 blastocyst-like model and its validation by primary trophoblasts

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Study question: Are the *in vitro* developed 3D-Sw71 blastocyst-like model and the primary trophoblast 3D model interchangeable?

Summary answer: We confirmed that 3D-Sw71 model can be successfully replaced by 3D primary trophoblast model.

What is known already: Overcoming the challenges and ethical considerations of manipulating human embryos, researchers use cell lines to resemble blastocyst morphology and behavior. We have constructed 3D-Sw71 blastocyst-like model from non-cancerous trophoblast cell line. Like human blastocyst, our Sw71 spheroids undergo compactization and cavitation, have similar shape and size and attach to endometrial epithelium, invade extracellular matrix and migrate between endometrial stromal cells. Models'Sw71 cells have the hybrid epithelial-mesenchymal phenotype of the extravillous trophoblasts (EVT), important for building functional placenta. 3D-Sw71 models have the unique invariant HLA-profile of EVTs (HLA-G+, HLA-C+) providing tolerogenic maternal-embryo immune recognition. Sw71-2D and 3D -cultures produced CD63-positive exosomes.

Study design, size, duration: To validate our models *in vivo*, we performed direct comparison between 3D Sw71 spheroids and analogous spheroids built from isolated primary chorion-derived trophoblasts (1st trimester of pregnancy). The comparison was done in context of the following criteria: generation/ differentiation, morphology (shape, size), function, invariant HLA profile, presence of hybrid phenotype and exosomes secretion. For each condition of validation at least 3 independent experiments were conducted.

Participants/materials, setting, methods: 3D-Sw71 spheroids were constructed from Sw71 cells and 3D primary trophoblasts spheroids – from trophoblasts from placenta of pregnant women, directed to elective early pregnancy termination. Trophoblast cells were isolated by enzymes treatment and Percoll gradient separation. The generation and migration of the spheroids was monitored by live cells imaging and EMT and HLA molecules were detected by FACS and immunocytochemistry. The exosomes isolated by ultrafiltration were characterized by TEM, dot-blot analysis and immunogold staining.

Main results and the role of chance: From pure trophoblast cultures (> 80%, assessed by FACS) were generated long-lived clones, cells from which were used for 3D primary trophoblasts spheroids construction. Our results showed that in both 3D culture settings we obtained stable, round shaped, multilayered and relatively symmetric spheroid structures followed by compaction (12–24 h) and formation of single differentiated spheroid (24-48 h) with an intact periphery and easy to manipulate. The primary trophoblasts 3D spheroids resemble 3D blastocyst-like structures in shape and size. Primary trophoblast spheroids migrated successfully in the same time frame as 3D in vitro blastocyst-like structure (BLS), (48 hours). The trophoblasts cells of the primary 3D spheroids have hybrid phenotype (Vim+Ck7+) as Sw71 cells of 3D-Sw71 BLS. These expressed the invariant HLA-G and -C molecules as well. The HLA molecules were proven also *in situ* in early human placenta by IHC. Sw71 and primary trophoblasts secrete exosomes, proven by TEM as 20-120 nm EV, positive for CD63 (exclusive exosome marker). In conclusion, 3D-Sw71 blastocyst-like structure represent a valuable model for in vitro trophoblast and implantation studies as well as trophoblasts-immune cells interactions.

Limitations, reasons for caution: Primary trophoblasts cultures have limited viability, which makes difficulty to perform more than three independent experiments for all conditions. In general, manually transferring 3D spheroids to new media conditions is very laborious, time-consuming and not amenable to high-throughput screenings.

Wider implications of the findings: Mimicking the implanting embryo has huge implications for a plethora of studies, such as toxicity and drug screens, development of implantation-promoting compounds. 3D-Sw71 model has a potential to integrate additional components such as immune cells, endothelial cells/vessels and bioengineered *in vitro* models of the uterine wall/glands to uncover human implantation.

Trial registration number: not applicable

Abstract citation ID: dead093.867
P-524 Fertility and subsequent pregnancy outcomes among women with tubal ectopic pregnancy treated with methotrexate

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Study question: What are the future fertility prospects and subsequent pregnancy outcomes among women with tubal ectopic pregnancy treated with methotrexate?

Summary answer: Pregnancy post-ectopic pregnancy occurred in 53% of women within 12 months following methotrexate treatment. Among those who became pregnant, ectopic pregnancy recurrence occurred in 16.8%.

What is known already: Concerns regarding ectopic pregnancy recurrence and future fertility predominate when counselling women about management options for tubal ectopic pregnancy treatment. Current UK National Institute for Health and Care Excellence guidance is based on low quality evidence and estimate long-term ectopic pregnancy recurrence rate of ~18.5%. Further, they state that there are no differences in rates of subsequent pregnancy or ectopic pregnancy recurrence between ectopic pregnancy management methods. However, 30% of women managed with methotrexate experience treatment failure and require rescue surgery and subsequent pregnancy outcomes in this group are poorly understood.

Study design, size, duration: The data for this study were derived from a UK multicentre RCT of methotrexate and gefitinib versus methotrexate and placebo for treatment of ultrasound diagnosed definite or probable tubal

ectopic pregnancy with pre-treatment hCG ≥1000 IU/L and ≤5000 IU/L (GEM3). The trial found adding gefitinib to methotrexate was not superior to placebo. This analysis reports trial participant follow-up data at 12-months examining post-treatment fertility, subsequent pregnancy outcomes and participant characteristics associated with ectopic pregnancy recurrence.

Participants/materials, setting, methods: Trial participants of GEM3 (as described above).

Participants were contacted to provide follow-up data 12 months after randomisation to treatment. Where telephone contact was unsuccessful, electronic health records were reviewed to collect pregnancy outcome data. Post-treatment fertility and pregnancy outcomes were summarised using descriptive statistics and compared between groups using a chi-squared test, $p<.05$ signified statistical significance.

Main results and the role of chance: Pregnancy follow-up data was obtained for 283/327 trial participants. (167 participants were successfully contacted by telephone; the electronic health records were reviewed for 116 participants). 52.7% (149/283) of the trial participants became pregnant in the 12-month follow-up period. There was no difference in subsequent pregnancy rates between 'methotrexate only' and 'methotrexate and rescue surgery' groups. The surgical approach (salpingectomy vs salpingotomy) did not affect subsequent pregnancy rates. Among women who had a pregnancy in the follow-up period, a live birth was recorded in 65% of women (n = 93/142, n = 7 missing), any pregnancy loss (miscarriage, ectopic pregnancy, stillbirth, termination of pregnancy or molar pregnancy) was recorded in 43% of women (n = 59/137, n = 12 missing). Recurrent ectopic pregnancy was reported in 16.8% of women (n = 22/131, n = 18 missing). There was no difference in rates of live birth, pregnancy loss or recurrent ectopic pregnancy between methotrexate only and methotrexate and surgery groups or between type of surgical management groups (salpingectomy vs salpingotomy).

Limitations, reasons for caution: This study only reports on a 12-month follow-up period and this should be considered when interpreting comparatively low post-treatment pregnancy rates. We did not record which couples were trying-to-conceive post treatment and were therefore unable to stratify results by this variable or report subfertility rates.

Wider implications of the findings: This prospective dataset strengthens current understanding of the likelihood of ectopic pregnancy recurrence. Furthermore, it provides reassurance that women with tubal ectopic pregnancy managed with methotrexate requiring rescue surgery have similar post-treatment fertility and pregnancy outcomes to those successfully treated with methotrexate.

Trial registration number: ISRCTN67795930

Abstract citation ID: dead093.868
P-525 A second euploid embryo transfer is more likely to succeed if there was a previous confirmed euploid implantation.

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Study question: Does the outcome of the first, euploid transfer affect the success rate of the second PGT-A transfer cycle?

Summary answer: Live birth rate is higher in the second euploid transfer if the first euploid transfer results in at least a biochemical pregnancy versus no implantation.

What is known already: There is limited evidence on counselling patients for a second euploid embryo transfer based on previous outcomes. The existing literature is reassuring showing very high implantation rates even following two or more successive failed euploid transfers. Achieving implantation is a major step towards success. The association between a PGT-A embryo transfer cycle where implantation was achieved (at least biochemical pregnancy, miscarriage or live birth) with the outcome of the subsequent embryo transfer is interesting to explore and incorporate in patient counselling.

Study design, size, duration: This is a retrospective analysis of patients who underwent PGT-A treatment in a single centre from 2015-2021. Live birth rate was the main outcome of interest. Pregnancy rate and miscarriage rate were secondary outcomes. Means and standard deviations were used for demographic parameters. Embryos were biopsied at blastocyst stage for PGT-A and next generation sequencing was used. Embryo transfers were performed with uniform protocols by different, experienced clinicians.

Participants/materials, setting, methods: Patients who had their second euploid embryo transfer were included in the analysis. Outcomes were assessed in relation to the outcome of the first euploid cycle; whether at least a biochemical pregnancy was achieved versus a negative pregnancy test. Patients with uterine anomalies / suboptimal endometrial thickness were excluded. Protocols for endometrial preparation and add-ons were individualised and were not uniform for the two cycles. Protocols included both medicated cycles and ovulatory cycles.

Main results and the role of chance: 331 women were included in the analysis undergoing their second euploid embryo transfer cycle. 152 had one previous euploid implantation (the first cycle resulted in a biochemical pregnancy, miscarriage, or live birth) (group 1) and 179 had one previous failed implantation of a euploid embryo (the first cycle resulted in a negative pregnancy test) (group 2). There was no significant difference between the two groups in patient demographic characteristics including age, body mass index and age at egg collection. No significant difference was documented for embryo quality, day 5 versus day 6 embryos or endometrial thickness. More cycles were medicated in group 1 compared to group 2. All patients had an elective single embryo transfer. Live birth rate was significantly higher for group 1 versus group 2, 58% vs 44%, $p = 0.013$, OR 1.75 (1.12-2.70). No significant difference was observed for pregnancy rate or miscarriage rate.

Limitations, reasons for caution: This is a retrospective analysis in a single fertility centre, therefore prone to bias and results cannot be generalised unless validated by larger prospective multi-centre studies. There are multiple possible cofounders such as background and duration of subfertility, endometrial preparation protocols and add-on interventions which cannot be accounted for.

Wider implications of the findings: These findings suggest that the first euploid transfer outcome is important for future euploid transfer cycles. Women who achieved at least a positive pregnancy test in the first cycle seem to have higher success rates in subsequent PGT-A transfer. Further research is warranted to explore this association for patient counselling.

Trial registration number: not applicable

Abstract citation ID: dead093.869
P-526 Modified Applebaum uterine scoring system to predict uterine receptivity in frozen embryo transfer(FET) cycles

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Study question: To evaluate modified Applebaum uterine scoring system in predicting the uterine receptivity and clinical pregnancy rate?

Summary answer: The modified version of Applebaum uterine scoring system has sensitivity of 0.9411 and specificity of 0.5869 in predicting the uterine receptivity in embryo transfer cycle.

What is known already: The endometrial receptivity have been accepted to be major limiting factors in the establishment of pregnancy. Different techniques have been developed to evaluate endometrial receptivity, such as endometrial receptivity array or histological dating. The advantage of Ultrasound is it's non-invasiveness, repeatability, real time monitoring and predictability.

Study design, size, duration: It is a prospective observational study. Study group comprises of 200 female with primary infertility over a period of 6 month.

Participants/materials, setting, methods: Exclusion criteria was patients associated with male sub-fertility or having uterine anomalies. The study was conducted at D.Y.Patil fertility centre, unit of Bloom IVF. All the patients

were given uterine scoring before FET based on Endometrial thickness, morphology, vascularisation, myometrial echogenicity, uterine artery PI and EDF, myometrial blood flow.

Main results and the role of chance: The overall pregnancy rate was 54%. A perfect score of 20 was associated with 97.4% pregnancy rate. Scores of 17 to 19 was associated with the pregnancy rate of 85.4% and scores of 14 to 16 was associated with the pregnancy rate of 28.6%. Score less than 13 is not associated with any pregnancy. The Modified Applebaum scoring system showed the sensitivity of 0.9411 and specificity of 0.5869 in predicting the outcome of the cycle.

On an individual basis, endometrial morphology, end diastolic blood flow(EDF) and Pulsatility index(PI) of uterine artery and myometrial blood flow were found to be significant for a pregnancy on the basis of Z score and p value of < 0.01

Limitations, reasons for caution: As there were no case of secondary infertility, Grade III/IV endometriosis, endocrine disorder and uterine anomalies. The scoring system might not replicate the same results.

Wider implications of the findings: As the sensitivity and specificity of this uterine scoring system is quite good. It could be a good tool in assessing endometrium before embryo transfer.

Trial registration number: Not applicable

Abstract citation ID: dead093.870
P-527 What is the influence of blastocyst hatching position on clinical pregnancy rates?

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Study question: Is there a relationship between the hatching zone in the blastocyst and its gestational potential?

Summary answer: Blastocysts that presented a hatching area opposite to the inner cell mass showed lower clinical pregnancy rates.

What is known already: Embryo implantation in the uterine endometrium is dependent on blastocyst hatching out of the zona pellucida, allowing the cells of the trophectoderm to establish contact with the uterine epithelium. Hatching begins with the continuous expansion of the blastocoel, thinning the zona pellucida. The blastocyst then exits through a rupture, called the hatching zone. In humans, trophoblast cells close to the inner cell mass (ICM) – polar trophoectoderm – rapidly proliferate and differentiate to follow endometrial invasion, being thus distinct from the remaining trophoectoderm. The association between the hatching zone (according to ICM localization) and implantation rates is scarce.

Study design, size, duration: This is a prospective observational cohort study with 588 patients that underwent in vitro fertilization (IVF) cycles with autologous oocytes and had a fresh or frozen hatching blastocyst embryo transfer between January/2018 and August/2022 in a private ART center. All embryos were cultured in a time-lapse system (Embryoscope, Vitrolife) and were fertilized by ICSI or IVF. Only embryos with known reproductive outcomes (the presence/absence of gestational sac and heartbeat) were included in this analysis.

Participants/materials, setting, methods: Embryos with evidence of natural hatching (either by penetration of trophectoderm cells, or by regular disruption of the trophectoderm followed by embryo extrusion) were included. Using the ICM as reference, blastocysts were classified according to its hatching position in four categories: mass, opposite to mass, intermediate (between mass and opposite) and multiple hatching. ANOVA, Fisher and chi-squared tests were applied properly for statistical analysis. $p < 0,05$ was considered significant.

Main results and the role of chance: The majority of embryos were classified as intermediate (n = 540, 77.4%), followed by opposite to mass (n = 88, 12.6%), mass (n = 50, 7.2%) and multiple hatching sites (n = 20, 2.8%), in a total of 698 embryos analyzed. Maternal age was similar between groups (36,22 ± 3,65; 36,03 ± 3,3; 36,07 ± 3,23; 37,35 ± 3,57, p = 0.35, for mass, intermediate, opposite to mass and multiple hatching respectively). The majority of embryos presented hatching by penetration of trophoectoderm cells (n = 685, 98.1%), regardless of hatching position and mode of insemination (IVF or ICSI). Biopsied embryos were included since they had reached natural hatching before trophoectoderm biopsy. There was no difference in the proportion of euploid (64%) and non-biopsied (36%) embryos transfers according to hatching position subgroups (68% vs 32%; 64.3% vs 35.7%; 63.3% vs 36.4%; 50% vs 50% for mass, intermediate, opposite and multiple hatching respectively, p = 0.56). Positive pregnancy rate were lower in the opposite to ICM group (47.7%, p = 0.04) when compared to intermediate group (59.3%). Positive pregnancy rates in mass and multiple hatching sites were 54% and 55% respectively. Clinical pregnancy rate were also lower in the opposite to ICM group (45.5%, p = 0.04) when compared to intermediate group (57%). Clinical pregnancy rates in mass and multiple hatching sites were 52% and 50% respectively.

Limitations, reasons for caution: Two experienced senior embryologists classified the blastocyst hatching position; however, operator subjectivity cannot be excluded. The influence of the ICSI hole in the hatching type (penetration or disruption) and position is debatable; nevertheless, both types and all hatching position classification were present in ICSI and IVF insemination.

Wider implications of the findings: The hatching zone of the embryo is potentially a variable of attention for embryo selection. The proliferation and differentiation of trophoblast cells opposite to the inner cell mass may affect the ability of the embryo to progress in the apposition, adhesion and, mostly importantly, invasion of the uterine endometrium.

Trial registration number: Not applicable.

Abstract citation ID: dead093.871
P-528 Intra-individual variability of serum progesterone level on the day of frozen blastocyst transfers in hormonal replacement therapy cycles

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Study question: Is there a significant intra-individual variability of serum progesterone level on the day of theHormone Replacement Therapy Frozen Embryo Transfer(HRT-FET) between two consecutive cycles?

Summary answer: No significant intra-individual variability of progesterone level measured the day of transfer was shown between two consecutive HRT-FET cycles.

What is known already: In frozen embryo transfer under HRT, a minimum P level around the day of embryo transfers necessary to optimize reproductive outcomes. Indeed, in a previous study, we have found that women with a serum P level ≤9.8ng/ml on the day of the single autologous blastocyst transfer had lower live birth rate. Moreover, some clinical factors have been described as influencing the P level on the day of the embryo transfer (i.e weight, gravidity, Ethnic origin,tobacco consumption...). Variations between patients raise the question of intra-individual variation in P measurement, in addition to the existence of inter-individual variation.

Study design, size, duration: We conducted an observational cohort study at the university-based reproductive medicine centre of our institution that focusing on women who underwent at least two consecutive single autologous HRT-FET of blastocyst, between January 2019 and March 2020.

Participants/materials, setting, methods: Patients undergoing two consecutive single autologous blastocyst HRT-FET using exogenous estradiol and vaginal micronized progesterone for endometrial preparation were included.The serum progesterone level was measured in the morning of the FET, in a single laboratory. The two progesterone measurements performed

the day of the first (FET1) and the second FET (FET2) were compared to evaluate the intra-individual variability of serum P.Paired statistical analyses were performed, as appropriate.

Main results and the role of chance: Two hundred and sixty-four patients undergoing two consecutive single autologous blastocyst HRT-FET were included. The mean age of the included women was 35.0 ± 4.2 years old. No significant intra-individual variability was observed between FET 1 and FET2 (Mean progesterone level after FET1: 13.4 ± 5.1ng/ml versus after FET 2: 13.9 ± 5.0; p = 0.08).Characteristics of embryo transfers were similar between the first and the second FET. Forty-nine patients (18.6%) had discordant progesterone levels (defined as one progesterone measurement > and one ≤ to the threshold of 9.8ng/ml) between FET1 and FET2. 37/264(14%) women had a high intra-individual variability (defined as a difference in serum progesterone values >75 e percentile (6.0ng/ml)) between FET1 and FET2. No specific clinical parameter was associated with a high intra-individual variability nor a discordant P measurements.

Limitations, reasons for caution: This study is limited by its retrospective design. Likewise, only women who underwent autologous blastocyst HRT-FET with micronized vaginal P were included.

Wider implications of the findings: No significant intra-individual variability was showed and the serum progesterone level seems to be reproducible in more than 80% of case. This study suggests that the serum progesterone level measured the day of the first transfer could be used to individualize luteal phase support on subsequent cycles.

Trial registration number: not applicable

Abstract citation ID: dead093.872
P-529 High Live Birth Rates After Low Dose Immunization Therapy (LIT) Pre and Post Pregnancy With Partner Lymphocytes In Patients With Recurrent Miscarriage (RM)

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Study question: Could Lymphocyte Immunization Therapy effectively improve pregnancy and live birth rate in Unexplained Recurrent Miscarriage patients?

Summary answer: The use of paternal lymphocytes pre and post pregnancy significantly increases the pregnancy success and Live Birth Rate In Patients With Recurrent Miscarriage (RM)

What is known already: RM affects 1–2% of couples who attempt to conceive and has been defined as three consecutive pregnancy losses within 20 weeks of pregnancy confirmation. Although many potential causes have been established for RM, about 50% of these remain idiopathic and unexplained owing to immunology factors. Meta-analyses have observed better effectiveness and safety of LIT in treating couples with RM shown improvement in pregnancy outcomes. Still, results are conflicting due to different screening criteria and therapeutic protocols. Objective of present study is to evaluate effectiveness of low dose LIT in patients with uRM and Th1/Th2/Treg paradigm disorders

Study design, size, duration: In self-controlled, prospective study, after IEC clearance and obtaining Informed consent, 19 RM patients underwent immunization with partner lymphocytes from August 2021 to October 2022. Inclusion criteria were at least three clinical pregnancies that culminated in abortion before 20 weeks of gestation or at least three cycles of IVF/ICSI with at least two PGT+ embryos and >8mm endometrium during ET and no pregnancy, normal coagulation tests, autoimmune antibodies negative, normal couple karyotyping.

Participants/materials, setting, methods: Maternal BMI of 30 and age of 45 was the upper limit with peripheral blood Th1/Th2/Treg cells proportions and concentrations of TNF-a, IFN-g, TGF-β1, IL-2, IL-4, IL-6, IL-10, and blocking antibodies were detected using flowcytometry/ELISA as per Seragen's Immuno profiling platform. 30-50ml paternal blood was used to purify lymphocytes which were intradermally administered to the wife before Embryo Transfer and post-pregnancy confirmation.

Main results and the role of chance: Proportion of Th1 cells was significantly decreased while the proportions of Th2 cells and Treg cells were significantly increased in immunotherapy patients after treatment. In addition, the concentration of TGF-β1 in serum was significantly higher after immunotherapy than before. The concentration of TNF-a, IFN-g was significantly high before therapy were significantly decreased after therapy. LIT effectively induced the production of blocking antibodies in all the patients. 16/19 (84%) uRM patients underwent ET post LIT became pregnant and 4 of the pregnant patient had delivered healthy babies (21%). 42% (8/19) of patients have reported uneventful ongoing pregnancies crossed >14weeks. 4 patients who were pregnant for more than 12-14 weeks had miscarriages (21%). 1 patient (5%) did not conceive. 1 patient (5%) conceived spontaneously and crossed 20 weeks of uneventful gestation. 1 patient (5%) opted to try to conceive naturally hence ET is not planned. The cumulative positive uneventful pregnancy outcome was 68%, approximately the same as the figures reported earlier. LIT is associated with high live birth rates, especially in women with RM. It not only ameliorates patient's cellular immune function but also further increases patient's pregnancy success rate with high safety, which is worthy of clinical application and promotion.

Limitations, reasons for caution: This prospective self-control study with small sample size lacks randomized control group. Beneficial effects observed in this study are in sync with published data. Larger study with patients recruited based on inclusion criteria of unexplained recurrent miscarriage due to immunological factors is proposed for recommending LIT as routine therapy.

Wider implications of the findings: Our study findings supports LIT as a beneficial treatment for RM/RIF in IVF patients. LIT may be considered safe and effective therapy in individual cases and based on the immunoprofiling (Th1/Th2/Treg paradigm disorders), after all other potential causes of RM or RIF have been ruled out.

Trial registration number: Not applicable

Abstract citation ID: dead093.873
P-530 Which evacuation method for missed abortion is preferred by women with recurrent pregnancy loss?

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Study question: Is medical or surgical evacuation for missed abortion (MA) preferred by women with recurrent pregnancy loss (RPL)? Which factors are determining for the women's preference?

Summary answer: Sixty-seven percent of the participants preferred surgical evacuation. Side effects and a history of unsuccessful medical evacuation in their first MA were determining factors.

What is known already: Guidelines currently recommend medical rather than surgical evacuation after MA. The success rates defined as adequate evacuation of the uterine cavity after MA without further intervention are reported to be 80% and 97% for medical and surgical evacuation, respectively. Medical evacuation is associated with heavier and longer duration of bleeding and higher intensity of pain. Surgical evacuation increases the risk of uterine perforation and Asherman's syndrome. Studies have found that women are more satisfied and have better subsequent mental health when they can decide themselves which method they would choose.

Study design, size, duration: An online questionnaire was sent October 5th, 2022, to 157 RPL patients who had experienced both medical and surgical evacuation for MA admitted to a center for RPL between January 2016 and September 2022. Eighty-seven (55.4%) patients completed the survey before deadline after being sent reminders. Sixty (69%) of the women had received a medical evacuation and 27 (31%) surgical evacuation for their first MA.

Participants/materials, setting, methods: The mean age of the respondents was 37 years (SD ± 0.5) and they had had at least three pregnancy

losses. The questionnaire featured questions regarding the women's experiences with medical and surgical evacuation, preferred evacuation method if they would have another MA, and determining factors for their preference. Categorical variables were compared using $\chi 2$ test and Wilcoxon Signed Rank Test. Analyses were conducted to determine associations between potential determining factors for preferred evacuation method.

Main results and the role of chance: A significantly higher intensity of pain was experienced, and analgesics were required for a significantly longer period after a first medical evacuation compared to a first surgical evacuation ($p < 0.001$ and $p = 0.006$). Bleeding, pain, sick leave, and mental discomfort had a significant impact on the choice of another evacuation method at the time of next MA in women who experienced a medical abortion first. As many as 40% had experienced failure of the first medical evacuation with need for subsequent surgical evacuation for complete evacuation; this was confirmed by consulting the patient's medical records. The odds ratio (OR) for preferring medical evacuation at next MA was 3.3 if the women had given birth before ($p = 0.011$). The OR for preferring medical evacuation at next MA was 0.3, if the women had received medical evacuation for their first MA ($p = 0.015$). There were no statistically significant associations between preferring medical evacuation and the following factors: BMI, age at first evacuation and menstrual pain. Two thirds (66.7%) of the women would prefer surgical evacuation for their next MA, while the remaining women (33.3%) would prefer medical evacuation.

Limitations, reasons for caution: The response rate of 55.4% was lower than expected 70% which implicates a risk of sampling bias such as a predominance of patients with a history of complicated evacuations responding to the questionnaire. Due to the retrospective design, recollection bias could also impact the results.

Wider implications of the findings: In this study population, medical evacuation was associated with a stronger experience of side effects and a higher failure rate than expected, which may explain why more women preferred surgical evacuation for their next MA. This may suggest current evacuation recommendations for MAs should become more individualized than currently.

Trial registration number: Not applicable

Abstract citation ID: dead093.874
P-531 Effectiveness and safety of uterine septum division: An updated systematic review and meta-analysis

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Study question: To evaluate whether division of uterine septum improves reproductive outcome as compared to no intervention and is a safe procedure.

Summary answer: While septal division doesn't seem to improve live birth and clinical pregnancy rates or reduce miscarriage rates, it has improved preterm birth and malpresentation rates.

What is known already: Septate uteri are common congenital uterine malformations and are seen in about 1% of women. While most women with septate uterus have normal reproductive outcome, studies have reported increased risk of adverse reproductive outcome including miscarriages in women with septate uterus. Hysteroscopic metroplasty or hysteroscopic transcervical division of the uterine septum has been regularly done with an intention to improve the outcome, although evidence on its effectiveness is conflicting. A systematic review of controlled studies done in 2014 has shown a potential benefit, but later studies including a recent randomized controlled trial (RCT) has shown no benefit.

Study design, size, duration: In this systematic review and meta-analysis, Medline and Cochrane Library databases were searched from commencement to December 2022. We identified 13 eligible studies of which 12 were non-randomised controlled studies and one RCT, comprising 1855 women.

Participants/materials, setting, methods: We included comparative studies of septal division versus non-intervention in women having septate uterus with a history of infertility or recurrent miscarriage. The outcomes assessed were live birth, clinical pregnancy, miscarriage, preterm birth, malpresentation and complications. The Newcastle-Ottawa Quality Assessment Scale was used for quality assessment. Statistical analysis was performed using Review Manager 5.4.1 software. Pooled odds ratios (OR) with 95% confidence intervals (CI) were computed using random effects models.

Main results and the role of chance: The live birth rates were similar for the septal division and non-intervention groups (OR: 1.48; 95% CI 0.86-2.56). The clinical pregnancy rates (OR: 1.24; 95% CI 0.64-2.39) and miscarriage rates (OR: 0.61; 95% CI 0.35-1.06) were also similar. The preterm birth rates (OR: 0.67; 95% CI 0.47-0.95) and malpresentation at birth (OR: 0.34; 95% CI 0.23-0.53) were lower in patients managed surgically. The complication rates were reported in four studies and were ranging between 2.6% and 9.4% with the main reported complication being uterine perforation.

Limitations, reasons for caution: While the review included comparative studies, only one was RCT. High level of heterogeneity between studies was observed. Many had different inclusion and/ or exclusion criteria and used different diagnostic modalities and criteria.

Wider implications of the findings: The findings of our review help in counselling women seeking septal division, particularly of lack of benefit in improving live birth rates or reducing miscarriage, but may reduce preterm births and malpresentation. Due to the heterogeneity of the included studies and low-quality evidence, large multicentre RCTs are warranted.

Trial registration number: not applicable

Abstract citation ID: dead093.875
P-532 Understanding the utility of hysteroscopic endometrial peeling in women with implantation failure

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Study question: Does hysteroscopic endometrial peeling improve reproductive outcomes in women with implantation failure (IF) undergoing a single euploid embryo transfer (SEET)?

Summary answer: Women with IF may benefit from intraoperative endometrial peeling prior to a SEET.

What is known already: Preimplantation genetic testing for embryonic aneuploidy (PGT-A) has been suggested as a strategy to improve implantation rates in women with IF. However, after controlling for the genetic status of the embryo, implantation rates still vary from 40-70%, suggesting that other factors aside from the ploidy of the embryo contribute with the cause of IF. We previously demonstrated that hysteroscopic endometrial peeling enhances implantation in women with IF. However, our study was limited by only including frozen embryo transfers of unscreened embryos. We aim to analyze the clinical utility of hysteroscopic endometrial peeling in women who underwent a SEET.

Study design, size, duration: A retrospective, cohort study included infertile patients with IF diagnosis who underwent endometrial peeling through hysteroscopy and subsequently a SEET from January 2016 to December 2022. Only the first transfer after the hysteroscopic endometrial peeling was included in the analysis.

Participants/materials, setting, methods: Women <41 years, with IF, normal saline sonogram, and no previous uterine surgeries were segregated into two groups: A) 66 patients underwent hysteroscopic endometrial peeling, which consists of removing the superficial endometrial layer of the whole uterine cavity with hysteroscopic biopsy forceps, and B) 38 controls who did not undergo surgical endometrial peeling. All patients underwent a subsequent SEET.

Main results and the role of chance: In total 104 women were included in the cohort. No differences were found in age, body mass index, baseline FSH, AMH, baseline antral follicle count, previous number of stimulation/IVF cycles,

number of embryos transferred, and, embryonic quality among cohorts. Evaluating the endometrium, no differences were observed in the endometrial pattern, however, the endometrial thickness was thicker in group B (8.4 ± 1.5mm vs 9.2 ± 1.3mm, p = 0.02). When analyzing the subsequent SEET cycle, women in group A had higher implantation (51.8% vs 40.6%, p = 0.001) and clinical pregnancy rates (63.5% vs 31.4%, p = 0.009). No difference was found in clinical loss rates (9.4% vs 10.1%, p = 0.06) among cohorts. Of the patients who underwent surgical endometrial peeling, 18.1% (12/66) had the following incidental intraoperative findings: mild intrauterine and/or cervical adhesions (5/66, 0.07%), endometrial polyps (10/66, 15.1%), polypoid endometrium (8/66, 12.1%). 64.3% of the patients with incidental findings became pregnant.

Limitations, reasons for caution: This study is limited by its retrospective nature. Additionally, in patients with noted uterine pathology, appropriate surgical management was administered in the same setting, this could have biased the results. Future randomized controlled trials are needed.

Wider implications of the findings: This study continues to support the possible benefit of mechanically peeling the superficial endometrial layer through hysteroscopy to increase implantation rates in women with IF who undergo a SEET.

Trial registration number: NA

Abstract citation ID: dead093.876
P-533 Comparison of Endometrial Receptivity Array (ERA) with Preimplantation genetic testing (PGT) versus ERA alone in improving outcomes in Recurrent implantation failure.

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Study question: Whether addition of Preimplantation Genetic Testing for aneuploidy (PGT-A) to Endometrial Receptivity Array (ERA) helps in improving success rates in Recurrent Implantation Failure (RIF) patients.

Summary answer: Addition of PGT-A intervention to ERA in RIF patients did not improve outcomes.

What is known already: RIF causes great emotional stress and financial burden for the infertile couple. Successful implantation requires a synchronous interaction between a competent blastocyst and a receptive endometrium. Though ERA, as a measure to assess window of implantation (WOI), is around a decade old, its role in improving success rates in patients with RIF is still controversial. PGT-A, as a tool for selecting euploid embryos, though has shown to be beneficial in women with advanced maternal age, its role in improving success rates in RIF needs further evaluation.

Study design, size, duration: This is a multicenter retrospective cohort study carried out from September 2015 till September 2022. 285 patients with RIF with previous failed transfers with ≥ 3 good quality embryos were included.

Participants/materials, setting, methods: 285 patients with RIF were divided into 2 groups. Group 1 underwent PGT-A and Personalised Embryo Transfer (pET) guided by ERA and Group 2 underwent only ERA followed by pET. In Group 1 patients, embryo biopsy was done on day 5 and day 6 blastocyst before cryopreservation and NGS platform was used to detect euploid embryos. Both groups then underwent ERA followed by pET in the next cycle.

Main results and the role of chance: In total of 285 RIF patients studied, 59.2% patients showed displaced WOI. 91.7% ERA reports were pre-receptive. Group 1 included 137 patients and Group 2 had 148 patients & baseline characteristics were similar in both the groups. Group 1 had more self transfer cycles, i.e. 76.7% self and 17.2% donor oocytes, as compared to Group 2 with 40.2% self and 30.7% donor oocyte cycles. The implantation rate (IR), live birth rate (LBR), miscarriage rate and cumulative LBR were similar in both the groups. Higher Pregnancy rate (PR) in Group 2 (70.9% vs 57.83%) could be attributed to higher donor cycles in this group. Multiple gestation rate was also higher in Group 2 (31.49% vs 11.21%) as 2 blastocysts were transferred in most cycles in group 2 compared to Single embryo transfer(SET) in most cycles in Group 1. As PR was higher in ERA group, subgroup analysis of self gamete transfers and donor oocyte transfers was also done, which showed similar PR, IR and LBR in both the groups. Hence, PGT failed to show any value in improving pregnancy outcomes in RIF.

Limitations, reasons for caution: Retrospective study design.

Wider implications of the findings: The role of PGT in improving clinical outcomes in RIF patients needs to be studied in larger prospective studies. In our study, ERA as an intervention improved outcomes in RIF.

Trial registration number: not applicable

Abstract citation ID: dead093.877
P-535 DNA methylation abnormalities induced by advanced maternal age in villi in early pregnancy prime a high-risk state for spontaneous abortion

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Study question: What's the epigenetic effect of advanced maternal age (AMA, >=35 at delivery) on human villus in early pregnancy and its relationship with spontaneous abortion (SA)?

Summary answer: AMA-induced local changes in DNA methylome would perturb villous transcriptome and trophoblast cellular function, which may partly explain increased risk of SA in AMA pregnancy.

What is known already: The proportion of AMA in living births has increased in many countries over recent decades. However, AMA has been regarded as an independent risk factor for many pregnancy complications, including SA, which are closely associated with placental dysfunction in early pregnancy. Currently, researches about the epigenetic influence of AMA has been extremely limited, and only one study in mice tried to explore the DNA methylation alterations of eight ICRs in AMA placenta. The DNA methylation pattern of the human placenta in AMA pregnancy is still blank, and a comprehensive genome-wide investigation of AMA's influence is urgently needed.

Study design, size, duration: We profiled the DNA methylome of 24 human chorionic villi samples (CVSs) from early pregnancies in AMA and young maternal age (YMA) and 11 CVSs from early spontaneous abortion cases, and the transcriptome of 10 CVSs from AMA and YMA pregnancies. Trophoblast cellular impairment were investigated by repressing target gene in trophoblast cell lineage.

Participants/materials, setting, methods: CVSs from early pregnancies were collected, the DNA methylome were profiled, using reduced representation bisulfite sequencing (RRBS), and the transcriptome were profiled. with mRNA sequencing(mRNA-seq). Single-cell villous transcriptional altas presented the expression patterns of targeted AMA-/SA-related genes. Cellular function experiment werewas performed after the knockdown of *GNE* expression in HTR8-S/Vneo cells.

Main results and the role of chance: AMA-induced local DNA methylation changes, defined as AMA-related differentially methylated regions (DMRs), might be derived from the abnormal expression of genes taking part in DNA demethylation, such as *GADD45B*. These DNA methylation changes were significantly enriched in the processes involved in Notch signaling and extracellular matrix organization, and reflected in the transcriptional alterations in the corresponding biological processes and specific genes, too. Furthermore, the DNA methylation level of special AMA-related DMRs not only significantly changed in AMA, but also showed more excessive defects in CVS from spontaneous abortion (SA), including four AMA-related DMRs whose nearby genes were overlapping AMA-related differentially expressed genes (DEGs) (*CDK11A*, *C19orf71*, *COL5A1*, and *GNE*). The decreased DNA methylation level of DMR near *GNE* was positively correlated with the down-regulated expression of *GNE* in AMA. Single-cell altas further revealed the comparatively high expression of *GNE* in trophoblast lineage, and the knockdown of *GNE* in HTR8-S/Vneo cells significantly impaired cellular proliferation and migration. Our study provides valuable resources to investigate AMA-induced epigenetic abnormalities and bring a new insight for explaining the increased risks of pregnancy complications in AMA pregnancy.

Limitations, reasons for caution: The samples size was still small, a larger cohort study would be helpful to verify our findings in future. Meanwhile, though the high co-relationships between the expression of specific DEGs

and the DNA methylation level of nearby DMRs, the detailed mechanisms in gene expression regulation need to be further explored.

Wider implications of the findings: It deepened our understanding of the influences of AMA and provided novel evidence to develop strategies for the diagnosis, providence and even therapy for pregnancy complications in AMA.

Trial registration number: not applicable

Abstract citation ID: dead093.878

P-536 The comparison of luteal phase support in modified natural cycle-frozen embryo transfer in young patients with high quality embryos -vaginal versus vaginal and subcutaneous progesterone.

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Study question: Does adding subcutaneous progesterone to vaginal administration affect pregnancy outcomes in young patients undergoing modified natural-frozen embryo transfer (mNC-FTET) with top-good quality, single blastocyst ?

Summary answer: Adding subcutaneous progesterone supplementation to vaginal administration after frozen-thawed embryo transfer in modified natural cycles does not improve live birth rates.

What is known already: Vaginal progesterone supplementation during frozen-thawed embryo transfer in modified natural cycles during luteal phase may increase the rate of live birth rate and decrease the rate of miscarriage.

Study design, size, duration: 245 participants were included in this retrospective cohort study conducted at a single IVF center between November 2020and January 2022.The primary outcome was the live birth rate; secondary outcomes included pregnancy, clinical pregnancy and biochemical and clinical abortion rates.

Participants/materials, setting, methods: 245 women aged 20-35 years in mNC-FTET cycles with a single top-good quality blastocyst embryo transfer were included. Patients with fibroids, synechiae, recurrent implantation failure, recurrent pregnancy loss were excluded. After thawing embryos were evaluated for quality, hCG(Ovitrelle-Merck) triggered; 129 patients received 200 mg vaginal progesterone (Lutinus-Merck)(group I), while 116 received 200 mg. vaginal and 25 mg subcutaneous progesterone (Progestan-Dex-Kocak Pharma) (group II). The main outcome was live birth rate(LBR), secondary outcome was miscarriage rate.

Main results and the role of chance: Baseline demographics and background characteristics were similar in the study groups. The live birth rate in group I is 86 of 106 (66.6%) compared 74 of 116 (63.7%) in group II (odds ratio 1,135, 95% confidence interval [CI]: 0.670-0.922, P = 0.637). There were 106 pregnant patients out of 129 in group I (82.17%) and 93 pregnant patients out of 116 in group II (odds ratio 0.877, 95 CI: 0.462-1,666, P = 0.68). The clinical pregnancy rate is 95 out of 129 patients (73.6%) in group I and 90 out of 116 patients in group II (odds ratio of 0.807, 95% confidence interval [CI]: 0.449-1.45, P = 0.474). The rate of biochemical abortion in group II was 10 of 129 patients (7.75%) and 4 of 116 (3.45%) (odds ratio 2.35, 95 CI: 0.717–7.718, P = 0.687). The clinical abortion rate was 6 of 129 patients (4.6%) and 6 of 116 patients (5.17%) in group II (odds ratio 2.35, 95 CI: 0.717-7.718; P = 0.687). According to primary and secondary outcomes, there were no significant differences between the groups.

Limitations, reasons for caution: To avoid bias, patients older than 35 years were excluded. Subcutaneous progesterone may be beneficial for older patients undergoing euploid embryo transfer. To reduce confounding factors, the number of patients was limited.

Wider implications of the findings: For luteal phase support, both vaginal and subcutaneous progesterone supplementation did not increase live birth rates following frozen-thawed embryo transfer in modified natural cycles in young patients.

Trial registration number: not applicable

Abstract citation ID: dead093.879

P-538 The association between Clinical Pregnancy Rate in IVF-Cycles and Endometrial Receptivity based on a Novel Ultrasonographic Endometrial Receptivity Scoring System

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Study question: Our study was aimed towards determining whether Ultrasound based assessment of Endometrial features like blood flow, echo-pattern and thickness, can correlate with IVF pregnancy rates.

Summary answer: It was found that Clinical Pregnancy Rate in IVF cycles improved as the Ultrasound based Endometrial Receptivity Score increased.

What is known already: Establishing a successful pregnancy depends on the endometrium and embryo. Suboptimal endometrial receptivity account for one-third of implantation failures. Despite the in-depth understanding of the processes associated with embryo-endometrial crosstalk, little progress has been achieved for diagnosis and treatments for suboptimal endometrial receptivity. Continuously mature embryo laboratory operation technology and embryo culture technology have significantly improved the quality of embryos. In addition, because of its accuracy and non-invasiveness, transvaginal ultrasound is widely used in the field of assisted reproduction, not only for monitoring follicles, but also for evaluating endometrial receptivity and this technique can help in achieving improved pregnancy rates.

Study design, size, duration: This was a prospective study carried out from 2020 to 2022 in which 275 participants were recruited. Exclusion Criteria were the following: congenital uterine anomalies or acquired uterine diseases, submucosal myoma, intrauterine adhesion, uterine effusion and adenomyosis, hydrosalpinx; endometriosis; pre-implantation genetic test cycles, freeze-thaw embryo transfer cycles which all transferred embryos were non-high quality embryos. That is to say, FET cycles with the transfer of at least one high-quality embryo were included in the analysis.

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Participants/materials, setting, methods: 275 patients underfoing FET cycle were included in this prospective study carried out from January 2020 to December 2022. Endometrial preparation protocols included natural ovulatory cycles, ovulation induction cycles, and hormone replacement treatment cycles. Transvaginal ultrasound monitoring of endometrial thickness and the diameter of the dominant follicle began on the 10-12th day of menstrual cycle. Embryo transfer was timed accordingly and frozen embryo transfer carried out.

Main results and the role of chance: A total number of 275 patients of FET were analyzed. It was found that only the echo of the endometrial central line was different between the pregnant group and non-pregnant group. Other parameters, such as endometrial thickness, volume, endometrial peristalsis, or the endometrial blood flow were not statistically different between the two groups. Then, according to the relationship between the different groups and the clinical pregnancy rate, a score of 0 to 2 was respectively scored. The sum of the scores for the six items was the patient's endometrial receptivity score. It showed that the clinical pregnancy rate increased as the endometrial receptivity score increased, and when the receptivity score reaches at least 5, the clinical pregnancy rate is significantly improved (58.2% versus 40.4%, P = 0.001). The echogenicity of the endometrial functional layer in the pregnancy group was more homogeneous, but it did not reach statistical significance. Other parameters, such as endometrial thickness, volume, the presence or absence of endometrial peristalsis, the direction of endometrial peristalsis, or the endometrial blood flow were not statistically different between the two groups.

Limitations, reasons for caution: In the absence of data of live birth rate, we considered clinical pregnancy rate as a proxy outcome to confirm receptive endometrium. Moreover, the sample size of the study was relatively small, and it is necessary to further expand the sample size to verify our conclusions.

Wider implications of the findings: We developed an endometrial receptivity scoring system and demonstrated its validity. It may aid clinicians in choosing the useful marker in clinical practice and for informing further research. As Ultrasonography is a simple and non-invasive diagnostic tool, the cost of the IVF cycle will also reduce.

Trial registration number: NOT REQUIRED

Abstract citation ID: dead093.880

P-539 Modelling embryo implantation in vitro using 3D 'instant' assembloids and human blastocysts

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Study question: Can embryo implantation be recapitulated *in vitro* using human blastocysts and a new 3D model of the human endometrium representing the architecture of the tissue?

Summary answer: We present an innovative implantation model consisting of blastocysts attaching on endometrial assembloids containing stromal cells, gland-like epithelial organoids, and overlaid with an epithelial monolayer.

What is known already: A successful pregnancy denotes the implantation of an embryo to the receptive, luminal lining of the uterus, the endometrium. The tissue transforms into the semi-permanent decidua which provides an optimal microenvironment and nourishes a healthy embryo. The transformation of the tissue is driven by a differentiation process, known as decidualisation, which accounts for the change of tissue-resident stromal cells to specialised decidual cells or senescent decidual cells. Simultaneously, endometrial glands of epithelial origin become secretory and provide histotrophic nutrition to the implanting embryo. Perturbations in this process of decidualisation may also result in an implantation failure or a pregnancy loss.

Study design, size, duration: Endometrial 'instant' assembloids, consisting of gland organoids and primary stromal cells in collagen hydrogels, were established from freshly isolated cells from endometrial biopsies. Additional cell types were incorporated including immune and endothelial cells creating a layer of epithelial cells recapitulating the luminal epithelium. Hormonal stimulated assembloids were further co-cultured with human blastocysts at 37 °C, 5% O_2 and 6% CO_2. Attachment of the blastocysts to the luminal epithelium was assessed upon overnight co-culture.

Participants/materials, setting, methods: Endometrial biopsies (n = 3) were obtained from consenting hormonally stimulated, healthy oocyte donors. After informed consent, human embryos (n = 14) were donated to research after 5 years of cryopreservation. Decidualisation was monitored by RT-qPCR analysis using epithelial and stromal cells from undifferentiated or decidualised assembloids. Attachment of the embryos (n = 11) to the model was assessed by disturbance of the liquid and this was further confirmed by immunofluorescence antibody labelling.

Main results and the role of chance: 'Instant' assembloids mimic the endometrium morphologically and functionally since hormonal stimulation results in the induction of decidual stromal (*PRL*, *SCARA5* and *DIO2*) and epithelial (*PAEP* and *SPP1)* marker genes. The presence of immune and endothelial cells was confirmed with the induction of *IL2RB* and *VWF* respectively. Immunofluorescent antibody labelling confirmed the similarity in morphology of the model to the human endometrium. Aiming to mimic implantation, assembloids underwent a 3-day long differentiation in a chemically defined medium, 8-bromo-cAMP, estradiol, and a progestin, that induced *in vitro* decidualisation. Following the 3-day treatment, instant assembloids were overlaid with single epithelial cells, isolated from instant assembloids which had undergone prolonged decidualisation treatment to imitate the physiology of the endometrium. Following a 24-hour long incubation period, human hatched day 6 blastocysts were positioned on the top epithelial layer of the instant assembloid. Immunofluorescent antibody labelling using makers for the epiblast (NANOG), primitive endoderm (GATA4) and epithelial cells (E-cadherin) were used to visualise the co-culture. Our findings signify the newly established model as the most advanced method to study embryo implantation *in vitro*. The uniqueness of the model is owned by the preservation of endometrial cell types and the potential to model disease through patient specificity.

Limitations, reasons for caution: The proposed system is an innovative method to recapitulate apposition and adhesion during implantation. Our results are preliminary and further experiments will be performed to increase our repeats and therefore reliability. Moreover, due to the complex nature of the co-culture system, imaging remains a challenge that requires further optimisation.

Wider implications of the findings: The embryo-assembloid co-culture system may provide a useful tool to aid our understanding of the mechanisms of implantation. Future work will include the labelling of the embryos with a nuclear dye to enable tracking using time-lapse microscopy. We further aim to expand our protocol to study the process of invasion.

Trial registration number: N/A

Abstract citation ID: dead093.881

P-540 Improper placentation due to skewed Oct4-Cdx2 expression may be a leading factor in idiopathic recurrent pregnancy loss (RPL) cases from Assam, India.

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Study question: Does the differential expression of key embryonic stem cell (ESC) markers due to upstream signaling cascade(s) deregulation influence RPL pathogenesis in the studied population?

Summary answer: Imbalanced Oct4-Cdx2 expression linked differential ESCs polarization due to β-catenin signaling deregulation during the peri-implantation period impacts RPL pathogenesis in the studied population.

What is known already: RPL affects approximately 2-5% of women globally and around 7% Indian women in reproductive age group; with 50% of case etiology being unclear. Proper placentation is indispensable for establishment, continuation, and success of a pregnancy; and it depends largely on a healthy balance between apoptosis and stem cell differentiation that aids proper trophoblast invasion. These differentiation events are tightly controlled by the interplay of oxygen tension, hormones, growth factors, other signaling molecules and most importantly by transcription factors including those deciding the stem cell lineage like Oct4, NANOG, and Cdx2, wherein, β-catenin is known to enhance Oct-4 activity in ESCs.

Study design, size, duration: The sample size was predicted using *Raosoft* software utilizing the prevalence rate of RPL. Overall 87 women with 2 or more pregnancy losses before completion of 20 weeks of gestation were enrolled as RPL cases with informed consent, whereas, for the control group, 107 women volunteers undergoing medical termination of pregnancy (MTP) were enrolled as gestation-week-matched comparative study group for experimental data analysis.

Participants/materials, setting, methods: Products of conception (POC) was collected from the MTP and RPL cases. POCs were collected in 2vials for every subject: (i) 4% buffered formalin for fixation and preparation of microscopic slides for immunofluorescence (IF) based protein expression of Oct4 and Cdx2; (ii) RNA later solution for site specific transcriptional analysis of *Oct4, NANOG, Cdx2*, and *β-catenin* utilizing Realtime PCR. The difference in expression was analyzed using SPSS statistical software.

Main results and the role of chance: IF based site specific protein expression data confirmed higher expression of OCT4 in RPL cases compared to MTP controls, whereas CDX2 expression was found to be low in RPL compared to MTP subjects. The Realtime PCR amplification of cDNA using *β-actin* as internal normalization control for *Oct4* (25.76 ± 10.17 folds) and *NANOG* (18.32 ± 6.67folds) showed increased expression whereas the *Cdx2* expression was downregulated (0.487 ± 0.186 folds) in RPL cases compared to MTP controls, indicating a shift towards pluripotency which is a non-favorable environment for the ongoing pregnancy since cell polarization for initiation of differentiation to different cell lineages is required at that time of gestation. The cdx2 and oct4 expression correlated inversely and significantly {Pearson's correlation= −0.838, p = 0.009; Spearman's rho= −0.786; p = 0.016}. The realtime PCR amplification of *β-catenin*, the key molecule involved in direct enhancing of *Oct4* expression during gestation was found to

be increased in RPL cases compared to MTP controls. Statistically, the *β-catenin-Oct4* expression correlated positively significantly.

Limitations, reasons for caution: Being a patient based study, the present study is limited to being an associative study only; and therefore secondary validation of the data for causal association using cell lines or suitable model system futuristically may add valuable scientific inputs and aid clinical intervention modalities for controlling RPL.

Wider implications of the findings: As the majority of RPL pathogenesis etiology is undefined limiting the possibilities of clinical interventions therefore we presume the present data can layout possible therapeutic interventions by regulating key regulators influencing skewed ESC profile aiding proper placentation leading to successful pregnancy in a subpopulation of cases with RPL history.

Trial registration number: Not applicable

Abstract citation ID: dead093.882

P-541 Does the IVF embryo remain at the site of deposition in successful IVF cycles?

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Study question: Does the placement of the IVF embryo differ from the implantation site?

Summary answer: At five weeks the implanted embryo has not moved in the lateral plane of the endometrial cavity but is slightly lower in the vertical plane.

What is known already: We previously showed that vertical placement of embryos approximately 9 mm from the fundus and equidistant from the tubal ostia has the highest implantation rate. Whether the embryo migrates from the point where the droplet is deposited before implantation is controversial. We used 3D ultrasound to compare the point of placement with the location of the gestational sac (GS) at 5 weeks gestational age (GA).

Study design, size, duration: Prospective analysis of 292 transfers of single egg donation blastocysts (2018-2020) and 139 resultant pregnancies in which the position of the GS in the coronal plane at 5 weeks was compared to that of the embryo flash immediately post transfer. A Voluson 6 with a 4D RAB4-8 transabdominal probe imaged the embryo transfer and a 4D RIC-5-9 transvaginal probe imaged the pregnancy scan (GE Healthcare, Austria).

Participants/materials, setting, methods: Consecutive patients with a normal uterine cavity undergoing egg donation in a single private IVF Center. Embryo transfers were performed under 2D US guidance using a soft Wallace Catheter. A 3D volume of the uterus was acquired immediately post-transfer. A second 3D scan was performed at 5 weeks GA. Distances of the embryo flash and the center of GS from the fundus, ostia and internal cervical os (ICO) were recorded and compared using SPSS.

Main results and the role of chance: Of 292 transfer procedures, 139 pregnancies who had the first ultrasound in our Center were included and analysed. The mean distance between the embryo flash and the uterine fundus was significantly less than the distance between the center of the GS and the uterine fundus [(9,49 ± 3,03 vs 11,02 ± 3,9 mm respectively ($p < 0,05$)]. The mean distances between the embryo flash and the right and left ostium were 16,66 ± 4,96 and 16,69 ± 4,61 mm respectively, with no significant differences from the same distances taken from the center of the GS.

Limitations, reasons for caution: As the image is of the embryo flash and not the embryo, this might have been misleading.

Wider implications of the findings: These findings extend and support the proposal of an optimal point of embryo placement for pregnancy. The apparent vertical difference in the implantation of the embryo could be due to uterine growth or it could be consequence of uterine peristalsis. This bears further study.

Trial registration number: not applicable

Abstract citation ID: dead093.883

P-542 How should we prepare the endometrium in euploid embryo transfer cycles? Modified natural or artificial endometrial preparation?

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Study question: Does modified natural endometrial preparation in single euploid frozen-thawed embryo transfer (FET) cycles affect chance of pregnancy and risk of miscarriage compared to artificial?

Summary answer: The rate of clinical pregnancy and live birth increases while the rate of miscarriage decreases in modified natural FET cycles compared to artificial FET cycles.

What is known already: Corpus luteum produces growth factors, angiogenic factors and vasoactive substances as well as hormones. These substances play role for initial placentation. Early maternal endocrine milieu resulting exogenous estrogen and progesterone could damage placentation, which may in turn cause miscarriage. High estradiol levels might also be responsible for decreased pregnancy rates (Wu et al 2021).

Study design, size, duration: This retrospective, single center study evaluated 1890 frozen single euploid embryo transfer cycles in women between 20-43 years old from January 2017 to September 2021. The study is based on data obtained from Istanbul Memorial Hospital, ART and Reproductive Genetics Center. FET cycles were analyzed in two groups according to different endometrial preparation protocol. Group A (n = 1335): modified natural FET cycles (mNat/FET) and Group B (n = 555): artificial FET cycles (AC-FET).

Participants/materials, setting, methods: Only good prognosis patients with ages between 20-43 years old were included. Exclusion criterias were women age 44 and above, recurrent abortion history, BMI>35 kg/m ^{2}, endometrial factor, uterin factor (adenomyosis, mullerian anomaly) history. NGS was used to study trophoectoderm biopsy material in all cases. Patients demographics, cycle characteristics and pregnancy outcomes were analyzed.

Main results and the role of chance: We analyzed the effect of endometrial preparation methods on pregnancy outcomes between the two groups after excluding confounding factors in single euploid embryo transfer cycles. There were no significant differences in patient demographics and cycle characteristics such as age, body-mass index, infertility duration, previous IVF attempts, AMH level, daily gonadotropin dosage used, number of oocytes obtained, mature oocytes and fertilized oocytes between the two groups. There was no significant difference between the morphologic grading of the transferred embryos in both groups. However, all pregnancy outcomes were statistically significantly different in two groups. In group A, biochemical pregnancy rate (76.9% vs. 73.9%, p:0.04), clinical pregnancy rate (71% vs. 65.7%, $p < 0.01$), live birth rate (65.4% vs. 50.5%, $p < 0.01$) were significantly higher than in group B. Also, biochemical pregnancy loss rate (7.7% vs. 11%, p:0.01), clinical pregnancy loss rate (6.7% vs. 21%, $p < 0.01$), second trimester pregnancy loss rate (0.8% vs. 1.5%, p:0.04) were significantly lower compared to group B.

Limitations, reasons for caution: The limitation of our study include the fact that this was a retrospective analysis. Prospective randomised studies are necessary to evaluate the differences between the two groups according to pregnancy outcomes.

Wider implications of the findings: This study shows that pregnancy outcomes are better in patients undergoing mNat/FET even after controlling for confounding factors when comparing mNat/FET and AC-FET in single euploid FET cycles. As a result, in appropriate patients, mNat/FET with higher pregnancy rate and live birth rate should be preferred as much as feasible.

Trial registration number: Not applicable

Abstract citation ID: dead093.884

P-543 Higher maternal age alters the insulin/IGF system and intracellular lipid metabolism of the endometrium and in the preimplantation embryo – insights from the rabbit model.

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Study question: Our aim was to analyse the embryo-maternal-interaction, focusing on the insulin/IGF-system and lipid metabolism on day 6 of pregnancy in reproductive young and old rabbits.

Summary answer: Advanced maternal age has a decisive effect on maternal and embryonic insulin/IGF-signalling and lipid metabolism, leading to higher embryo loss already during preimplantation period.

What is known already: The reproductive potential in women declines with age. Molecular and biochemical mechanisms involved in age-related infertility and their impact on embryo-maternal-interaction during early pregnancy are still not completely understood. However, establishment of a successful pregnancy depends on the physiological condition of the mother and a continuous dialogue with the developing embryo. The insulin/IGF-system plays a pivotal role in this embryo-maternal crosstalk, connecting maternal insulin/IGF with embryonic metabolism, cell proliferation and differentiation.

Study design, size, duration: We used the rabbit as reproductive model to investigate maternal age-related alterations in embryo-maternal-interaction during preimplantation period. Compared to humans, the rabbit shares high similarities in early embryo development, gastrulation and lipid metabolism (DOI: 10.1530/REP-12-0091 and DOI: 10.1093/ajcn/62.2.458S). A total of 50 young (16-20 weeks) and 31 old (<108 weeks) sexually mature, female rabbits were used for analysis at day 6 post coitum.

Participants/materials, setting, methods: We measured mRNA and protein levels of target genes of the insulin/IGF system and lipid metabolism by quantitative PCR, Western Blot and ELISA. Therefore, maternal blood, endometrium and blastocysts were analysed from reproductive young (16 to 20 weeks) and old (over 108 weeks) rabbits on day 6 of pregnancy. Blastocysts and ovulation points were counted and only blastocysts at the pre-gastrulating stage 1, separated in embryoblast (EB) and trophoblast (TB), were used for further analyses.

Main results and the role of chance: At day 6 of pregnancy, reproductive old rabbits had a lower amount of embryos. Maternal serum insulin and IGF levels were reduced in reproductive old rabbits, accompanied by a paracrine upregulation of IGF1 and its receptors in the endometrium. Preimplantation blastocysts adapted to hormonal changes by reducing IGF1 and IGF2 levels in both embryonic compartments, EB and TB, while the expression of embryonic IGF receptors was unchanged.

Furthermore, an increase of fatty acids metabolism was observed in the ageing endometrium, indicated by the higher level of carnitine palmitoyltransferase 1 B (CPT1B) and elevated expression of fatty acid binding and transport proteins. These results are in line with lower level of fatty acid synthesis (FASN) in the endometrium from old, gravid rabbits, the key enzyme of fatty acid synthesis. Embryonic fatty acid uptake and b-oxidation were increased in EB and TB, too. The observed changes in embryonic and maternal lipid metabolism are caused by the alterations of the transcription factors cAMP-responsive element binding protein (CREB) and peroxisome proliferator-activated receptor (PPAR) α and γ in endometrium and embryos from reproductive old rabbits.

Limitations, reasons for caution: Rabbit embryogenesis reflects human development only during blastocysts formation. Therefore, statements are limited to the embryogenesis stages investigated.

Wider implications of the findings: The results of the current study are in accordance with the common literature, showing that advanced maternal age affects developmental competence of embryos. Our study points out that advanced maternal age alters lipid metabolism and insulin/IGF-signalling, which are two crucial components in embryo-maternal-interaction.

Trial registration number: not applicable

Abstract citation ID: dead093.885
P-545 Efficacy, safety and acceptability of a vaginal tampon for the collection of biospecimen samples: a systematic review and meta-analysis

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Study question: Are self-collected vaginal tampons, as a biospecimen collection tool, comparable to current gold-standard methods in their efficacy, safety and acceptability?

Summary answer: Tampon efficacy for detection of gynaecological abnormalities was comparable to that of gold-standard methods in addition to being widely accepted with minimal reported adverse effects.

What is known already: Women's services have poor attendance. Fear and embarrassment surrounding gynaecological health remain prevalent. Numerous long term sequalae can result from lack of gynaecological care include pelvic inflammatory disease, infertility and malignancy.

Self-collection vaginal tampon samples have the potential to increase screening and diagnosis rates for multiple gynaecological conditions. Although the menstrual tampon as a biospecimen collection device has been demonstrated to be able to identify various gynaecological abnormalities, currently, possibly due to its novelty and lack of information on validity, they are not routinely used.

Study design, size, duration: Systematic review was conducted following PRISMA guidelines and prospectively registered with PROSPERO (*CRD42022374857*). A primary search through online databases was conducted, identifying 151 studies. Following this, title and abstract screening, full-text review and forward and backward citation searching were conducted in respective order, leading to a total of 92 studies for analysis.

Participants/materials, setting, methods: PubMed, MEDLINE, Cochrane Library, Scopus, and Web of Science were searched for eligible studies. All primary studies reporting the acceptability, efficacy or safety of vaginal tampons as a biospecimen collection device in comparison to other conventional methods of collection were included. Data was stratified based on which quantitative and/or qualitative variables were reported, baseline characteristics, and the abnormality being screened for. The Modified Newcastle Ottawa scale was used to assess risk of bias.

Main results and the role of chance: This systematic search identified 92 eligible studies, exploring HIV, HPV, HSV, Chlamydia trachomatis, Neisseria gonorrhoeae, cervical cancer, endometrial cancer, bacterial vaginosis, Candida albicans, Actinomyces israelii, Gardnerella vaginalis, Atopobium vaginae, and Trichomonas vaginalis. Menstrual tampons were compared to both clinician and self-collected samples including an endocervical swab, cervical brushing, cervicovaginal lavage, various tissue biopsies and urine samples.

Detection of most gynaecological abnormalities did not profoundly vary between tampon collection versus gold-standard method. Self- collected tampon specimens were widely accepted with factors including lower pain scores, a greater willingness to use, convenient accessibility (especially in developing areas), and avoidance of embarrassment being reported.

Very few papers reported adverse side effects and in those that did, vaginal tampon collection methods were regarded as less traumatic with a decreased likelihood of bleeding as compared to other methods including cervical swabs.

Limitations, reasons for caution: The effect of tampon sampling prior to other specimen collection methods could contribute to results that favoured tampon collection. A biopsy was not performed in some studies, leading to an unknown true disease status.

Wider implications of the findings: The use of menstrual tampons in clinical practice may help overcome barriers to accessing healthcare and participation in screening, necessary for preventing avoidable gynaecological conditions and their associated long-term complications. Further research is required to explore the optimisation of tampon specimen collection, including transportation and storage requirements.

Trial registration number: Not applicable

Abstract citation ID: dead093.886
P-546 History of caesarean births and risk of miscarriage - secondary analysis of three randomised controlled trials

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Study question: Does the history of caesarean births influence miscarriage risk in subsequent pregnancies?

Summary answer: In women with a history of 3 or more miscarriage(s), the subsequent miscarriage risk may increase with the presence and number of previous caesarean births.

What is known already: History of caesarean birth increases the risk of complications in subsequent pregnancies, including risks of spontaneous preterm birth. The evidence of association between caesarean births and subsequent miscarriage risk is scant and conflicting.

Study design, size, duration: We performed a secondary analysis of the dataset from three multicentre, prospective, double-blind, and placebo-controlled randomized trials.

Study registration, duration, size, population, and intervention are as follows:

1) PROMISE; ISRCTN92644181; 2010 - 2013, 826 women with a history of unexplained recurrent miscarriages, progesterone.

2) PRISM; ISRCTN14163439; 2015 - 2017, 4038 women who experienced vaginal bleeding in early pregnancy, progesterone.

3) TABLET; ISRCTN15948785; 2011 - 2016, 940 euthyroid women with thyroid peroxidase antibodies, and miscarriages or infertility history, levothyroxine.

Participants/materials, setting, methods: The primary outcome for this analysis was miscarriage rate, defined as loss of pregnancy <24 weeks of gestation. RR with 95% CI was estimated using the Poisson regression model and adjusted for age, BMI, ethnicity, smoking status, and previous miscarriages. Random-effects two-stage individual participant data meta-analysis approach was used to account for treatment allocation effects. Subgroup analysis was conducted in women with a history of repeated miscarriages (3 or more) at randomisation.

Main results and the role of chance: After excluding pregnancies with incomplete data, we included 5,039 pregnancies in the final analysis. The overall miscarriage rate was 24.9% (1256/5039).

In the non-specific population of women, there was no association between the history of caesarean births and subsequent miscarriage risk (aRR 1.00, 95% CI 0.86 to 1.17; 5,039 pregnancies).

In contrast, for the subgroup of women with a history of 3 or more miscarriage(s) at randomisation, the miscarriage rate appears to increase with the history of caesarean births, although with some degree of statistical uncertainty (aRR 1.10, 95% CI 0.97 to 1.26; 1172 pregnancies). The association became significant when the analysis was categorised according to the number of previous caesarean births. With 1 previous caesarean birth, the miscarriage risk increased by 3% (aRR 1.03, 95% CI 1.00 to 1.06; 1080 pregnancies). This risk was increased by 37% with 2 previous caesarean births (aRR 1.37, 95% CI 1.03 to 1.83; 971 pregnancies), therefore indicating the presence of a biological gradient of effect which increases our confidence in the above findings.

The history of previous caesarean births may be a valid prognostic risk factor for future miscarriage risk in women with a history of recurrent miscarriage.

Limitations, reasons for caution: Further analysis by the gestation at miscarriage (early vs. late), the type (elective vs. emergency), indication or cervical dilatation status at caesarean births is required to understand the possible mechanism behind this association and to better identify women at increased risk of miscarriage(s) related to caesarean births.

Wider implications of the findings: Our findings suggest that a history of caesarean births may be associated with a small increased risk of subsequent miscarriage risk in a subgroup of women with a history of recurrent miscarriage(s). This information may assist women and healthcare providers to reach more informed decisions regarding the mode of delivery.

Trial registration number: The study is registered with the OSF registry (ve5j4).

Abstract citation ID: dead093.887

P-547 Recurrent miscarriage and the risks of stroke and type II diabetes later in life: a Mendelian randomization analysis

Abstract withdrawn by the authors

Abstract citation ID: dead093.888

P-548 Randomized controlled trial evaluating efficacy of autologous platelet –rich plasma therapy for patients with recurrent implantation failure in frozen-thawed embryo transfer after PGT-A

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Study question: To evaluate if the intrauterine perfusion with autologous PRP enhances frozen-thawed embryo transfer effectiveness in patients with recurrent implantation failure (RIF) after PGT-A.

Summary answer: The intrauterine perfusion (IP) with autologous PRP should be considered perspective, effective and safe therapeutic tool for patients with RIF

What is known already: Despite keen development of embryology, routine implementation of preimplantation genetic testing and assessment of endometrial receptivity, recurrent implantation failure (RIF) remains a challenging dilemma for fertility specialists. As it contains significant growth factors involved in delicate process of implantation, platelet –rich plasma (PRP) therapy should promote endometrial receptivity and improve assisted reproductive technology outcomes. Moreover, being autologous, PRP is not expected to trigger adverse immune event in the patient and is therefore perceived to be therapeutically safe. Pilot study conclusions have been inspiring however there is strong need for randomized controlled trials.

Study design, size, duration: Study type: Interventional.

Study design: randomized controlled study
Intervention Model: Parallel Assignment
Masking: open-label
Study size: 232
Duration: 40 months (July, 2019 - October, 2022)
Multicenter trial: 3 ART clinics

After obtaining institutional review board approval, 232 women aged 28 - 42 years were involved.

Matching criteria: RIF, normal karyotype, absence of uterine factors of infertility, availability of euploid embryos after PGT-A

Participants/materials, setting, methods: 2 groups of patients: study group (N = 118): single intrauterine perfusion with 2.0 ml of PRP on day 10 -11 of menstrual cycle; Control group (N = 114): no therapy. Endometrium preparation was executed according to standardized protocol of hormone replacement therapy. PRP preparation was carried using patented tubes. In all cases SET was performed. Primary outcome measure was clinical pregnancy rate. Secondary outcome measures were pregnancy loss rate, endometrial thickness and adverse event.

Main results and the role of chance: The clinical pregnancy rate was higher in the study group (63.55% *vs* 38.59%) (χ^2=14.462, OR = 2.775, 95% CI 1.630 - 4.722, $p < 0{,}001$). The endometrium thickness before intervention didn't between groups (7.3 *vs* 7.4 mm), however, endometrium thickness measured just before embryo transfer was significantly higher in the study group (10.5 *vs* 8.4 mm, Student's t-test value: 11.87; number of degrees of freedom f = 230; Critical value of Student's t-test=1.972, at significance level $\alpha = 0.05$). The pregnancy rate loss did not differ between groups (χ^2=0.033, OR = 0.908, 95% CI 0.324 – 2.546, $p > 0{,}05$).

No adverse event was noted.

Limitations, reasons for caution: The number of recruited participants was limited. Nevertheless, these data show statistically significant positive effect on ART outcomes. Further investigation and meta-analysis are required.

Wider implications of the findings: According to obtained results intrauterine perfusion with PRP should be recommended in FET cycles in patients with RIF.

Given proven promotion effect on endometrial thickness the intrauterine perfusion (IP) with autologous PRP should be considered perspective and safe management method for patients with thin endometrium.

Trial registration number: 4765

Abstract citation ID: dead093.889

P-549 Endometrial eubiosis vs dysbiosis: what role does the abundance of Lactobacillus play in endometrial receptivity?

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Study question: Is there an influence of Lactobacillus relative abundance on the expression profile of a set of genes related to endometrial receptivity?

Summary answer: The abundance of Lactobacillus genus in endometrium does not affect key genes related to endometrial receptivity determination.

What is known already: Typically, endometrial eubiosis has been determined by a Lactobacillus genus dominant presence (at least 90%), whereas a lower presence of this genus has been considered as endometrial dysbiosis. However, in recent years this belief has been called into question due to the theory that an abundance greater than 50% could already be considered as "Lactobacillus dominance". Nevertheless, there are few studies that analyze the influence of the relative abundance of this genus on endometrial receptivity.

Study design, size, duration: A total of 61 patients (average age= 39.04 years) were included in the study. Patients went through an endometrial biopsy for microbiome testing. Endometrial samples were classified according to their abundance of Lactobacillus genus: 43 samples with <90% (average age= 39.4 years) and 18 samples with=>90% (average age= 38.1 years), and a gene expression analysis of a 48 genes-panel related to endometrial receptivity was performed.

Participants/materials, setting, methods: RT-qPCR was performed for detection of certain microorganisms and pathogens in endometrial biopsies. Samples were selected based on the absence of pathogens and the presence of a regular profile of the rest of microorganisms, and classified according to their abundance of Lactobacillus. Gene expression of 48 genes related to endometrial receptivity was analyzed by microfluidic RT-qPCR. Data was analyzed by Delta-Delta Ct method, and Fold change was determined using Wilcoxon test.

Main results and the role of chance: There are no significant differences ($p > 0.05$) in terms of gene expression profile of a 48 genes set related to endometrial receptivity by the current threshold (Lactobacillus=>90% vs Lactobacillus <90%). According to this result, the abundance of Lactobacillus would not affect the window of implantation. In addition, of total samples with receptivity evaluation (N=49), 76.9% of the Lactobacillus >90% group are receptive, whereas in the case of the Lactobacillus <90% group, the proportion of receptive endometria is 63.8%.

Limitations, reasons for caution: Results could be affected by sample size and by typification, since some Lactobacillus species which are not considered beneficial may be responsible for the genus dominance in samples. In addition, further research on the expression analysis of other genes and the influence of different microorganisms are required.

Wider implications of the findings: Abundance of Lactobacillus in endometrial samples does not seem to affect 48 genes related to receptivity. Endometrium functionality does not have to be related to implantation window, at least based on the abundance of Lactobacillus with a 90% cutoff. It would be necessary to evaluate if pathogens could alter it.

Trial registration number: Not applicable

Abstract citation ID: dead093.890

P-550 Comparison of progesterone levels according to the type of vaginal micronized progesterone used for luteal phase support in artificial-cycles for endometrial preparation in embryo transfer.

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Study question: Are there differences in the ongoing pregnancy rate (OPR) using Cyclogest® versus Progeffik® at a dose of 800 mg daily as luteal phase support?

Summary answer: The administration of Cyclogest® as luteal phase support shows higher ongoing pregnancy rates (OPR) in a cycle of hormone replacement therapy (HRT) with own oocytes

What is known already: The importance of serum progesterone levels around the time of embryo transfer in patients undergoing FET under artificial endometrial preparation has been well established; some studies have shown that optimizing serum progesterone levels on the day before, leads to an improvement in live birth rates (LBR). However, to date, no study has examined which vaginal preparation reports better serum progesterone levels and its impact on OPR.

Study design, size, duration: This prospective randomized clinical trial was performed between September 2019 and June 2022 and enrolled 490 patients scheduled for cryopreserved ET after an artificial endometrial preparation cycle with estradiol valerate and micronized vaginal progesterone. They were divided into two groups, those who used Progeffik® and others with Cyclogest® total dose of 800mg. SPLs were measured the day before (P+4), the day of the transfer (P+5) and the day of BHCG (P+19) and compared the OPR.

Participants/materials, setting, methods: Patients between 18-40 years of age with BMI <30 and triple-layer endometrium > 7,5mm underwent transfer of one blastocysts with own oocytes. The study was carried out in the assisted human reproduction unit of the Virgen de Valme University Hospital (Seville). Ethical approval was granted and formed consent was obtained. The primary endpoint is to assess which vaginal progesterone preparation gives better ongoing pregnancy rates.

Main results and the role of chance: The 484 patients were randomized (1:1) into two groups. Progeffik was administered to 149 patient and Cuyclogest to 335 patient. The characteristics of the patients were comparable between groups with similar causes of fertility and type of IVF. Regarding the results of serum progesterone levels measured according to the type of micronized vaginal progesterone used to support the luteal phase, we obtained for day P+4 (13,69 for Progeffik® vs 15.89 for Cyclogest®), while for day P+5 (11.95 vs 17.69) and on day P+19 (13.69 vs 15.41), the results obtained after the administration of Cyclogest® being significantly higher with a p-value of 0,01. When assessing the ongoing pregnancy rate, they were also significantly higher with the administration of Cyclogest® versus Progeffik® (59.7% vs 48.3%) with a p value of 0,024.

Limitations, reasons for caution: The newborm rate needs to be validated as it has not exceeded 9 months since the study.

Wider implications of the findings: This study demonstrates the statistically significant superiority of Cyclogest-type vaginal micronized progesterone administration over its competitor Progeffik used for luteal phase support, being a more convenient way of administration for women since it's simplicity.

Trial registration number: 0388-N-22

Abstract citation ID: dead093.891

P-551 Association between the length of in vitro culture and mode of ART and the initial se-hCG rise in ongoing singleton pregnancy

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Study question: Does the length in vitro and mode of ART (fresh and frozen cycles) impact the se-hCG rise in cycles with ongoing single implantation pregnancy?

Summary answer: Length in vitro and mode of ART alter the hCG. Blastocysts in FET induced higher hCG than FET clevage stage embryos and fresh blastocyst transfers.

What is known already: Human chorionic gonadotropin (hCG) is produced by placental trophoblasts providing the first measurable sign of pregnancy. Factors, such as multiple pregnancies, advanced maternal age and gender of the fetus are known to influence hCG levels. Other factors such as mode of ART and length in vitro have been considered to alter the hCG kinetics but with conflicting results. Some studies have shown that hCG levels are higher after fresh blastocyst transfer compared with fresh cleavage stage embryo transfer (ET). Other studies found frozen-thawed embryo transfer (FET) results in higher hCG compared with fresh ET, other studies concluded the opposite.

Study design, size, duration: Multicenter historical cohort study based on clinical data. The study included 5271 women undergoing in vitro fertilization (IVF), intracytoplasmic sperm injection (ICSI), insemination (IUI) or FET with single ET followed by hCG measurement day 15-19 after ovulation induction (IVF/ICSI/IUI/natural cycle FET) or progesterone administration (estrogen/progesterone substituted FET cycles). All included cycles resulting in single implantation and ongoing pregnancy verified by ultrasound in week 7-8. Data was collected between January 2014 and December 2021.

Participants/materials, setting, methods: Data was prospectively collected from the Danish Medical Data Centre used by three public fertility clinics, Copenhagen University hospitals. The association between length in vitro (no in vitro period (IUI), day2/3, day5, day6) and mode of ART (IVF/ICSI, FET) and the initial hCG rise was tested both with linear regression and multivariable regression. The results were based on mean serum hCG and presented in percentage with the corresponding 95% confidence interval (CI).

Main results and the role of chance: After accounting for the pre-defined exclusion criteria (i.e. oocyte donation, pre-implantation genetic testing, more than one gestational sac), 5251 cycles were included in the study. 2122 were FET, 2521 were fresh ET and 608 were IUI. The initial hCG rise was overall lower for fresh ET compared to IUI (no in vitro period). For cleavage stage ET, hCG was 18% (95% CI: 13% - 23%, $p < 0.001$) lower, and for blastocyst transfer 23% (95% CI: 18% - 28%, $p < 0.001$) lower. In FET, hCG was 26% (95% CI: 13% - 40%, $p < 0.001$) higher for blastocyst transfer compared to cleavage stage ET. When comparing blastocyst transfer in FET vs. fresh ET cycles, hCG was 33% (95% CI: 27% - 45%, $p < 0.001$) higher in FET cycles. Stratifying FET cycles in natural and substituted cycle did not alter the result. All results remained significant after adjusting for referral diagnosis, women age and treatment center.

Limitations, reasons for caution: It cannot be excluded that the higher level of hCG in IUI pregnancies is due to an additional vanished implantation.

Wider implications of the findings: The mechanisms in the embryo and endometrial interplay are far from understood. The present data add to the knowledge regarding this, pointing towards alteration in the implantation window in fresh stimulated cycles. Studies following the more detailed hCG rise are needed for further elucidating how ART affect the early implantation.

Trial registration number: Journal-nr.: 21019857

Abstract citation ID: dead093.892

P-552 Pilot study to determine efficacy of L. rhamnosus BPL205, L. plantarum BPL207 and L.crispatus BPL209 in normalizing the vaginal environment (study PROSALVAG)

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Study question: Can the use of orally *L.rhamnosus* PBL205, *L. plantarum* BPL207 and *L.crispatus* BPL209 reestablish the vaginal environment?

Summary answer: The use of oral probiotics changes the infectious microbiological vaginal environment to a healthy one, consequently the vaginal pH is reestablished.

What is known already: The normal flora of the reproductive tract includes a variety of *Lactobacillus* species that promote a healthy environment for the embryo during the preimplantation period, so that *Lactobacillus* spp. are considered to promote ideal environment for implantation. Live birth rate correlates directly with the presence of *Lactobacillus* and inversely with the existence of bacterial vaginosis.

Alterations of the vaginal flora such as bacterial vaginosis are related to an increased risk of miscarriage. It has been shown that women with an unbalanced vaginal microbiome are 1.4 times less likely to achieve pregnancy after IVF treatment compared to women with normal microbiome.

Study design, size, duration: Prospective pilot study to assess the effect of 10 weeks of use of an oral probiotic containing *Lactobacillus rhamnosus* BPL205, *Lactobacilus plantarum* BPL 207 and *Lactobacillus crispatus* BPL209 supplemented with vitamin D, in vaginal health reestablishment. 21 women assisting to a fertility clinic and with indication of vaginal microbiota restoration and low number of vaginal *Lactobacillus* were recruited. After 5 and 10 weeks of supplementation, the reestablishment of vaginal health was study.

Participants/materials, setting, methods: Women with indication of vaginal microbiota restoration, low number of vaginal *Lactobacillus* and with the informed consent signed, were asked to take a nutraceutical with probiotic and vitamin D once a day for 10 weeks. Cytology, vaginal pH and pain were analyzed at 5 and 10 weeks. In those patients with some kind of infection diagnosed the levels of TNF-α was analyzed after 5 weeks of probiotic use.

Main results and the role of chance: All patients started the study with vaginal pH > 4,5. After the use of oral probiotic containing *Lactobacillus rhamnosus* BPL205, *Lactobacilus plantarum* BPL 207 and *Lactobacillus crispatus* BPL209 supplemented with vitamin D (oral probiotic) during 5 weeks, 66,7% of the patients obtained a pH < 4,5. After the use of oral probiotic during 10 weeks all patients achieve a pH < 4,5.

At the beginning of the study all patients result in yeast or vaginosis profile in their cytology, in except one. After 5 weeks with oral probiotic, 73,7% of them turns into a healthy vaginal flora essential to control pathogen proliferation (Döderlein flora: more of two hundred species with Lactobacillus predominance). After 10 weeks this percentage increase to 89,5%.

There is a statistically significant reduction in Menstrual pain from an intensity of 6,1 ± 1,8 (mean ± standard deviation) to an intensity of 3,8 ± 3,5 at week 5 and to a 1,5 ± 3,0 at week 10 ($p = 0,01$ and $P < 0,0001$ respectively).

In patients diagnosed with chronic endometritis at the beginning of the study ($n = 5$) TNF-α levels decrease from 8,14 ± 1,8 to 5,59 ± 2,3 after 10 weeks using the oral probiotic in study ($p = 0,02$).

Limitations, reasons for caution: The main limitation was the number of patients recluted. We have present the preliminary results of a study that

continue recruiting patients in order to diminish the limitation of the study. Also, the number of parameters studied is being increased

Wider implications of the findings: The use of *L.rhamnosus* PBL205, *L. plantarum* BPL207 and *L.crispatus* BPL209 reestablish the healthy vaginal environment of women with an unhealthy ones. This allows better environmen for fertility and t to control pathogen proliferation. An improvement in inflammatory conditions in that patients with chronic endometritis is also observed.

Trial registration number: Not applicable

POSTER VIEWING
NURSING AND MIDWIFERY

Abstract citation ID: dead093.893
P-554 Examination of Mobile Applications Specific to Infertile Couples Through the "APPLICATIONS" Scoring System in Holistic and Utility Perspectives

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Study question: Does mobile applications (apps) developed for infertile couples have a holistic and utility characteristic?

Summary answer: Many mobil apps developed in the field of fertility have limited features and very few relevant, understandable, and holistic apps were present for infertile individuals.

What is known already: In an increasingly technological world, mobile apps offer promising alternatives to support couples' fertility treatment. Infertile couples can use these apps with aim to manage lifestyle, learn about fertility, follow clinical appointments, receive psychological support, or to seek peer support. For increasing efficiency of this applications, holistic approach should be considered. Human is a whole with biological, physiological, psychological, social and spiritual dimensions and in fertility treatment process all dimensions are highly crucial. Thus, the purpose of this study was to evaluate the utility and holistic perspectives of apps developed for infertile couples by using "APPLICATIONS" mobile app scoring system.

Study design, size, duration: The study was a systematic evaluation of apps related to fertility and it was conducted via mobile applications platform. To determine relevant apps search was conducted on Google Play and Apple App Store by using "infertility", "fertility", "in vitro fertilization", "IVF", "sterility" and "fecundity" key words.The search was conducted twice in 1-year intervals, in February 2021 and March 2022. In total from 1917 reached apps; 1841 apps were excluded, and 76 eligible app were evaluated.

Participants/materials, setting, methods: Mobile apps were independently evaluated by two authors via using APPLICATIONS scoring system. The APPLICATIONS scoring system consists objective criteria such as comprehensiveness (holistic approach), fee, subscription, use of evidence-based information, purchasing, connection, advertising, text search area, interplatform suitability, use of images/shapes or videos, and app-specific features are objective scoring criteria and subjective criteria for instance subjective presentation and ease of navigation in the app. Consequently the maximum score of APPLICATIONS scoring system is 17.

Main results and the role of chance: The study reached 1917 applications from the search with its keywords. Seventy-six mobile applications meeting the inclusion criteria were analyzed according to the "APPLICATIONS" scoring system. Of the 76 applications in the analysis, 18 were available only in the Apple App Store (23%), 28 were available only in the Google Play Store (36%), and 30 were available on both platforms (41%). Among analyzed app, we determined that one app received 16 points out of maximum 17 points, and two apps received 15 points each. Also, the lowest scoring app earned 4 points. According to overall composite scores, evaluable apps in our study showed scores in the middle range of possible scores with an average score of 9 out of 17, indicating insufficient quality in mobile apps. Besides that, only six apps (%7.8) meet all holistic comprehensiveness criteria and received full scores. Of 76 mobile applications, 31.5% were developed by a healthcare professional, 31.5% by a software company, and 38% by an unknown entity. In addition, 48% of the applications evaluated were specific to infertile couples, 45% were specific to infertile women, and 7% were specific to infertile men.

Limitations, reasons for caution: Because of the nature of the digital environment, mobile apps are in a continuously changing and rapidly developing internet environment, and currently, newly developed mobile apps were not present in the analysis. This was the limitation of our study.

Wider implications of the findings: Using mobile health apps can help infertile couples to improve treatment outcomes. Healthcare professionals should be aware of the widespread use of apps and correct the faulty practice recommendations that patients may encounter, and couples should be guided to the correct practices.

Trial registration number: not applicable

Abstract citation ID: dead093.894
P-558 The importance of sexual desire for the sexual health of infertile women and men starting their fertility clinic journey

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Study question: Is the sexual desire of infertile women and men correlated with their intercourse frequency and sexual satisfaction?

Summary answer: Both partner's sexual desire is moderately correlated to intercourse frequency and their own sexual satisfaction and weakly correlated to the sexual satisfaction of their partner.

What is known already: Reviews showed that infertile couples have worse sexual health than the general population. Qualitative studies explained that infertile patients feel less attractive and consider intercourse a 'duty', associated with failure. Fertility clinics offer diagnosis and treatments but have yet to start caring for sexual health. The Pleasure&Pregnancy-programme, combining psychosexual education with communication, mindfulness and sensate focus exercises, recently proved to increase the sexual desire of infertile women pursuing natural conception. Whether improving sexual desire can be expected to increase the intercourse frequency and sexual satisfaction of women and men about to start fertility treatment had yet to be explored.

Study design, size, duration: A cross-sectional cohort of 140 heterosexual couples ($n = 280$; response rate 51%) was surveyed between 2019 and 2022. Couples filled out a package questioning their sexual health over the past four weeks at a chosen moment between their first fertility clinic consultation and the end of their diagnostic workup. This package included a questionnaire to be filled out by each partner individually and a couple questionnaire. Non-responders received were reminded.

Participants/materials, setting, methods: Sexual desire and satisfaction of women and men was assessed with subscales of the following valid and reliable questionnaires: Female Sexual Function Index in women (FSFI; the higher, the better) or International Index of Erectile Function in men (IIEF; the higher, the better). Intercourse frequency of couples was assessed with a sexual activity event log. Spearman rho's correlations assessed associations between sexual desire, sexual satisfaction and coital frequency.

Main results and the role of chance: Responding women and men were in their early thirties and had tried to conceive naturally for 18.2 months, on average. The sexual desire of women (and of men was not correlated ($r = 0.116$, $p = 0.178$). Couples had sexual intercourse seven times per month (7.12 ± 4.03), on average. The sexual desire of both women and men was moderately correlated to couple's intercourse frequency (♀: $r = 0.402$, $p < 0.001$; ♂: $r = 0.426$, $p < 0.001$). The sexual desire of women was also moderately correlated to their own sexual satisfaction ($r = 0.481$, $p < 0.001$)

and weakly correlated to the sexual satisfaction of their male partner ($r = 0.270$, $p = 0.001$). The sexual desire of men was significantly but weakly correlated to their own sexual satisfaction ($r = 0.361$, $p < 0.001$) and the sexual satisfaction of their female partner ($r = 0.239$, $p = 0.005$).

Limitations, reasons for caution: The sexual health of the included couples is currently followed-up during fertility treatment. Linear mixed models, taking account of dyads and of multiple assessments, would allow analysing the impact over time of women's sexual desire whilst taking account of the sexual desire of her male partner and vice-versa.

Wider implications of the findings: Sexual desire seems important for a couple's coital frequency and both partner's sexual satisfaction. Examining whether a six-month sexual health programme that improves women's sexual desire, could in the longer term improve intercourse frequency and especially sexual satisfaction or prevent the deterioration thereof would be interesting.

Trial registration number: not applicable

Abstract citation ID: dead093.895
P-559 Attitudes and experiences regarding fertility education among health and physical education teachers and Yogo teachers in upper secondary schools in Japan.

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Study question: What are the attitudes/experiences of Japanese health and physical education teachers (HPETs) and Yogo teachers (YTs) in upper secondary schools (USSs) toward fertility education?

Summary answer: Twenty-three fertility facts were considered; teachers' perceptions of the need to teach USS students were thoroughly high, while teaching experiences were comparatively low.

What is known already: In Japan, in the 2018 commentary on the national curriculum standards for health and physical education, teaching content on fertility was included for the first time. There are very few studies of school-teachers who teach fertility, both nationally and internationally. In Japan, only one study has examined the fertility knowledge of teachers, and their knowledge was found insufficient. No studies have identified the experiences and awareness of HPETs and YTs* toward fertility education in the context of the revision of the curriculum guidelines. *YTs are specially licensed teachers in Japan to facilitate children's development through health education in schools.

Study design, size, duration: This study was designed as a cross-sectional questionnaire survey. The target groups were HPETs and YTs working in all 68 public USSs in Kumamoto, Japan. Questionnaires were mailed to the public USSs in January 2020, and targeted persons were asked to post completed questionnaire in three weeks; 145 HPETs (response rate; 53.3%) and 46 YTs (52.3%) participated in this survey. The study protocol was approved by the Ethics Committee (approval number: 28-10).

Participants/materials, setting, methods: We selected 23 fertility facts. Teachers were asked what USS students should know by graduation, whether the teachers have teaching experience, and how much they understood these fertility facts. Simple aggregation was carried out. To assess the differences between HPETs and YTs, a chi-square analysis and a Mann–Whitney U test were conducted. The significance level was set at 5%.

Main results and the role of chance: In our study, 122 of HPETs (84.1%) were men and all the YT were women. Fertility facts that over 70% of teachers believe USS students should know by graduation concerned "STIs", "smoking", "aging" and "girls' thinness or obesity" as risk factors of infertility, as well as "most fertile period within menstrual cycle", "irregular menstruation", "excessive exercise for girls", "reasons for infertility" and other facts. On "irregular menstruation", "girls' thinness or obesity" and "PCOS and endometriosis" YTs showed greater motivation than HPETs ($p<.05$).For more than half of the fertility facts, significant differences were identified. HPETs were more experienced at teaching about "women's aging" and "men's aging" as infertility risk factors, "reasons for infertility", "frequency of sperm production", "risks of advanced pregnancy", "relationship between advanced pregnancy and perinatal mortality" and other facts than YTs ($p<.01$). Meanwhile, YTs were more experienced to teach as to the effects of "irregular menstruation" and "PCOS and endometriosis" on fertility than HPETs ($p<.01$). Fertility facts that few teachers have taught, despite many teachers believe their students should know by graduation, were "frequency of infertile couple", "the incidence of child's disease by male aging" and "PCOS and endometriosis" and other facts.

Limitations, reasons for caution: The limitations of this study are that participants were all working at public USSs in only one prefecture in Japan, and the return rate was just over 50%. Therefore, caution should be taken when generalising the results to the general population of Japanese HPETs and YTs.

Wider implications of the findings: It was suggested that for HPETs and YTs to be able to conduct fertility education, seminars and teaching materials such as guidebooks and online video materials must enable them to understand fertility facts based on scientific evidence and have an ability to teach them appropriately.

Trial registration number: none

POSTER VIEWING
PSYCHOLOGY AND COUNSELLING

Abstract citation ID: dead093.896
P-561 Perceptions About Infertility and Medically Assisted Reproduction (MAR) in Eastern Europe: Knowledge, Awareness and Concerns by Young Adults (18-30).

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Study question: How do young adults in the four Eastern European countries (North Macedonia, Slovenia, Kosovo and Albania) perceive and understand infertility issues and the MAR techniques?

Summary answer: Overall, young participants in the study in the four countries acknowledged that they are not familiar with MAR techniques available and technical processes involved.

What is known already: There is little comprehensive research about perceptions, knowledge, and concerns among young people about MAR and infertility, and even less in these four countries. The unfamiliarity about MAR and infertility demonstrates that there is little understanding of medical issues and little understanding about the success rates.

Study design, size, duration: Part of the European Project B^2-InF team conducted a multi-country qualitative study in Albania, Kosovo, Slovenia and North Macedonia with young adults (18-30) in 2021 assessing sociocultural, gender and legal perspectives related to MAR and information provided by the MAR clinics. Between 10-15 interviews were conducted in each country. Data was collected in native language, transcribed and translated in English.

Participants/materials, setting, methods: The B^2-InF team carried out and analyzed a total of 50 interviews with young people living in these 4

European countries. A thematic analysis was performed using Atlas.ti software. The study used purposive sampling technique in order to capture heterogeneity of young participants (gender, age, residence, marital status/relationship, sexual orientation, education and religion)

Main results and the role of chance: Young adults perceive infertility as a topic that is not discussed very much in public. The individuals affected by it tend to keep it private, reluctant to discuss it within their social environment which contributes to the taboo of infertility and may limit access to MAR techniques. Despite this, many individuals, male and female, face infertility problems, including data n these countries.

In all four countries, young people agree that infertility imposes great pressure on both males and females. In certain countries, religion affects the use of MAR techniques, whereas LGBT people are faced with stigmatization while using MAR techniques.

Young interviewees reported general knowledge about MAR treatments and specifically, certain techniques they are familiar with, such as in vitro fertilization or artificial insemination. In addition, surrogacy was a process that many participants were familiar with.

However, all young interviewed participants claim that more information about MAR is needed and they are not confident about where they should search for it.

Limitations, reasons for caution: This study is first of its kind in the MAR research body and its results are useful for policy-makers dealing with (in)fertility. However, information provided by the young participants in these 4 countries would serve as an overview of gaps and concerns about MAR techniques.

Wider implications of the findings: The results of this study are used to develop National Guidelines aimed for policy makers and MAR clinics to improve information about infertility among young people.

Trial registration number: not applicable

Abstract citation ID: dead093.897
P-562 Elective egg freezers' disposition decisions: A qualitative study

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Study question: What are the factors that influence elective egg freezers' (EEF) disposition decisions towards their surplus frozen oocytes?

Summary answer: Achieving motherhood or dealing with grief if motherhood was not achieved, the complexities of donating to others, and a lack of information and professional advice.

What is known already: Most women who undergo EEF do not use their oocytes. Consequently, there is an abundant, but unquantified, number of women with surplus oocytes in storage globally. Many women are deciding about the disposition of their surplus oocytes due to storage limits in countries such as Australia, Belgium, Finland, and Taiwan. However, no studies have examined the factors that influence EEF oocyte disposition decisions. Research exploring factors relevant to embryo disposition and planned oocyte donation may not be relevant. Consequently, women are making the challenging and stressful decision regarding the fate of their oocytes with limited research available to support them.

Study design, size, duration: Thirty-one structured interviews took place in Australia between October 2021 and March 2022. Recruitment was via: Facebook (paid advertising, posts on relevant groups and organisation sites), newsletters and emails from universities and professional organizations, emails to eligible patients from an IVF clinic, and snowballing. A reflexive thematic approach was planned; data collection and analysis occurred concurrently. Recruitment occurred until the process of analysis did not identify any new themes and saturation have been reached.

Participants/materials, setting, methods: Eligible participants (EEF with surplus frozen eggs, 18+, living in Australia) were interviewed and included women who had previously made a disposition decision (n = 7), were currently deciding (n = 6), or who not yet considered the decision (n = 18). Interviews took place on recorded teleconference, were transcribed verbatim and anonymised. Transcripts were iteratively coded via NVivo and analysed, and themes developed inductively. The researcher reflected on their subjectivity with co-authors to ensure accuracy and clarity of data interpretation.

Main results and the role of chance: Six inter-related themes were identified related to the decision-making process: 'decisions are dynamic'; 'triggers for the final decision'; 'achieving or not achieving motherhood'; 'conceptualisation of oocytes'; 'the impacts of egg donation on others'; and 'external factors affecting the final disposition outcome'. All women reported a type of trigger 'event' for making a final decision (e.g. completing their family). Women who achieved motherhood were more open to donating their oocytes to others, wanting to share the joy of motherhood, but were concerned about the implications for their child (e.g. donor-conceived half-siblings) and also felt responsibility for potential donor children. Women who did not achieve motherhood were less likely to donate to others due to the grief of not becoming a mother, often feeling alone, misunderstood, and unsupported. Reclaiming oocytes (e.g. taking them home) and closure ceremonies helped some women process their grief. Donating to research was viewed as an altruistic option as oocytes would not be wasted and did not have the "complication" of a genetically linked child. Decisions were often made based on misinformation and a lack of knowledge of the available disposition options and their consequences, with few women seeking professional advice on their decision.

Limitations, reasons for caution: Most participants had not considered the decision and their stated intentions may not reflect their final decision. Women who had previously made disposition decisions were difficult to recruit despite comprehensive study advertising. Other limitations were the use of convenience sampling and conduct of interviews via teleconference (due to COVID).

Wider implications of the findings: Due to a lack of understanding of the disposition options, their impacts, and women not seeking professional advice, decision support (e.g. counselling, decision aids) is suggested. Counselling should occur at least at the beginning and end of the process, address disposition options, impacts, grief, and gaining support from others.

Trial registration number: Not applicable

Abstract citation ID: dead093.898
P-564 factors influencing men's attitudes and behaviours regarding family building decisions: a systematic review and global perspective

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Study question: What factors influence men's attitudes and behaviours regarding family building decisions?

Summary answer: The attitudes and behaviours of men regarding family building decisions are influenced by a combination of social and personal factors.

What is known already: Men have an important role to play in the decision-making process regarding family building. However, research on this topic has historically focused on women. Furthermore, existing research focuses primarily on data from high-income countries with limited perspectives from men from low- and middle-income countries. This study aimed to explore the factors influencing men's attitudes and behaviours regarding family building decisions across low-, middle-, and high-income countries.

Study design, size, duration: A systematic review was conducted via a search on PubMed, Psych Info and Web of Science databases using the following keyword combinations; fertility AND intention OR desire OR pregnancy AND childbearing OR family building OR reproductive decision making AND attitudes OR motivations OR desires OR behaviours AND parenthood OR fatherhood OR men. Study designs were either qualitative, quantitative or mixed-methods.

Participants/materials, setting, methods: Studies were included if they examined men's attitudes and behaviours regarding family building decisions, involved only male participants or male and female participants if the results for male participants were reported separately. Male participants undergoing fertility treatment, participants with or without children, or homosexual participants were included. Studies from any country, published between years 2010-2022, and in English language only were included.

Main results and the role of chance: A comprehensive search yielded 1745 articles, with studies being excluded if they involved female participants only, results were aggregated for studies including male and female participants and studies involving participants undergoing surrogacy or adoption. As a result, 22 studies were included in this review. From the 22 included studies, 2 main themes were derived; personal and social factors. The personal theme consisted of factors at the individual level related to finance, education, health, age, sexuality, masculinity, knowledge and other personal factors. The social theme related to wider issues, including social pressure, social support and marital status. Across included studies, the most common personal factor influencing men's attitudes and behaviours regarding family building decisions was financial issues, that is, being financially stable/secure. The most common social factor across included studies was discovered to be support, that is, receiving support from family, society and workplace. Half of the included studies reported the stability of men's relationship with their partner as a factor that influences their intention for fatherhood. Interestingly, masculinity was a recurring theme, with men reporting fatherhood as being an expression of masculinity and a way to fulfil their masculine roles and identity within their family, society and community.

Limitations, reasons for caution: Of the 22 studies included in this review, 8 of the studies involved young participants of ages ≤25 years, thus results obtained from these studies were not representative of the attitudes and behaviours of all adult men regarding family building decisions.

Wider implications of the findings: This is the first review to include studies of men from a combination of low-, middle- and high-income countries. Understanding men's attitudes and behaviours regarding family building decisions can help raise and promote fertility awareness among men, thereby helping men achieve their desired reproductive intentions.

Trial registration number: not applicable

Abstract citation ID: dead093.899
P-565 Egg donor perceptions of anonymity and ancestry testing in the United States and Spain

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Study question: How do compensated egg donors in two different cultural and regulatory settings view anonymous donation and understand the implications of consumer ancestry testing?

Summary answer: Spanish and U.S. egg donors differ in their desire for anonymity, awareness of consumer ancestry testing, and the implications of ancestry testing for maintaining anonymity.

What is known already: In the literature, many have expressed concern that without the promise of anonymity people would be unwilling to donate eggs and sperm. A related concern is that the rise in consumer ancestry testing will mean the end of anonymous donation and therefore contribute to a reduction in donors.

Study design, size, duration: This is a mixed-methods study drawing upon surveys and interviews with oocyte donors in the United States (341) and Spain (126). The study was conducted between the years of 2018 and 2022 and included participants from multiple fertility clinics throughout Spain and the United States.

Participants/materials, setting, methods: This is a multi-sited study. Participants include current and former compensated oocyte donors who completed an online REDCap survey. Text boxes were provided in the survey so the participant could elaborate where appropriate. A subset of donors (200 U.S. and 76 Spain) in each location agreed to participate in a semi-structured, open-ended interview with one of the investigators. Interviews were conducted in person or over Zoom in the participant's language of choice.

Main results and the role of chance: Of 341 U.S. respondents, nearly two-thirds (214, 63%) preferred that open or known donation rather than anonymous. Of the Spanish respondents, 38% stated they would prefer non-anonymous donation, 50% were unsure, and 11% stated they would not want non-anonymous donation. Both groups, 178 US (52%) and 57 Spain (51.4%), equally expressed a desire to someday meet the people born from their eggs or would be open to contact to share medical information. Of the U.S. donors, only 17 (5%) expressed a desire for no future contact with the people born from their donations, while 9 (8.7%) of the Spanish donors expressed desire for no future contact. U.S. donors were almost unanimously aware of the existence of consumer ancestry testing and 66 (19%) had attempted to use such tests to either find their donor-conceived offspring or make themselves available to be found. Among 111 Spanish respondents, 24 (21.6%) were not aware that consumer ancestry testing exists or that it could be used to find them, but 57 (51.35%) expressed a desire to be found if it were to become more widely used in Spain. Findings indicate that egg donors in both locations are mostly open to the idea of non-anonymous donation.

Limitations, reasons for caution: Study limitations include a potential bias in the survey sample as it is possible that people who participate in research might be more open than those who do not. We attempted to ameliorate this possibility by recruiting participants from a wide range of clinics, practices, and other sources.

Wider implications of the findings: Findings indicate that concerns surrounding the impact of consumer ancestry testing and the loss of anonymity for donors are overestimated. While there are cultural differences surrounding donation in the U.S. and Spain, assumptions surrounding oocyte donors' desires for anonymity are not well-aligned with donor sentiments in either location.

Trial registration number: not applicable

Abstract citation ID: dead093.900
P-566 Is the seminal oxidative stress the mirror of psychological stress perceived by infertile men?

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Study question: To assess the association between anxiety and depression scores and the levels of oxidative stress in seminal plasma of Tunisian infertile men.

Summary answer: Depression in hypofertile men is associated to higher levels of catalase in seminal plasma and thus to oxidative stress.

What is known already: Within the last decades, the knowledge concerning the link between psychological and oxidative stress in infertile men has been surging. In a limited number of studies aiming to elucidate the psychological aspect of male infertility, data related to increased incidence of depression and anxiety have been reported. It has also been reported that anxiety and depression may trigger the production of reactive free oxygen radicals leading to disruption of the balance between free radicals and antioxidants semen properties.

Study design, size, duration: This was a cross-sectional study performed in the Laboratory of Cytogenetics and Reproductive Biology of Fattouma Bourguiba University Teaching Hospital (Monastir Tunisia). A total of 282 patients were assessed for levels of anxiety and depression and evaluated for

semen parameters. Among these, 105 patients were assessed for levels of superoxide dismutase (SOD) and 51 patients were evaluated for the levels of catalase in seminal plasma from September 2022 to January 2023.

Participants/materials, setting, methods: Were included patients addressed for semen quality assessment. All patients with a previously diagnosed psychological disorder, those who presented severe depression and/or anxiety symptoms or had a stressful life event were not included. Informed consent was obtained from all the participants. The subjects were evaluated for anxiety and depression symptoms using the valid Arab version of the HAD (Hospital Anxiety and Depression) scale. Semen analysis and results interpretation were performed according to 2021 WHO guidelines.

Main results and the role of chance: The mean HAD-D (depression) and HAD-A (anxiety) scores were of 6.56 ± 3.07 (IIQ [4-8]) and 7.94 ± 3.73 (IIQ [5-10]) respectively. The mean levels of SOD and catalase were of 42.32 ± 24.08 and 19599.82 ± 12745.36 respectively. The results showed that patients exhibiting elevated HAD-D scores have higher levels of catalase in seminal plasma compared to those with normally ranging HAD-D scores (29856.07 ± 15904.51 VS 17968.14 ± 11564.79 respectively; $p = 0.02$). However, SOD levels were similar between the two groups and no correlation was found between seminal oxidative stress as assessed by catalase and SOD levels and both HAD-D and HAD-A scores.

Limitations, reasons for caution: The main limitation reason could be related to the limited number of patients evaluated for levels of catalase in seminal plasma (N = 51). Furthermore, assessing hormone's levels (LH, FSH and testosterone) would be of great interest in elucidating the implicated pathways leading to oxidative stress and impaired semen quality.

Wider implications of the findings: Our results shed the light on the elevated levels of the catalase in patients with high depression scores. The absence of correlation between antioxidant enzymes and psychological well-being of infertile patients is a reassuring finding. However, awareness and recognition of depressive symptoms in infertile men is crucial when managing infertility.

Trial registration number: not applicable

Abstract citation ID: dead093.901
P-567 The role of self-criticism in the relationship between infertility-related stress and anxiety and depression symptoms in women facing infertility

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Study question: Can self-criticism help to explain the relationship between infertility-related stress and anxiety and depression symptoms in women facing infertility?

Summary answer: Women with higher infertility-related stress presented also with higher anxiety and depression symptoms and their self-criticism attitudes help to explain this relationship.

What is known already: Infertility is a demanding and potentially stress-inducing medical condition, which can trigger anxiety and depression symptoms in women. Feelings of being inadequate, failing to achieve a major life goal, and experiences of shame might develop dysfunctional strategies such as self-criticism attitudes. Self-criticism has been associated with poor mental health outcomes. However, in the field of infertility, the role of self-criticism in the relationship between infertility-related stress and anxiety and depression symptoms has never been explored.

Study design, size, duration: This cross-sectional study was conducted between December 2021 and March 2022. Women (in a heterosexual relationship), having an infertility diagnosis and/or trying to conceive for more than 12 months and aged between 18 and 45 years were invited to participate in the study. Data were collected through an online platform, after the dissemination of the study on social media created specifically for this purpose, through a snowball strategy.

Participants/materials, setting, methods: The sample was composed of 130 women.Participants completed a self-reported questionnaire including demographic, health data, and measures of infertility-related stress (COMPI), self-criticism (FSCRS), and anxiety and depression symptoms (DASS-21). A mediation model using PROCESS was used to test whether the relationship between infertility-related stress and anxiety and depression symptoms is mediated by self-criticism. Psychological support was used as a control variable since preliminary analyses revealed that this variable has a multivariate effect on the outcomes.

Main results and the role of chance: Women were on average 34 years old and the majority were married. Seventy-three percent had already undergone fertility treatments and the most frequent diagnosis was unexplained infertility and female factor. About 30% were having psychological support. The results obtained through the mediation models showed significant direct effects on the relationship between infertility-related stress and anxiety ($b = .13$, SE = .05, $p < .01$) and depression symptoms ($b = .30$, SE = .05, $p < .001$), indicating that higher levels of infertility-related stress are associated with higher levels of anxiety and depression symptoms. Significant indirect effects were also identified between infertility-related stress and depression symptoms (SE = .02; IC de 95 % [.0167, .1124]) and between infertility-related stress and anxiety symptoms (SE = .05; IC de 95 % [.0100, .0906]), through self-criticism showing that this relationship can be explained by self-critical attitudes.

Limitations, reasons for caution: Due to the nature of our sample, the results need to be interpreted with caution. The cross-sectional design does not allow to draw causal directions; further longitudinal studies exploring the role of these variables are needed and exploring the role of possible confounders. Studies including men are needed as well.

Wider implications of the findings: This work highlighted the role of self-criticism as explaining the relationship between infertility-related stress and anxiety and depression symptoms. Psychological support aiming to promote self-compassion (in contrast with self-criticism) attitudes might help to develop more functional strategies and contribute to better mental health outcomes in women facing infertility.

Trial registration number: NA

Abstract citation ID: dead093.902
P-568 Why menopause education is needed

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Study question: What do women know and think about the menopause?

Summary answer: Women reported a lack of their own and their health professional's knowledge of the menopause. Overall they had negative attitudes.

What is known already: Female fertility decline is strongly linked to the age a woman will go through the menopause. The menopause is defined as having been for one year without a period. The menopause causes the end of fertility. But women start to lose their fertility approximately 8-10 years before the menopause. Studies have shown that women are not educated about the menopause, and neither are their health professionals. This lack of education may result in infertility, childlessness, misdiagnosis of symptoms and seriously affect wellbeing and treatment. It is time to find out what women of all ages feel about the menopause.

Study design, size, duration: We conducted a mixed methods study: two anonymous, online surveys using multiple choice and open-ended questions on Qualtrics. One survey was designed for women under 40 years old and the other for women over 40. Both surveys were promoted using the social

media of some of the authors (Twitter, Instagram, Facebook and Linkedin). The under 40s survey was live between 8/2/2022 to 15/3/2022 and the over 40s survey was live between 19/5/2021 and 26/5/2021.

Participants/materials, setting, methods: The study had ethical approval from UCL Research Ethics Committee ID no: 9831/005. The qualitative data was analysed thematically. 738 participants completed the under 40s survey. 2933 completed the over 40s survey and the data was analysed in three groups: 950 perimenopausal women, 934 postmenopausal women and 1049 who classed themselves as other which included those who were neither peri or post menopausal.

Main results and the role of chance: We asked women how informed they were about the menopause. Approximately 45% of women under 40 reported that they were not informed at all. For women over 40, we asked how informed they felt they had been before the age 40; 57.6% of the perimenopausal and 53.5% of the other group answered 'Not informed at all,' which was significantly greater than the post menopause group; 45.2% (422/934) ($p<.05$). Most women were happy about no longer menstruating, although some expressed sadness regarding fertility loss.

Most women thought that the menopause should be taught at school, but over 80% in both surveys had received no menopause education at school themselves. The perimenopause group was significantly more likely to select 'School' (79.2%, 752/950) and 'Apps such as period trackers and fertility apps' (50%, 475/950) than the other two groups ($p<.05$).

In the free text question, across both surveys the main themes were lack of education of the women, and of their health professionals and the negative narrative of the menopause. Women over 40 said that their lack of knowledge had caused many issues as they were often unaware that they were in the perimenopause. They felt their health professionals did not support them.

Limitations, reasons for caution: As the surveys were online and promoted using social media, only certain women in the population had access. The women were mostly highly educated. Women who did not have internet access, social media or did not know English to an adequate level would not have had access to the survey.

Wider implications of the findings: Most women had limited knowledge and negative attitudes towards menopause, leaving them unprepared. Improved menopause education is required to ensure women understand their fertility, to improve perimenopause quality of life and present a more positive narrative of life postmenopause. Health professionals training needs to incorporate education of the menopause.

Trial registration number: NA

Abstract citation ID: dead093.903
P-569 Unraveling parenthood intentions of 1700 adolescents in 2022, Flanders (Belgium)

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Study question: What are the current attitudes in Flemish adolescents aged 15-19 towards having children?

Summary answer: While the majority of adolescents want to have children, 25% have ambiguous feelings about having children and 10% indicate a wish to remain childless.

What is known already: In the past decades, a very positive attitude towards having children has been reported in young people. In the adult population, most people desire to have children or are already parents, but the group of people who choose to remain childfree is increasingly visible and vocal. The current generation of adolescents is more concerned about environmental impacts than previous generations.

Study design, size, duration: An anonymous online survey of multiple choice and open-ended questions was offered to adolescents in Flanders (Belgium). Forty – four schools in Flanders participated and 1700 adolescents completed the questionnaire from February 2022 to June 2022. We performed a qualitative analysis of answers in open ended questions.

Participants/materials, setting, methods: Adolescents aged 15 – 19 years old, attending the last two years of secondary school in Flanders (Belgium). Most pupils had the opportunity to complete the questionnaire during class, others chose to complete the questionnaire in their own free time.

Main results and the role of chance: The majority of pupils would like to have children (60.2%), a considerable proportion is still undecided (24.7%) and 10.8% report they do not want children. Significantly more boys than girls would like to have children (67% versus 61.7%, $p < 0.01$). Having a positive representation of children and the desire to build a family in the future are the most important reasons for wanting children. Adolescents who have ambiguous feelings or who do not want children are rather pragmatic in their decision-making. They may consider factors such as financial stability, career goals and personal development before deciding to have children. Other reasons for having an ambiguous or negative attitude towards parenthood reflect the sense of uncertainty in association with the current global situation and socio-political situation. Our data do not allow us to conclude whether the observed decline in the desire for parenthood is a temporary phenomenon closely linked to current events, or a more permanent one.

Limitations, reasons for caution: Participants were predominantly girls and attended general secondary education. The questionnaire has only been distributed in schools in Flanders and not in the entire country of Belgium due to administrative constraints. Pupils with a positive attitude towards having children may have been more likely to participate in the study.

Wider implications of the findings: Although reproductive intentions can change over the course of a lifetime, our results suggest that the future fertility rate may decline due to an increasing segment of voluntarily childfree people. A balance may be necessary between removing practical barriers toward parenthood, while acknowledging the legitimacy of other life plans.

Trial registration number: not applicable

Abstract citation ID: dead093.904
P-570 Which fertility education interventions have been delivered to adolescents and are effective in increasing fertility knowledge? A systematic review

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Study question: Which fertility education interventions have been delivered to adolescents and are effective in increasing fertility knowledge? A systematic review

Summary answer: Adolescents' fertility knowledge is low, particularly when it comes to individual factors. Educational interventions are perceived as useful and can increase fertility knowledge.

What is known already: The importance of fertility education is now well-recognized, but most research has focused on young and emergent adults. Targeting these populations has often resulted in negative public opinion feedback including pressure to have children, with studies showing that increasing young women's knowledge leads to higher anxiety levels. Despite recent calls for including fertility education within the reproductive health education framework at an age where prevention could be more effective, there are no systematic reviews on the effects of these interventions in adolescents.

Study design, size, duration: We are conducting a living systematic review based on a registered protocol (PROSPERO CRD42022345551, registered 26 September 2022). A literature search from 01.01.2010 to 29.09.2022 and an update has been planned. Databases included ISI Web of Science, PubMed, PsychArticles and PsychInfo. Preferred reporting items for systematic reviews and meta-analyses (PRISMA) guidelines were followed.

Participants/materials, setting, methods: A search was conducted using combinations of MeSH terms and keywords, (e.g. "adolescents"; "fertility knowledge"). Studies in English, Spanish, French and Portuguese, with both qualitative and quantitative methods, were eligible. Two reviewers independently selected studies and extracted data. A third researcher solved conflicts. A narrative synthesis approach was used.

Main results and the role of chance: Our search identified 5127 records after removal of duplicates, and 427 met eligibility criteria for full text analysis. However, <30 were included in the review (update planned). Studies included adolescents from all continents, with most participants from USA. Results revealed that adolescents have a low understanding of fertility risk factors, particularly individual ones when compared to environmental factors. The majority of adolescents agree that fertility knowledge should be included in sex education curricula and perceive this awareness as relevant for future decisions regarding family formation and existent options. The preferred source of information is online digital media, but adolescents acknowledge that information has to be delivered via several sources and that literacy is built through repeated and progressive exposure.

Limitations, reasons for caution: Included studies were limited to those published in peer review journals. The analyzed studies over-represent interventions in Western countries. There was no sufficient data to perform meta-analysis. Although these limitations suggest a cautious approach to data interpretation, the studies found constitute the best available evidence.

Wider implications of the findings: Our findings indicate that fertility education is not included in most reproductive health programs or interventions, and that evidence of effective interventions is scarce. Further prospective multicountry studies including fertility knowledge as main outcome are warranted to allow meta-analysis and compare results concerning preferred learning method and treatment options.

Trial registration number: not applicable

Abstract citation ID: dead093.905
P-571 Endometrial cortisol and estradiol levels as indicators of pregnancy prognosis and their association with State-Trait Anxiety Inventory (STAI) psychological questionnaire punctuations in IVF patients

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Study question: How anxiety and stress questionnaires punctuations and endometrial steroid biosynthesis metabolites levels are associated with embryo implantation and pregnancy outcomes in IVF patients?

Summary answer: Those patients with high cortisol and low estradiol endometrial levels, were stressed according to STAI State punctuations and showed lower rates of implantation and pregnancy.

What is known already: Although IVF treatments are known to be a source of stress, its relevance in reproductive success remains controversial, mainly due to the lack of accurate biomarkers. Steroid hormones, such as progesterone and estrogens, are essential for reproductive physiology, and directly related to common stress biomarkers such as cortisol. Nowadays, clinical stress evaluation is exclusively performed via highly subjective psychological questionnaires, not being standardized in the clinical setting. In this study, we measured endometrial levels of main steroid hormones -including cortisol-, stress psychological questionnaires, and reproductive outcomes for determining the relationship among potential stress biomarkers and their influence on fertility.

Study design, size, duration: In this prospective cohort study, a total of 55 IVF patients were included (<45 years old, no uterine or systemic pathologies and good quality embryos). All endometrial biopsies were collected in mid-secretory phase for metabolites measurement between 2019 and 2021. Also, these patients were psychologically evaluated by stress related questionnaires the same day of biopsy collection or days later in the same cycle. Reproductive outcomes were compared between groups established according to stress biomarkers thresholds.

Participants/materials, setting, methods: Concentration of eleven selected steroid metabolites was measured by ultra-performance liquid chromatography-tandem mass spectrometry. A psychological questionnaire was also assessed to measure patients' stress and anxiety (STAI). According to metabolites' concentration distribution, Barnard's test was applied to compare the proportion of patients with successful and unsuccessful treatments at different thresholds. A Wilcoxon test was performed for mean metabolites' levels comparison between stressed and unstressed patients. A p-value of 0.05 was stablished as significant.

Main results and the role of chance: All patients with high endometrial cortisol levels (>12.5 ng/g; n = 3) failed to implant (100% vs 45.8% non-implanted in > 12.5 ng/g and <12.5 ng/g patients respectively, p-value=0.047), while the totality of patients with high endometrial estradiol levels (>0.9 ng/g; n = 5) achieved embryo implantation and on-going pregnancy (100% vs 50% implanted in > 0.9 ng/g and <0.9 ng/g patients respectively, p-value=0.038). Those patients with high cortisol levels, all not pregnant, had also low estradiol levels and high stress state punctuation (STAI State punctuations $\geq$ 60). Conversely, patients with high estradiol levels, all pregnant, had also low cortisol levels and were not stressed (STAI State punctuations < 60). Stratification of patients into stressed (STAI State>60) and unstressed (STAI State<60) independently of their implantation results, revealed that 27% of stressed women presented high cortisol levels, while 100% of unstressed patients had low cortisol levels. In the same way, 42% of unstressed patients had high estradiol levels, while 100% of stressed patients presented low estradiol values. Hence, mean values differences of cortisol (10.54 vs 3.05 ng/g) and estradiol (0.55 vs 4.10 ng/g) between stressed and unstressed patients were not significant (p-value=0.08862 and p-value=0.2327, respectively). No relevant associations were observed with stress as a trait.

Limitations, reasons for caution: Due to the heterogeneous and non-normal metabolites' concentration distribution among patients, this study would need a largest sample size for having a representative population in the different subgroups. Although we have used molecular and psychological validated stress biomarkers, a causal relationship between stress and reproductive outcomes should be further explored.

Wider implications of the findings: This study highlighted cortisol and estradiol as potential endometrial biomarkers that better reflect pregnancy prognosis. Further investigation is needed to find if other psychological questionnaires molecularly recognize stressed patients as a noninvasive diagnosis associated to reproductive outcomes. These patients would benefit from psychological counseling to improve their reproductive success.

Trial registration number: Not applicable

Abstract citation ID: dead093.906
P-572 The occupational challenges reported by UK embryologists: stress, fatigue, and burnout

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Study question: To determine the prevalence of occupational stress, fatigue, and burnout reported by UK embryologists and their perceived impact of work conditions on wellbeing.

Summary answer: The surveyed UK embryologists reported low somatic symptom severity and moderate perceived stress, but high levels of burnout and overall stressful working conditions.

What is known already: High levels of occupational stress, fatigue, burnout, and occupational health issues have been reported among embryologists in the Spanish, US, and international surveys. These issues were associated with embryologist's occupational challenges and work conditions. Most (58.3%) of the previous UK survey participants reported work-related health issues, including stress/mental health problems (27.8%). However, that study did not evaluate stress and burnout utilizing the same standardized measures as recent US and international surveys, both of which identified considerably higher burnout than the Spanish survey. The present study will address this gap and identify issues that concern UK embryologists using our survey toolkit.

Study design, size, duration: A cross-sectional, mixed methods web-based survey was sent via email to 253 of an estimated ~400 UK embryologists working in Human Fertilisation and Embryology Authority (HFEA)-licensed UK ART/IVF clinics and private practices in January 2023. Participants self-reported their stress levels, fatigue, burnout, and work conditions (cryopreservation, technology, overtime work, doing double-work, employer understanding, etc.).

Participants/materials, setting, methods: Proportions across the Maslach Burnout Inventory-General Survey (MBI-GS), Perceived Stress Scale (PSS), Patient Health Questionnaire (PHQ-15), and a single-item work unit grade (A–F), and customized occupational and sociodemographic questionnaires were calculated using descriptive statistics. Welch's *t*-test and ANOVA to compare PSS and PHQ-15 scores between the groups categorized by occupational questions, and Pearson's correlation coefficients and multivariate analysis to cross-correlate statistically significant and biologically important parameters will be utilized once the survey is closed.

Main results and the role of chance: To date, of 253 embryologists, 104 (41%) completed the survey (mean age 34.3 years, 88% female); 77% worked in private/for-profit, 17% government, 5% corporate, and 1% academic settings. A total of 60% of the respondents reported symptoms of burnout on the MBI exhaustion dimension and 42% on MBI cynicism, and 68% of them reported being unable to cope with their workload. The PSS showed moderate perceived stress, and the PHQ-15 showed low somatic symptom severity, with 38% reporting fatigue. Additionally, 2% experienced constant, 13% high, 33% moderate, 37% mild, and 15% no anxiety. Moreover, 53% reported the current cryopreservation processes caused anxiety, 63% reported technology would ease the burden on the lab staff, and 74% reported technology would lessen their stress as an embryologist. Regarding their workplace environment and culture, 80% reported working overtime, 57% found themselves doing double work due to a lack of technology integrations and analog record, and 80% felt their employers did not understand their occupational challenges. When asked to rate their work unit safety, 38% of embryologists gave their laboratories grade of A (excellent), 50% B (very good), 10% C (acceptable), 3% D (poor), and 0% F (failing).

Limitations, reasons for caution: This study is limited by the self-reporting nature of the data collection via on-line surveys, which precluded interviewing or follow-up questions of embryologists.

Wider implications of the findings: Overall, work-related health issues and occupational challenges reported by UK embryologists align with results from recent surveys and could be addressed by organizational enhancements and technology improvements. Lower levels of job-specific stress and burnout among them compared to their colleagues might be due to HFEA-provided structure/certainty to their professional responsibilities.

Trial registration number: NCT05708963

Abstract citation ID: dead093.907

P-573 What are Greek adults' and teenagers' knowledge and attitudes to having children?

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Study question: What is the level of fertility awareness and attitudes to having children among Greek teenagers and adults?

Summary answer: Fertility awareness among Greek teenagers and adults is limited with fundamental misconceptions which may be defining attitudes and may impact family planning and reproductive autonomy.

What is known already: According to the Organization for Economic Co-operation and Development, Greece is among the countries with the highest maternal age at first birth worldwide, while the total fertility rate has decreased alarmingly. The shift towards delayed parenthood is attributed to the lack of education concerning fertility issues and family planning options within the reproductive age population. This leads to dramatic misinterpretations regarding reproductive dynamic and respective choices. This study aimed to present current data and identify knowledge gaps. This will indicate where future initiatives should be focused to improve fertility awareness and education while respecting reproductive autonomy and individualism.

Study design, size, duration: This was a mixed methods study using an anonymous, online questionnaire. A 41-item questionnaire for adults and a 46–item questionnaire for teenagers were developed originating from a validated questionnaire from a previously published survey conducted in the UK. A total of 780 respondents completed the survey, which was live between the 11th and 26th of May 2022.

Participants/materials, setting, methods: Participants were adults and teenagers aged 17-45, who had not yet had children but wanted to in the future. Online Survey Software & Tools for WEB design was employed to generate a friendly-format questionnaire for the respondents. The methodology employed was via CAWI (Computer Assisted Web Interviews). The questionnaire addressed demographics, knowledge on fertility matters, opinions and attitudes towards childbearing. Respondents were also offered the "prefer not to say", and "don't know" options.

Main results and the role of chance: The ideal age to have the first child differed significantly between men and women (32.33 ± 4.50 vs 30.64 ± 3.94; $p < 0.001$), as did the ideal age to have competed a family (38.71 ± 5.18 vs 36.97 ± 4.71; $p < 0.001$). Teenagers preferred to have completed their family at a younger age than the adults (33.82 ± 5.87 vs 37.64 ± 4.96; $p < 0.001$). The desired children number was 2.30 ± 0.7 for men, 2.37 ± 0.71 for women and 2.38 ± 0.7 for teenagers. One third overestimated dramatically the start of fertility decline identifying it as age 46. Over 50% of men and teenagers were not aware of the timing of a women's fertile window. Women seemed to be more informed on fertility and choose the physician as the educational resource (69%, 261/392), while men were mainly informed from their partners, and choose internet as the educational resource (67%,163/244). Women appeared more concerned with their fertility (191/392 vs 64/244) and felt more pressure to have children compared to men (135/392 vs 54/244; $p = 0.02$) mainly by their family. Interestingly, relating to reasons that my affected the decision to have children, the most common response for women was "I am not financially ready" (45%, 175/392), compared to men's "I am ready to have children now" (39%, 96/244).

Limitations, reasons for caution: The fact that more women than men were included in the teenagers' group posed a limitation. This study portrays knowledge and attitudes of a population of reproductive age that wants to have children, and hence cannot reflect on knowledge and attitudes on fertility of the general population.

Wider implications of the findings: Findings identify misinterpretations that may jeopardize family-planning, and lead to unintentional childlessness and age-related infertility. The desired number of children was greater than the actual number reported by OECD. This data calls the scientific community to enable informed reproductive choices by working interdisciplinary towards all-inclusively educating the general population.

Trial registration number: Not applicable

Abstract citation ID: dead093.908
P-574 Qualitative evaluation of fertility education for emerging adults in Denmark – a pilot study

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Study question: What are emerging adults' attitudes towards fertility education?

Summary answer: Emerging adults find it relevant and important to learn about fertility from health professionals during high school.

What is known already: Women and men in many countries increasingly postpone family building. Studies have shown that people tend to underestimate the decline in fecundity with advancing age, overestimate the success rate of fertility treatment, and have insufficient knowledge on how to protect their fertility potential. Postponement of parenthood may be a consequence of insufficient knowledge about fertility. Fertility awareness interventions take a preventative focus with the goal of reducing future infertility and promoting informed decision-making so that people can meet their family building goals. Studies among emerging young adults in Denmark show they desire fertility education during their youth educational training.

Study design, size, duration: A fertility education intervention was conducted with the aim of teaching 250 emerging adults from high schools about fertility. The intervention was an interactive two-hour educational session provided by health professionals at Rigshospitalet, Copenhagen, Denmark. The participants were educated in female and male fertility, fertility decline, myths about fertility and reproductive sustainability. Around one week later, semi-structured qualitative focus group interviews were conducted with some of the participants to evaluate the intervention.

Participants/materials, setting, methods: The study participants (n = 20) were single or cohabiting men and women from six different high schools in Copenhagen, Denmark. Study participants were between 18 and 19 years old. The six focus group interviews were audiotaped, anonymized and transcribed in full. Data were analyzed using qualitative content analysis following the method by Graneheim and Lundman.

Main results and the role of chance: Overall, the participants found it very important and relevant to learn about fertility, and they wanted to know more about fertility and family building than was possible during this short intervention. The participants wanted to learn about fertility when they were at high school, so they have the information they need prior to starting their family in the future. They saw the importance of understanding the choices they make today and the effects this can have on their future fertility potential. It was important for them that the educational intervention was not a scare campaign, but an open discussion about fertility. Study participants asked for solutions at a structural societal level to make it easier for younger people in the 20'ies to start a family if so desired. Some of the emerging adults found the teaching language was too heteronormative, and this made it difficult for them to see themselves included. Engagement during the intervention and the possibility to ask questions to the teachers was important for the study participants. After the educational intervention, some of the participants started to reflect about their fertility, and some changed their intentions regarding their ideal age to have their first and last child.

Limitations, reasons for caution: The study participants had all chosen to be a part of this study. Attitudes towards family building, how we talk about fertility and family building is associated with societal and cultural context. Hence, emerging adults in other societal and cultural settings may have different needs and opinions on fertility education.

Wider implications of the findings: This study contributes to the understanding and implementation of future fertility awareness educational interventions and campaigns targeted to and effective among emerging adults. Hence, the findings can be useful in the process of increasing fertility awareness in this population.

Trial registration number: N/A

Abstract citation ID: dead093.909
P-575 What do Portuguese women know about fertility preservation options?

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Study question: What do Portuguese women know, and what attitudes do they reveal regarding fertility preservation?

Summary answer: There is a lack of knowledge concerning fertility preservation options and a solid desire to access more information.

What is known already: Fertility preservation techniques increase the possibility of conceiving a biological child later, whether for social or medical reasons, but it should be noted that they do not ensure success. Previous studies showed an insufficient knowledge of the population regarding cryopreservation techniques, their implications, and their benefits. Additionally, a high percentage of participants who do not consider using fertility preservation stated that it might be related to a lack of knowledge regarding fertility issues. It has been pointed out that people would like more information about fertility preservation and consider that reliable sources of information should be available to the public.

Study design, size, duration: Cross-sectional study. Participants' recruitment was set through online advertisement, using social media platforms and private messages, and participants were solicited to share the study link with two more women (Exponential Non-Discriminative Snowball Sampling method). The online advertisement comprised detailed information about the study's aims and procedures, inclusion criteria, and the voluntary and anonymous nature of the participation. Data collection took place online between March and May 2022.

Participants/materials, setting, methods: 257 women aged 18-45. Participants completed a 9-items questionnaire. These questions addressed: a) knowledge concerning fertility preservation (e.g., Are you aware of the existence of any of the following fertility preservation options?); b) attitudes towards fertility preservation (e.g., Would you consider any of the following fertility preservation techniques?); and c) whether they would like to get more information on this topic (e.g., Would you like to receive more information about fertility preservation options?).

Main results and the role of chance: Eighty-eight (34.2%) participants were aware of the oocytes and embryos cryopreservation techniques, 60(23.3%) only knew the oocytes cryopreservation, 58(22.6%) were aware of oocytes, embryos, and ovarian tissue cryopreservation, 37(14.4%) did not know any of the previously mentioned techniques. Most women (n = 177; 68.9%) were not actively considering fertility preservation, 36(14.0%) would consider oocyte cryopreservation, 34(13.2%) would be willing to pursue it, but would need more information, 9(3.5%) would consider embryos cryopreservation and 1(0.4%) would consider ovarian tissue cryopreservation. Of those contemplating fertility preservation, 45(17.5%) would do it to prevent the effect of age on fertility, 21(8.2%) would do it due to the absence of a

partner. Regarding the concerns about fertility preservation, the participants were worried about the costs (n = 26; 10.1%), age (n = 25; 9.7%), hormonal injections and other fertility drugs (n = 10; 3.9%) and the desire for spontaneous pregnancy (n = 8; 3.1%). The participants not considering preserving their fertility mentioned the main reasons were that they never thought about it (n = 56; 21.8%), did not want to get pregnant (n = 34; 13.2%), age (n = 32; 12.5%), the desire for spontaneous pregnancy (n = 28; 10.9%) and the costs (n = 16; 6.2%). Most participants agreed that fertility and fertility preservation information should be provided during medical consultations or at school.

Limitations, reasons for caution: The recruitment and data collection process (social media and online survey) have limitations, such as sampling bias, self-selection concerns, or under-representation of the population (e.g., exclusion of participants not using social media or online platforms), limiting the chance of making generalisations.

Wider implications of the findings: Making available more information regarding fertility preservation may warrant that more women have the opportunity to consider this option and make informed decisions regarding their reproductive life.

Trial registration number: not applicable

Abstract citation ID: dead093.910
P-576 The perspective of patients and company leaders regarding fertility support in the workplace in Portugal

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Study question: Do Portuguese workers have fertility-friendly policies or initiatives in the workplace to help and support them reconcile work with their reproductive struggles and choices?

Summary answer: Most workers are not receiving adequate support. There's a need to educate Portuguese policymakers regarding reproductive struggles and the possible benefits of implementing fertility-friendly policies.

What is known already: With the European population ageing and birth rates getting lower, many companies are adopting family-friendly policies to encourage staff retention (Chand & Markova, 2018; Chzen et al., 2019). However, literature regarding reproductive health support and fertility-friendly policies, to help those who are infertile and struggling to conceive whilst being employees is scarce. Legislated support specific to combining work and fertility treatment is necessary to reduce psychological distress (Seenan & Akker, 2018). No study was found by the authors about this issue in the Portuguese population.

Study design, size, duration: Study I explores if Portuguese workers receive fertility benefits from and feel supported by their employers, using an online 29-item questionnaire. The data collection started on 20th March 2022 and ended on 4th Sep 2022. Study II explores how employers support their Portuguese employees' fertility plans. Both studies followed a cross-sectional design, using an online 12-item questionnaire. The data collection started on 1st April 2022 and ended on 18th May 2022.

Participants/materials, setting, methods: Study I has a sample of 107 Portuguese workers. The majority are above 35 years of age and have been trying to conceive for more than 24 months. Study II has a sample of 24 employers, including leaders, admin, and HR staff. The majority are working for a large company in a leadership position. Both questionnaires had multiple options, Likert-scale, and open-ended items. The data were analyzed using IBM SPSS Statistics, Version 28.0.

Main results and the role of chance: Study I: All participants reported their company does not have fertility-friendly policies, 72.9% reported difficulties reconciling trying to conceive with work life, 56.1% perceived their attempts interfere with career progression, and 55.1% decided not to inform their superiors about their journey. People who are not offered benefits by their employer experience significantly higher anxiety, disclose less information to their superiors and consider quitting their job more frequently than those who are offered benefits by their company. However, they also experience significantly lower concern/worry, which may be due to increased awareness of fertility issues, pressure not to fail, unwanted interactions, and/or being treated differently.

Study II: 87.5% stated their company does not have fertility-related policies, 66.7% that theirs does not offer fertility-related support and 20.8% did not know whether theirs does. Leaders of medium/small companies report their companies to be significantly more flexible regarding schedules for consultations/exams and taking time off for mourning, and they also thought providing financial help for fertility preservation and fertility treatment to be more useful than leaders of large companies do. Respondents attribute moderate to high importance to the existence of fertility-related policies, but the data shows a lack of agreement regarding which benefits to offer.

Limitations, reasons for caution: Small sample sizes condition the extrapolation of the findings. Replications of the studies representative of different geographic areas and company types are required. Also, the association between organizational and individual benefits as outcomes of the implementation of fertility-friendly policies and initiatives in the Portuguese population is yet to be studied.

Wider implications of the findings: This is the first study that has tackled the issue of fertility-friendly policies and support initiatives in the workplace in the Portuguese context. We gathered data from both employers and employees, which allows the findings of the studies to provide a comprehensive view of the reality of this population.

Trial registration number: not applicable

Abstract citation ID: dead093.911
P-579 Preliminary data on family relations in anonymous oocyte donation families: children's perspectives at age 5 to 7 years

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Study question: Do children born in oocyte donation families perceive their relationships with their parents differently than children conceived trough ICSI?

Summary answer: No significant differences were found in the family relationships between children born in oocyte donation families (OACC) compared with controls (ICSI).

What is known already: Majority of the previous research concerning relationships in families using oocyte donation focused on the mothers' perspective. Little is known about how children in these families view their family relationships. Studies investigating young children's perspectives in donor-families (5-10 years old) indicate good parent-child relationships (Imrie, et al., 2021; Blake, et al., 2013; Casey, et al., 2013). Few of these studies involve young children conceived using anonymous oocyte donation.

Study design, size, duration: This study included 17 children born through anonymous oocyte donation in heterosexual parents and a comparison group of 13 children conceived ICSI using the heterosexual parents' own gametes. Data were collected between August 2021 and January 2023. The sample is part of a larger ongoing case-control-study investigating family relationships and the wellbeing of both parents and their children in families created by anonymous oocyte donation.

Participants/materials, setting, methods: Children were 5 to 7 years old (M = 6.13; SD = 0.5) and had been born after assisted reproduction (ICSI with or without using anonymous oocytes) with their two heterosexual parents. All children were invited to the hospital to undergo biomedical and psychological testing, including the Family Relationship Test (FRT; English version: Bene and Anthony, 1985; Dutch version: Baarda and van Londen, 1985). Multiple blind evaluators were used.

Main results and the role of chance: No significant differences were found between children born in oocyte donation families (OACC) compared with

controls (ICSI). Two-sampled-T-tests show no significant differences for (1) positive feelings (towards and received by children) to their mothers (OACC: M = 6.65, SD = 4; ICSI:M=8.85, SD = 4), fathers (OACC: M = 5.06, SD = 0; ICSI: M = 5.08, SD = 0) or themselves (OACC: M = 0.35, SD = 0.5; ICSI: M = 0.77, SD = 0), (2) negative feelings (towards and received by children) to their mothers (OACC: M = 1.47, SD = 0.5; ICSI: M = 1.92, SD = 4), fathers (OACC: M = 2.06, SD = 0; ICSI: M = 2.31, SD = 0.5) or themselves (OACC: M = 0.76, SD = 0; ICSI: M = 0.38, SD = 0) and (3) dependency to their mothers (OACC: M = 4.29, SD = 3; ICSI: M = 4.54, SD = 0), fathers (OACC: M = 2.82, SD = 1; ICSI: M = 2.69, SD = 2) or themselves (OACC: M = 0.53, SD = 0.5; ICSI: M = 0.85, SD = 0.5).

Limitations, reasons for caution: This study used multiple evaluators, which may induce a potential evaluators bias. The small sample size limits the validity of our findings to a whole population. As the study is still ongoing, the data to be presented will expand.

Wider implications of the findings: The findings are relevant to clinics offering anonymous oocyte donation, to (future) parents who used or who considerate using anonymous oocyte donation to create a family.

Trial registration number: not applicable

Abstract citation ID: dead093.912
P-580 Virtual reality as tool to reduce anxiety during embryo transfer.

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Study question: Is the use of virtual reality during embryo transfer effective as a measure to reduce anxiety during the procedure?

Summary answer: With the current evidence, it cannot be affirmed that virtual reality is useful as a tool to reduce anxiety during embryo transfer.

What is known already: The embryo transfer (ET), the last and crucial procedure of in vitro fertilization (IVF), generates anxiety, especially in couples with a history of reproductive failure. The latest evidence suggests that a high level of stress can influence reproductive results. Virtual reality (VR) seems to be a useful tool that helps to relieve pain, stress, anxiety and depression symptoms. VR has recently been proposed as a method to alleviate pain and emotional impact in other gynecological procedures such as hysteroscopy. It would be interesting to study the potential beneficial impact of the application of virtual reality to reduce anxiety during ET.

Study design, size, duration: This is an experimental and ramdomized study. A total of 128 infertile women undergoing ET were included, who were grouped into two groups based on whether or not they used VR glasses. In all of them, the level of anxiety was assessed according to validated questionnaires (STAI and GAD-7) along with other clinical parameters such as pain (VAS) or blood pressure. The recruitment period was from May to June 2022.

Participants/materials, setting, methods: 128 women between the ages of 18 and 40 who underwent vitrified or fresh embryo transfer were included. Patients under treatment with antidepressants or anxiolytics, drug users, or neurosensory deficits were excluded. In the experimental group, the usual ET procedure was performed applying VR (virtual reality class I medical device developed by Deepsen and Metronic®). The study was carried out in the Assisted Human Reproduction Unit of the Virgen de Valme University Hospital (Seville).

Main results and the role of chance: The 128 participants were randomized into two groups, 64 in each study arm: experimental group (exposed to VR) and control group (normal ET procedure). The characteristics of the patients were comparable between the two groups. Systolic blood pressure (120.62 ± 15.11 vs 126.04 ± 12.26), diastolic blood pressure (75.41 ± 9.58 vs 81.28 ± 9.28) and mean blood pressure (90. 14 ± 10.97 vs 95.54 ± 9.79) after embryo transfer were significantly ($p < 0.05$) lower in the experimental group compared to the control group. However, no differences were observed between the two groups in pain (VAS), blood pressure before the procedure, respiratory rate and oxygen saturation before and after ET. Anxiety level scores (STAI and GAD-7) were comparable between the two groups. Unlike the only comparable study in which a higher pregnancy rate was observed in the experimental group (although without statistical significance), in our study the pregnancy rate was higher in the control group (33.3% vs 43.8%). However, in our study the gestation rate that exceeded 6 weeks of pregnancy was higher in the experimental group (81% vs 71.4%), although without statistical significance.

Limitations, reasons for caution: The sample size is too small, making it difficult to find significant relationships. In addition, there are no previous studies that support the theory of the study.

Wider implications of the findings: There is only one previous comparable study evaluating the use of VR in assisted reproduction. Our findings suggest changes in blood pressure with the use of VR, although this is not the case in the rest of the parameters analyzed. A larger sample is needed to confirm these findings.

Trial registration number: Not applicable

POSTER VIEWING
REPRODUCTIVE ENDOCRINOLOGY

Abstract citation ID: dead093.913
P-582 Identification of microRNA in women diagnosed with premature ovarian insufficiency using urinary exosome in Korean population

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Study question: Would we able to identify specific microRNA which is specific in premature ovarian insufficiency?

Summary answer: Our study provides compelling evidence suggesting that microRNA-4516 would be a diagnostic marker in Korean premature ovarian insufficiency women.

What is known already: Premature ovarian insufficiency is diagnosed in women before the age of 40 years old, when there is no menstruation for at least for six months with hypergonadotropic hypogonadism in serum evaluation. It is a disease which ovarian functions are irreversible, but women visit outpatient clinic only when their ovarian functions are already diminished. Exosomes are functional vehicles which transport complex proteins, lipids and nucleic acids between cells, containing numerous microRNAs and it may include disease-specific miRNA signature that can be a valuable diagnostic tool.

Study design, size, duration: This was a prospective study including patients diagnosed with premature ovarian insufficiency(POI)/Turner syndrome and control individuals who visited outpatient clinic in Department of Gynecology in Gil Hospital, Gachon University from January 2019 to August 2021. We divided the patients into two groups depending on the time period of enrollment; Sequencing cohort and Validation cohort. In sequencing cohort, total 19 patients (7/7/5) were enrolled, and in validation cohort, total 46 patients (15/11/20) were enrolled.

Participants/materials, setting, methods: The patients in POI group were participants diagnosed with POI but they not Turner syndrome. After collecting first morning urine samples, we extracted exosomes and RNA sequencing was done based on microRNA library. We did real-time PCR using a selected hsa-miR-4516 for confirmation. Then we did confirmation study using POI mouse model (Cyclophosphamide + Busulfan).

Main results and the role of chance: Expression of hsa-miR-4516 detected via qRT-PCR analysis of RNA from the validation cohort exactly mapped the RNA sequencing results of the sequencing cohort. Moreover, it was significantly upregulated in patients with POI and Turner syndrome compared to the control group. The TaqMan PCR assay confirmed the

upregulation of hsa-miR-4516 expression in patients with POI and Turner syndrome. To evaluate miR-4516 as a biomarker of POI, we established a chemotherapy-induced POI mouse model by injecting the mice with CTX and BUS. Next, we assessed the expression of mmumiR-4516 in the ovaries of the chemotherapy-induced POI mouse model; mmu-miR-4516 expression was significantly increased in the ovaries but not in the uteri in the POI mouse model compared to the control. These results suggest that upregulation of mmumiR-4516 expression is associated with pathological changes in the ovaries.

Limitations, reasons for caution: We also observed inconsistencies between the sequencing and qRT-PCR results. The cause of POI is highly diverse, which can lead to discrepancies in exosomal miRNA expression between the cohorts. The cohort is small and limited compared to the validation cohort, and this may have caused inconsistencies between the cohorts.

Wider implications of the findings: We suggest that hsa-miR-4516 could be developed as a biomarker of POI within an easy and convenient diagnostic test using non-invasive sampling methods.

Trial registration number: -

Abstract citation ID: dead093.914
P-583 Is donor age a major factor in egg donation for clinical outcome?

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Study question: How significant is the age factor for clinical success of the controlled ovarian stimulation cycles (COS) in various age subgroups of the egg donors?

Summary answer: There is no significant negative impact of the donor age for clinical outcome in egg donation cycle.

What is known already: It was shown that the donor age was an independent factor for ovarian stimulation cycles outcomes, which could predict over 80% of IVF success. It also reported that there were no associations between donor oocyte harvest and probability of live birth, adjusting for donor age. Different studies showed that the donor age less than 25 years old doesn't predict better embryo development or clinical pregnancy outcome for egg donation cycles in the same age group of recipients.

Study design, size, duration: Three IVF centers having been involved in this study, data used from donors (n = 6319) examined between 2019-2022, they were divided into 3 age groups (A: 19-22, B: 23-29, C: ≥30 years). COS protocols were analyzed including different parameters: number of oocytes retrieved, antral follicle count (AFC), maturation rate (MR), utilization rate (UR) and follicle-to-oocyte index (FOI). The association between COS cycle parameters and the age characteristics was assessed by T-Student (parametric) or U-Mann-Whitney (non-parametric).

Participants/materials, setting, methods: In group A mean age 19-22 years , group B 23-29 years and group C - over 30 years donors 717, 1000, 1000 respectively COS protocols were analysed including different parameters such as: number of oocytes retrieved, maturation rate (MR), utilization rate (UR) and follicle-to-oocyte index (FOI).

Main results and the role of chance: Mean age in groups: A (n = 717) - 21,0 ± 1,1; B (n = 1000) - 26,3 ± 1,9 and in group C (n = 1000) - 31,6 ± 1,5. The mean number (SD) of oocytes harvested: 26,94 (12,40), 25,98 (12,03) and 23,22 (11,49) oocytes in groups A, B and C respectively. The same tendency with no statistical difference was observed for values of AFC: 33,24 (14,55), 32,41 (13,86) and 29,77 (12,98).

Values of FOI in A and B groups were higher than in C: 81,85 (17,37), 81,06 (18,54) and 78,24 (17,97) in groups A, B and C, respectively with statistically proven difference (p = <0.05) for both. MR was higher in C group (83 ± 15%) vs A (80 ± 16%) and B (81 ± 16%) with proven statistical significance (p = <0.05). Recipients of group A (average age 33,05) - received 94,55% FR, 66,15% used BR and % 57,84 CPR. Group B recipients (age average 35,08) - 92,88% FR, 61,69 used BR and 54,77% CPR. Recipients of Group C (average age 33,07) - 94,55% FR, 60,01% used BR and 59,05% CPR.

Limitations, reasons for caution: This study is limited by its retrospective nature and more studies are necessary to investigate not only COS outcomes but also the impact of the age related factors on pregnancy rates.

Wider implications of the findings: Our findings may bring a controversial spotlight on the doubtful issue of the relationship between donor age and clinical and embryo outcomes of the COS programs.

Trial registration number: not applicable

Abstract citation ID: dead093.915
P-584 Treatment of benign uterine bleeding in morbidly obese postmenopausal women with an aromatase inhibitor

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Study question: Can morbidly obese women with postmenopausal bleeding, a benign endometrial biopsy, and no response to an oral progestin be successfully treated using an aromatase inhibitor?

Summary answer: Postmenopausal uterine bleeding in morbidly obese women with benign endometrial histology and an elevated serum estrone level do respond to treated with an aromatase inhibitor.

What is known already: Morbidly obese postmenopausal women who have uterine bleeding and a benign endometrial biopsy are commonly not good surgical candidates, and need good medical treatment options especially when treatment with an oral progestin has failed. The etiology for uterine bleeding in these individuals can stem from elevated estrogen production from peripheral sources.

Study design, size, duration: This was a prospective, proof of concept study with no placebo control group. Ten morbidly obese postmenopausal women with elevated serum estrone levels and uterine bleeding for > eight weeks who had failed oral progestin therapy were treated with letrozole 2.5 mg/d and evaluated monthly for 3 months duration. At the first monthly follow-up visit, serum estrone levels were re-assessed. Vaginal bleeding was re-assessed at each monthly follow-up visit.

Participants/materials, setting, methods: This study was performed in an outpatient setting at a private health system. Inclusion criteria included postmenopausal uterine bleeding (not spotting), age > 50, postmenopausal status (based on an FSH level of > 40 mIU/mL and an estradiol < 25 pg/mL), an estrone level > 30 pg/mL, a BMI > 40 kg/m2, a benign endometrial biopsy, a normal pelvic ultrasound, and failure of progestin therapy. All women received letrozole 2.5 mg/d and were evaluated monthly.

Main results and the role of chance: Ten women were enrolled in the study. Their ages ranged from 51 to 68. Their mean BMI was 50.75 kg/m2. All of the women in the study had daily vaginal bleeding for > 8 weeks that was greater than spotting, and had additional co-morbidities that made them poor surgical candidates. Serum estrone levels pre-treatment ranged from 63 pg/mL to 550 pg/mL with a mean of 216 pg/mL. After 4 weeks of treatment with letrozole, 8 women had complete cessation of their vaginal bleeding which was sustained for the complete 3 months of follow-up. The two other women in the study had significantly reduced vaginal bleeding from treatment month one to 3 based on self-reporting. Serum estrone levels after 4 weeks of treatment with letrozole dropped by an average of 71% with a range of 50% to 93%. There were no serious adverse events, and no treatment tolerability complaints. Since there was no control group for comparison, the reductions in both vaginal bleeding and serum estrone levels could be from chance alone, including regression to the mean, and not treatment related.

Limitations, reasons for caution: This was a small, proof of concept study that was performed in a select population of patients. Although a very

favorable outcome was obtained in our study population, a much larger study might show both a reduced success rate in regards to vaginal bleeding and some significant adverse events.

Wider implications of the findings: The data from this study suggests that morbidly obese postmenopausal women with uterine bleeding and benign endometrial histology who have failed oral progestin therapy, are poor surgical candidates, and in whom IUD placement may be very difficult, may have another good treatment option.

Trial registration number: N/A

Abstract citation ID: dead093.916

P-585 Iron intake in relation to ovarian reserve among women seeking infertility treatment

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Study question: Is there an association between iron intake and ovarian reserve among women seeking fertility care?

Summary answer: Supplemental iron intake above 45mg/d is associated with lower ovarian reserve among women seeking fertility care.

What is known already: Although the literature regarding iron intake in relation to ovarian reserve is scant and inconsistent, some evidence suggest that iron may have gonadotoxic effects.

Study design, size, duration: Observational study including 582 female participants attending the Massachusetts General Hospital Fertility Center (2007–2019) enrolled in the Environment and Reproductive Health (EARTH) Study

Participants/materials, setting, methods: Iron intake was estimated using a validated food frequency questionnaire (FFQ). Markers of ovarian reserve included antral follicle count (AFC) (assessed via transvaginal ultrasound) and day 3 FSH, both obtained during the course of infertility evaluation. The association between iron intake and ovarian reserve was evaluated using Poisson regression models for AFC and quantile regression models for day 3 FSH adjusted for age, menstrual cycle characteristics, physical activity, carbohydrate intake and total energy intake.

Main results and the role of chance: Participants had a median age of 35 years and median total iron intake of 29 mg/d. Total iron intake was inversely related to AFC and this association was driven by intake of supplemental iron. Compared to women with a supplemental iron intake $\leq$20mg/day, women consuming 45-64mg/d of supplemental iron had a 17% (-35%, 0.3%) lower AFC and women consuming $\geq$65mg/d of supplemental iron had a 32% (-54%, -11%) lower AFC after adjusting for potential confounders (p, linear trend=0.003). Similarly, in multivariable-adjusted analysis, day 3 FSH levels were 0.9 (0.5, 1.3) IU/mL higher among women with a supplemental iron intake $\geq$65mg/d when compared to women with a supplemental iron intake $\leq$20mg/d (p, linear trend=0.02).

Limitations, reasons for caution: Iron intake was estimated using a method that relies on self-report and we had no biomarkers of iron status in our participants. Also, only 36 women consumed $\geq$45mg/d of supplemental iron.

Wider implications of the findings: Since all study participants were seeking fertility treatment, our findings may not apply to women in the general population. Although our findings are consistent with studies of women with iron overload, given the paucity of literature on this topic it is essential that this question is revisited in future studies.

Trial registration number: Not applicable, not a randomized trial

Abstract citation ID: dead093.917

P-586 Adding letrozole to gonadotropins during ovarian stimulation for IVF results in a similar cumulative live birth rate at lower costs following a failed IVF cycle

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Study question: Following a failed IVF cycle, does adding letrozole for the first 5 days of a repeated cycle have any advantage versus repeating the same protocol?

Summary answer: Following a failed IVF cycle, letrozole supplementation during ovarian stimulation for IVF results in a similar cumulative live birth rate (CLBR) with 22% less gonadotropins.

What is known already: Letrozole results in an increase of intraovarian androgen concentration, which augments follicular stimulating hormone (FSH) receptor expression on granulosa cells and follicular responsiveness to exogenous gonadotropins. Letrozole co-treatment with gonadotropins during controlled ovarian stimulation (COH) for IVF has been investigated mainly in poor and normo-responders with conflicting clinical outcomes. This is the first and largest study that investigates the effects of adding letrozole for the first five days of COH for IVF, in GnRH antagonist cycles, to a category of patients who had a prior failed stimulated IVF cycle using the same protocol.

Study design, size, duration: This is retrospective monocentric study of 426 patients, with a prior failed stimulated IVF cycle using an antagonist protocol, who underwent a second cycle between 2012 and 2017. In those patients, we studied the clinical outcomes of doing a repeated cycle, using the same protocol with the addition of 5mg of letrozole daily in the first 5 days of the cycle (Group A, N = 213) versus doing a repeated similar cycle without letrozole (Group B, N = 213).

Participants/materials, setting, methods: Groups A and B were matched for age, body mass index, anti-mullerian hormone and infertility diagnosis. Statistical analyses were carried out using student t test, Anova and linear regression models. The primary outcome was the CLBR. Secondary outcomes included the number of mature follicles, the number of mature oocytes (MII), the number of usable embryos, clinical pregnancy rates (CPR), live birth rates (LBR) and the total dose of gonadotropins administered.

Main results and the role of chance: This is the first study that investigates the effects of co-treatment with letrozole during COH for IVF in the category of patients who had a prior failed IVF cycle. Our results show that patients who received letrozole during COH with gonadotropins, versus gonadotropins alone, had a significantly lower number of MII oocytes (7 ± 4.6 vs $8,2 \pm 5.0$; $P < 0.01$). However, despite that, they had a similar number of usable embryos (1.8 ± 1.6 vs 1.9 ± 1.7; $P = 0.31$), CPR (23.5% vs 28.7%; $p = 0.22$), LBR per embryo transfer (16.9% vs 22.1%; $p = 0.17$) and CLBR (27.8% vs 25.3%; $p = 0.55$). A 22% lower dose of gonadotropins (3468 ± 1389IU vs 4442 ± 1657IU; $p < 0.001$) was needed to achieve similar pregnancy outcomes in the letrozole group versus the control group, resulting in lower treatment costs.

Limitations, reasons for caution: The retrospective nature of this study is its main limitation.

Wider implications of the findings: This study confirms that in women with a prior failed IVF cycle, co-treatment with letrozole results a similar cumulative live birth rate at a significant lower cost.

Trial registration number: NCT05677828

Abstract citation ID: dead093.918

P-587 effects of erzhi-tiangui granules to NEAT1 and apoptotic factors expression in granulosa cells of women with diminished ovarian reserve and Kidney-deficiency syndrome

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Study question: To investigate whether erzhi-tiangui granules (ETG) can reduce granulosa cells (GCs) apoptosis levels by regulating NEAT1 expression, thereby improving the cycle outcomes of patients.

Summary answer: ETG significantly improved oocyte quality and decreased apoptotic factor levels in GCs, which may be related to its intervention in NEAT1 expression.

What is known already: The mechanism by which diminished ovarian reserve (DOR) occurs is mainly due to extensive apoptosis of GCs, which leads to follicular atresia and reduced follicle numbers. It has been found that changes in NEAT1 expression in ovarian tissues can exert influence the extent of apoptosis of GCs, which leads to diseases such as POF and PCOS. While ETG, a clinical formula used to improve female fertility, can improve the quality of oocytes and embryos by down-regulating the expression of apoptosis-related effector proteins, thus improving clinical outcomes. Whether ETG improves clinical outcomes in DOR patients via NEAT1 hasn't been elucidated.

Study design, size, duration: This study was conducted in two separate parts from January 2020 to September 2021. Experiment I included 15 women each with normal ovarian reserve (NOR) and DOR, aiming to investigate the expression differences of NEAT1, key apoptosis proteins (Bcl-2 and Caspase-3). Experiment II, a randomized controlled trial, recruited 70 DOR patients, aimed to investigate if ETG could regulate the expression levels of Bcl-2 and Caspase-3 through NEAT1, thus acting to improve clinical outcomes.

Participants/materials, setting, methods: We detected expression levels of lncRNA NEAT1 and apoptotic proteins Bcl-2 and Caspase-3 of DOR and NOR women using RT-qPCR and Western-blotting, respectively. DOR patients were randomly assigned to ETG and control group, treated with ETG and placebo respectively. Clinical efficacy was confirmed by comparing two groups regarding changes in clinical and laboratory data. We also probed the changes in expression levels of NEAT1, Bcl-2 and Caspase-3 to investigate effects of ETG on GCs' apoptosis.

Main results and the role of chance: In Experiment I, compared to the NOR group, the expression levels of NEAT1 and Bcl-2 were down-regulated while those of Caspase-3 were up-regulated in the GCs of DOR patients ($P < 0.05$). In Experiment II, a total of 65 of 70 patients completed the study and were included in the outcome analysis. There were no significant differences between the two groups regarding baseline characteristics. Before treatment, there was no significant difference in the Kidney-deficiency syndrome scores between the two groups. After treatment, the ETG group had significantly lower Kidney-deficiency syndrome scores compared to the control group ($P < 0.01$). Also, Kidney-deficiency syndrome scores were significantly lower in the ETG group post-treatment compared with that pre-treatment ($P < 0.001$); there was no significant change in the control group ($P > 0.05$). There were no significant differences between the two groups in the duration of gonadotrophin stimulation, serum estrogenand progesterone levels on the trigger day, and normal fertilization rate. Compared to the control group, the ETG group had lower gonadotropin dosage and increased number of oocytes, transferable embryos and top embryos obtained ($P < 0.05$). Furthermore, the expression of NEAT1 was upregulated ($P < 0.05$), Caspase-3 expression was decreased ($P < 0.01$) in GCs from the ETG group than those in the control group ($P < 0.05$).

Limitations, reasons for caution: The small number of observed cases and the lack of long-term efficacy may lead to biased study conclusions. Further studies are also expected in the future to understand the relevant pathway mechanisms.

Wider implications of the findings: In this study, not only to judge the therapeutic effects of ETG by using clinical symptoms and clinical laboratory indexes as observations, but also to explore the molecular mechanism of its treatment of Kidney-deficiency type DOR based on NEAT1, which may be related to affecting the apoptotic process.

Trial registration number: No. 82274573, No. 82174429, No. ZR2021MH255, No. tsqn202103182

Abstract citation ID: dead093.919

P-588 A comparison of obstetrical and neonatal outcomes in patients with the hyperandrogenic states, polycystic ovarian syndrome(PCOS) and congenital adrenal hyperplasia(CAH): a retrospective population-database study.

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Study question: PCOS and CAH are associated with increases in pregnancy complications. Therefore, we evaluated differences in pregnancy and neonatal outcomes between women with PCOS and CAH.

Summary answer: When comparing these conditions PCOS was associated with pregnancy-induced hypertension and gestational diabetes, whereas CAH had more cesarean sections and small for gestational age infants.

What is known already: PCOS and CAH are causes of hyperandrogenism among women of reproductive age. Apart from causing hyperandrogenism, they also share characteristics such as menstrual irregularity, polycystic ovaries and insulin resistance with central adiposity. Several meta-analyses have associated PCOS with adverse obstetrical and neonatal outcomes with most studies finding an increased frequency of pregnancy-induced hypertension (PIH), preeclampsia, gestational diabetes (GDM), cesarean section (CS) and preterm delivery. CAH on the other hand, is associated with higher rates of CS and inconsistent evidence about increases in GDM, chorioamnionitis and congenital anomalies.

Study design, size, duration: A retrospective population-based study utilizing data from the Healthcare Cost and Utilization Project - Nationwide Inpatient Sample (HCUP-NIS), was performed. A dataset of all deliveries between 2004 and 2014 inclusively, was created. Within this group, all deliveries to women who had a diagnosis of PCOS or CAH and pregnancy were identified. Each subject had a delivery or maternal death to be included once per pregnancy.

Participants/materials, setting, methods: Descriptive analyses were performed to compare the demographic features among both groups, for which chi-squared tests were used. Multivariate logistic regression analysis was performed to calculate unadjusted and adjusted odds ratios (aORs) and corresponding 95% confidence intervals (CI) and correct for any difference in baseline demographics ($P < 0.05$) between the groups. According to the Tri-Council Policy statement (2018), institutional review board (IRB) approval was not required, given that data was anonymous and publicly available.

Main results and the role of chance: A total of 15,179 women with either PCOS or CAH were included. Approximately 98% of those women (14,881) were diagnosed with PCOS, while the rest 2% (298) had CAH. The adjusted analysis for race, obesity, previous CS, multiple gestation and IVF yielded the following results. For pregnancy outcomes PIH (aOR=1.76; 95% CI: 1.12-2.77; $p = 0.015$) and GDM (aOR=1.68; 95% CI: 1.12-2.52; $p = 0.012$) were more common among women with PCOS, whereas no statistically significant results were detected for rates of placenta previa, preeclampsia or eclampsia superimposed on pre-existing hypertension or eclampsia. After additional adjusting for PIH and GDM, the delivery outcomes showed CS to be significantly less common in the PCOS group (aOR=0.59; 95% CI 0.44-0.80; $p < 0.001$), with no significant differences concerning preterm premature rupture of membranes, preterm delivery, abruptio placenta, chorioamnionitis, operative vaginal delivery, hysterectomy, postpartum hemorrhage, wound complications, maternal death and need for transfusion. In neonatal outcomes, women with CAH had higher percentages of SGA neonates (aOR=0.32; 95%

CI 0.20-0.52; $p < 0.001$) and no difference in intrauterine fetal demise and congenital anomalies.

Limitations, reasons for caution: The limitations of our study are its retrospective nature and the fact that it relies on an administrative database. Furthermore, CAH type was not specified. Information on medication use and compliance was also lacking information about certain potential confounders such as parity, and duration of labor.

Wider implications of the findings: This is the first study to investigate the differences in obstetrical and neonatal outcomes between these two hyperandrogenic disorders and we noted that the majority of risks were similar, despite the difference in the pathophysiology of these conditions. Rates of certain complications differ and studies are needed to elucidate why.

Trial registration number: N/A

Abstract citation ID: dead093.920

P-590 Similar mean follitropin delta daily/total doses yield satisfactory ICSI outcomes in different subgroups of maternal age and body mass index: a "real-world" experience with Rekovelle®

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Study question: Can similar mean follitropin delta daily/total doses yield satisfactory ICSI outcomes in different subgroups of maternal age and body mass index (BMI)?

Summary answer: Similar mean follitropin delta daily/total doses yield satisfactory number of oocytes and maturity rates, and pregnancy rates in different subgroups of maternal age and BMI.

What is known already: Rekovelle® (follitropin delta) is a novel recombinant human FSH (rFSH) expressed from a host cell line of human fetal retinal origin (PER.C6®) and is the first commercially available rFSH product derived from human cell lines. Rekovelle® allows the individualization of the initial dose of gonadotropin using predictive response factors to COS, such as antimüllerian hormone (AMH) levels and body weight. In clinical practice different approaches can be adopted to obtain the most satisfactory response to controlled ovarian stimulation (COS). The objective of this study was to describe data on "real-world" Rekovelle® administration, regarding the response to COS and ICSI outcomes.

Study design, size, duration: This non-interventional study based on secondary use of data included patients undergoing ICSI in a private university-affiliated IVF center from Jan/2018 to Dec/2021. Each patient or cycle had to meet the following inclusion criteria: pre-menopausal women, undergoing COS with Rekovelle® (16 ug) daily for ICSI; diagnosed with infertility, or with partners diagnosed with male infertility factors, eligible for ICSI using fresh sperm from partner ejaculation; presenting with both ovaries.

Participants/materials, setting, methods: Ovarian response to stimulation and ICSI outcomes were described for 362 ICSI cycles. The primary outcome measures were the numbers of retrieved oocytes and maturity rates, and the secondary outcome were the ongoing pregnancy rates per fresh and per fresh and/or frozen-thawed embryo transfer. The results obtained with the population enrolled in the ESTHER-1 trial (extern), and a population stimulated with follitropin alpha 300 IU (on site) were recorded (data not shown, to be presented).

Main results and the role of chance: The mean total dose of follitropin delta administered in subgroups of age and BMI were as follows: age ≤35 y-old 156µg, 36-39 y-old 158 µg, and ≥40 y-old 157µg, and BMI: <18.5 165µg, 18.5-24.9 156µg, 25.0-24.9 159µg, and ≥30 171µg. Patients in the Follitropin delta group showed acceptable outcomes in different age and BMI groups (Table1). Adequate embryo morphokinetic development and euploidy rates were also observed, suggesting that the modified protocol does not interfere with oocyte/embryo competences and embryo implantation potential. This was further corroborated by satisfactory cumulative pregnancy rates.

Limitations, reasons for caution: Given its descriptive nature, we have focused on the number and maturity stage of retrieved oocytes instead of clinical ICSI outcomes. The use of historical cohort groups is another drawback. Despite the eligibility criteria for inclusion in the analysis, potential differences in the baseline characteristics cannot be ruled out.

Wider implications of the findings: Patients in different subgroups of age and BMI stimulated with similar mean follitropin delta daily and total doses showed satisfactory number of retrieved oocytes and maturity rates, and cumulative pregnancy rates without increasing OHSS, providing a rationale for conducting clinical trials to confirm the benefits of using this modified protocol.

Trial registration number: N/A

Abstract citation ID: dead093.921

P-591 Follicular fluid-derived exosomes rejuvenate ovarian aging through miR-320a-3p-mediated FOXQ1 inhibition

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Study question: Dose the follicular fluid-derived exosomes rejuvenate ovarian aging?

Variable	≤ 35 y-old	36 – 39 y-old	≥ 40y-old	BMI < 18.5	BMI 18.5 – 24.9	BMI 25.0 – 29.9	BMI ≥ 30.0
Female age	33.4 ± 1.4	37.5 ± 1.1	41.5 ± 1.7	37.3 ± 2.8	37.6 ± 3.3	37.0 ± 2.6	36.6 ± 2.6
FSH dose/oocyte (ug)	9.9 ± 10.2	15.4 ± 33.2	24.3 ± 46.6	12.1 ± 51.3	14.5 ± 37.4	14.9 ± 27.0	13.8 ± 12.5
OHSS (%)	10.6	4.2	2.6	0.0	4.6	10.0	0.0
Retrieved oocytes (n)	15.8 ± 11.3	10.3 ± 7.3	6.5 ± 5.6	13.7 ± 10.1	10.7 ± 9.5	10.6 ± 7.0	12.4 ± 9.2
Mature oocyte (%)	68.7	72.7	70.1	62.2	72.7	68.2	73.5
Blastocyst development (%)	57.6	46.1	42.2	60.0	51.4	50.0	41.2
Clinical pregnancy/transfer (%)	72.6	54.7	43.4	41.7	53.2	65	61.8
Miscarriage (%)	14.7	16.2	21.2	20.0	13.9	18.5	23.8
Ongoing pregnancy/transfer (%)	61.7	44.8	28.9	33.3	44.9	50.0	44.1

Summary answer: Follicular fluid-derived exosomes efficiently rescued ovarian function in aged mice, which was achieved via transfer of miR-320-3p to granulosa cells and subsequent Foxq1 inhibition.

What is known already: The ovary enters the malfunctional aging phase earlier and faster than other organs and systems. Ovarian aging is mainly characterized by a progressive decline in oocyte quantity and quality, which ultimately leads to female infertility. Various therapies have been established to cope with age-related ovarian dysfunction, among which exosome-based therapy has been considered a promising strategy that can benefit ovarian functions via multiple pathways.

Study design, size, duration: Ovarian granulosa cells of young C57BL/6J mice (6-8 w) and old C57BL/6J mice (40 w) were cultured in vitro. The follicular fluid exosomes of young mice and the same amount of PBS were respectively injected into the ovarian sac of old mice.

Participants/materials, setting, methods: Cell proliferation and apoptosis were detected by CCK8, flow cytometry and Western Blot. Mitochondrial function was assessed by measuring mitochondrial membrane potential, ATP levels, mtDNA copy numbers. The number of ovarian follicles were observed by HE staining. Senescence and telomere related gene expression levels were detected by RT-qPCR. 2-cell rates and blastocyst formation rates were observed after in vitro fertilization. The model mice were directly cooped up to observe the pregnat and litter sizes.

Main results and the role of chance: The follicular fluid exosomes from young mice can promote the proliferation of ovarian granulosa cells from old mice and improve mitochondrial function. When the follicular fluid exosomes from young mice were injected into the ovarian sac of old mice, the number of primordial and growing follicles in the ovaries of old mice were increased and the developmental ability of oocytes was improved.

Limitations, reasons for caution: Findings in mouse.

Wider implications of the findings: This study suggests that young follicular fluid exosomes may be involved in ovarian senescence by transferring miR-320a-3p targeting FOXQ1 into ovarian granulosa cells of aged mice.

Trial registration number: no

Abstract citation ID: dead093.922

P-592 Follicular GnRH agonist challenge test (FACT) to predict suboptimal response to GnRH agonist trigger in oocyte cryopreservation cycles

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Study question: Can follicular GnRH agonist administration predict suboptimal response to GnRH agonist trigger assessed by LH levels post ovulation trigger in non-medical oocyte cryopreservation program?

Summary answer: Follicular agonist challenge test (FACT) can serve as an adjunct pre-trigger, intracycle tool to predict adequate response to agonist trigger.

What is known already: Suboptimal response to GnRH agonist ovulation trigger refers to failure to retrieve the expected number of oocytes. It was demonstrated in up to 5.5% of cycles based on relatively low number of studies with heterogenous suboptimal response definitions and prevalence, no clear cut-offs and mainly post - stimulation tools to predict unsuccessful cycle. The most studied variable associated with suboptimal response was LH levels 10-12 hours after ovulation trigger below 12-15IU.

Study design, size, duration: We prospectively recruited all women that underwent non-medical fertility preservation in our tertiary university affiliated medical center between October 2020 and February 2022 and agreed to participate in the study. The study included 91 women.

Participants/materials, setting, methods: On day 2 to menstrual cycle, blood tests were drawn (basal Estradiol/E2, basal FSH/FSH2, basal LH/LH2, Progesterone/P2) and ultrasound (US) was performed. On that evening, the women were instructed to inject 0.2 mg GnRH agonist and arrive for repeated blood workup 10-12 hours later at the next morning, followed by a flexible antagonist protocol. On the morning after ovulation trigger blood tests were again taken and LH levels were compared to FACT LH levels.

Main results and the role of chance: LH levels following agonist ovulation trigger below 15IU occurred in 1.09 % of cycles and were predicted by FACT, $r = 0.57$, $p < 0.001$. ROC analysis demonstrated that FACT LH > 42.70 IU would predict LH post trigger of more than 30 IU with 75% sensitivity and 70% specificity, AUC= 0.81. LH levels post trigger also displayed significant positive correlation to basal FSH ($r = 0.35$, $p = 0.002$) and basal LH ($r = 0.54$, $p < 0.001$). LH levels post ovulation trigger were not associated with total oocytes number or maturity rate. The strongest correlation to the total number of retrieved oocytes and mature oocytes was progesterone level post ovulation trigger ($r = 0.74$, $p < 0.001$).

Limitations, reasons for caution: Limitation of our study include the relatively small sample size designed to detect 5% suboptimal proportion and might have been insufficient to allow identification of very rare suboptimal responses

Wider implications of the findings: Suboptimal response to agonist trigger, as assessed with post trigger LH levels is a rare event. FACT could serve as an adjunct pre-trigger, intracycle tool to predict adequate response to agonist ovulation trigger. Future studies should focus on optimization of agonist trigger efficacy assessment and prediction, especially in high responders.

Trial registration number: 0304-20-ASF

Abstract citation ID: dead093.923

P-593 developmental programming by maternal androgen excess is mediated by androgen receptor pathways

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Study question: How does maternal hyperandrogenism featured PCOS-pregnancy affects the placental and embryo development and further contributes to the development of PCOS-like phenotypes in adult offsping?

Summary answer: Maternal hyperandrogenism compromises PCOS-pregnancy and embryo development due to placenta dysfunction, leading to the subsequent development of anxiety-like behavior and/or impaired metabolism in adult offspring

What is known already: Women with PCOS suffer an increased risk of having miscarriage, preterm delivery, and perinatal mortality. The hostile *in utero* environment because of maternal hyperandrogenism likely play a detrimental role in embryo development as well as the disease susceptibility later in life. As according to previous findings, daughters of women with PCOS have 5 fold increased risk of getting PCOS diagnosis, while sons of women with PCOS suffer from metabolic diseases.

Study design, size, duration: We used a PCOS-like mouse model induced by continuous exposure to dihydrotestosterone (DHT) from prepuberty, which developed obesity, anovulation, and abnormal ovarian morphology to study the effects of maternal hyperandrogenism during pregnancy. In comparison, we also simultaneously treated PCOS-like females with flutamide, an androgen-receptor blocker. We examined embryos at E10.5 and E13.5 focusing on placentas in relation to embryo development. Their male and female offspring is also phenotyped until 6 months of age.

Participants/materials, setting, methods: To explore molecular mechanisms contributing to the developmental defects, whole genome bisulfite and bulk RNA sequencing of placenta were performed. Human Trophoblast organoid culture under different conditions was applied to further evaluate the

detrimental effects of maternal hyperandrogetism in placental development. With the help of trophoblast organoid culture system, possible treatment options are also going to be explored.

Main results and the role of chance: We found a lower pregnancy rate and impaired placentas together with defective embryonic development, which was partially prevented when co-treated with the androgen receptor blocker, flutamide. RNA sequencing of the placenta revealed that DHT severely interferes with placental and fetal development, which are prevented by the treatment with flutamide. In addition, the placenta from DHT-exposed dams showed impaired differentiation capacity of cell types located in the labyrinth. The hyperandrogenic maternal environment led to the development of anxiety-like behavior and impaired metabolism in adult male offspring and partial disturbance in the metabolism of female offspring later in life. The effect of hyperandrogenism on trophoblast differentiation and metabolic capacity is still under investigation.

Limitations, reasons for caution: Although androgen receptor pathway was found to be responsible for placental and fetal abnormalities and the development of adult phenotypes, and the treatment with flutamide prevented most of the unfavorable effects, the use of flutamide in clinic is tightly regulated and not suggested for women with PCOS.

Wider implications of the findings: Maternal hyperandrogenism greatly compromises PCOS-pregnancy and embryo development due to placenta dysfunction, leading to the subsequent development of unfavorable phenotypes in adult offspring. Such effects are mainly mediated by the androgen receptor pathway as the administration of flutamide partially prevents the compromised placenta and fetal development.

Trial registration number: not applicable

Abstract citation ID: dead093.924
P-594 A prevalence study of vitamin D insufficiency among infertile women in Sweden, its determinants and IVF outcome.

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Study question: What is the prevalence and the determinants of vitamin D insufficiency and do women with insufficiency have worse IVF outcome compared with them with sufficiency?

Summary answer: Women from Non-Nordic countries, with avoiding sun behavior and low intake of vitamin supplementation have higher prevalence of vitamin D insufficiency but comparable IVF outcome.

What is known already: Vitamin D seems to have an important role in human reproduction and infertility. Vitamin D insufficiency is highly prevalent around Europe, yet studies from north latitude countries, where sunlight exposure is undetectable during the winter, describing the prevalence and determinants of this condition in women with infertility lacking.

Moreover, there is still discrepancy in the existing evidence whether insufficient vitamin D status is associated with poor IVF/ICSI outcome and no published studies in this field exist from Nordic countries.

Study design, size, duration: Prospective cohort study that was conducted between September 2020 and August 2021 at Reproductive Medicine, Sahlgrenska University Hospital, Gothenburg in Sweden. Gothenburg is a city located in southwestern Sweden (latitude 57.7° N) with highly variating duration of daylight between seasons.

Participants/materials, setting, methods: Women with infertility who were scheduled for IVF/ICSI treatment were included. Venous blood samples for measurement of serum 25(OH) vitamin D concentration and questionnaires assessing vitamin D intake and sun exposure habits were collected from 265 women.

Main results and the role of chance: The overall prevalence of vitamin D insufficiency was 27%, ranging from 7% in summer to 33% in winter.

The odds for vitamin D insufficiency were higher for women originating from non-Nordic Europe (OR 3.33, 95% CI 1.26-8.80, adjusted p=0.015), Middle East (OR 14.46, 95% CI 5.20-40.20, adjusted p<0.001) and Asia (OR 6.46, 95% CI 1.79-23.27, adjusted p=0.004) compared with women originating from Nordic countries. It was more likely for women who did not use vitamin D supplements to have insufficiency compared with them using supplements (OR 3.48, 95% CI 1.69-7.20, adjusted p<0.001). The odds for vitamin D insufficiency were also higher for women staying "in the shade all the time" compared with women who were exposing themselves "in the sun all the time" (OR 3.65, 95% CI 1.43-9.27, adjusted p=0.007) and with them staying "both in the sun and shade" (OR 3.57, 95% CI 0.88-14.43, adjusted p=0.074).

The reproductive outcome of IVF/ICSI treatment between women with serum 25(OH) vitamin D insufficiency and sufficiency was comparable.

Limitations, reasons for caution: The use of a questionnaire has intrinsic limitations (recall bias, low accuracy of the provided information). Moreover, the questionnaire did not include questions about sun protection factor usage during sun exposure. Blood sampling was underrepresented during summer due to limited resources during the summer months with closed IVF clinics.

Wider implications of the findings: Pre-IVF/ICSI treatment vitamin D serum concentration do not seem to influence IVF success. However, information about vitamin D should target women from non-Nordic countries and to advise them for supplementation and sun exposure. The aim is to avoid vitamin D insufficiency and the associated adverse long-term health consequences.

Trial registration number: not applicable

Abstract citation ID: dead093.925
P-595 What to expect from "standard vaginal progesterone regimen" in Hormone Replacement Therapy Frozen Embryo Transfer (HRT-FET) – do different products result in different serum levels?

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Study question: Do luteal serum progesterone levels (P4) vary in relation to time of blood sampling, the type and dosing of vaginal progesterone products used for HRT-FET?

Summary answer: Significant differences in luteal serum P4 levels exist between time of blood sampling as well as between different vaginal progesterone products and the administration regimen.

What is known already: Mid-luteal P4 levels below 9-11 ng/ml have been shown to negatively affect reproductive outcomes in HRT-FET cycles. Formulations of vaginal progesterone products differ as progesterone can be suspended in vegetable hard fat, in a vegetable lipophile liquor, be prepared in an oil-in-water emulsion carrier, or mixed in a tablet. Moreover, dosing regimens vary from one to three times daily, and BMI, age, and parity affect serum P4 levels; importantly, no consensus exists in terms of optimal time point to measure P4 in relation to administration, the specific serum P4 cut-off level to use or the most optimal vaginal progesterone product.

Study design, size, duration: Cohort study including 488 HRT-FET blastocyst transfers performed from January 2020 to November 2022 in patients undergoing a "standard vaginal progesterone regimen". Each patient participated only once in the study. To compare the vaginal progesterone product used in the present study, we reviewed the most recent literature, including data from six studies, using three different products in six different regimens;

all studies were cohort studies, including between 227 -1150 patients undergoing a "standard" HRT-FET protocol.

Participants/materials, setting, methods: A total of 488 patients in a public Fertility Clinic underwent HRT-FET, including endometrial preparation with oral oestradiol (6mg/24hours), followed by vaginal micronized progesterone, 400mg/12hours (Cyclogest®). Blood sampling on the blastocyst transfer day was standardised to two to four hours after progesterone administration. In contrast, P4 was measured randomly on the day of pregnancy testing.

For comparison a review of the literature from six HRT-FET cohort studies using either Crinone®, Utrogestan® or "PIVET"-pessaries was performed.

Main results and the role of chance: The mean serum P4 (2-4 hours after administration) on the day of blastocyst transfer in our cohort was 15.4 ± 6.6ng/ml. On the day of pregnancy testing (random time points) serum P4 levels were divided into four groups, depending on the time from progesterone administration to blood sampling: <2hours, >2<4hours >4<6hours and >6hours.The mean P4 levels were 14.7 ± 6.3ng/ml, 15.6 ± 5.8ng/ml, 14.3 ± 4.9ng/ml and 12.9 ± 6.4, respectively, and a significant difference was seen, between P4 levels measured 2-4 hours and >6 hours after vaginal administration ($p = 0.03$).

As regards the product, the mean P4 levels in this study (Cyclogest®) was significantly different from the reviewed P4 levels of other "standard" HRT-FET cohorts, using three different products (Uterogestan®, Crinone® and the "PIVET-pessary") 12.1 ± 7.0ng/ml ($p < 0.001$), 7.6 ± 3.2ng/ml ($p < 0.001$) and 29.3 ± 20.3ng/ml ($p < 0.001$), respectively.

As regards the dose, a significant difference in P4 levels was seen between dosing regimens in two cohorts when using the same product (Crinone®) 90mg x 3 vs. 90 mg x 2; 11.3 ± 4.7ng/ml and 7.6 ± 3.2ng/ml, respectively, $p < 0.001$.

Interestingly, when comparing another product (Utrogestan®) used in different dosing regimens, 400mg x 2 vs. 200mg x 3, no difference in P4 levels was seen, 12.1 ± 7.0ng/ml and 11.3 ± 5.1ng/ml, $P = 0.09$.

Limitations, reasons for caution: In HRT-FET serum P4 levels reflect the exogenous administration, only, and P4 fluctuations depend on the route and number of administrations, as well as absorption and metabolization. The reviewed data used for this comparison between different progesterone products and regimens derive from cohorts with different characteristics and blood sampling timings.

Wider implications of the findings: Luteal serum P4 measurement is important, however, standardization of blood sampling in relation to the latest vaginal progesterone administration is mandatory to obtain correct levels. Clinicians should be aware of the significant differences in P4 levels related to dose and vaginal absorption between different types of vaginal progesterone products.

Trial registration number: EudraCT no.: 2019-001539-29

Abstract citation ID: dead093.926
P-597 Sexual function in women with Polycystic Ovary Syndrome: an updated systematic review and meta-analysis

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Study question: To present a comprehensive review of the evidence on sexual function in women with polycystic ovary syndrome (PCOS) compared to women without PCOS.

Summary answer: Women with PCOS report lower sexual function and sexual satisfaction compared to women without PCOS.

What is known already: Research focusing on the psychosocial aspects of PCOS or sexual function is fairly recent and data are limited. Data are often contradicting with studies reporting no differences in sexual function in women with PCOS and studies reporting more sexual problems in women with PCOS. A meta-analysis from our group in 2018 reported lower sexual function and lower sexual satisfaction in women with PCOS compared to women without PCOS.

Study design, size, duration: A systematic review and meta-analysis of the published literature was conducted.

Participants/materials, setting, methods: Eight databases were searched from inception to August 24th 2022. Two authors independently screened articles based on pre-defined inclusion (e.g. adequate PCOS diagnosis) and exclusion criteria (e.g. hyperandrogenism caused by other diseases),assessed their quality and graded the certainty of the evidence. Review Manager was used to perform all analyses.

Main results and the role of chance: Twenty-seven articles were included, of which 23 used questionnaires and four used visual analogue scales (VAS). Pooling FSFI scores showed worse sexual function across most domains in women with PCOS, including arousal (weighted mean difference [WMD]== -0.37, P= 0.002), lubrication (WMD= -0.53, P <0.001), and orgasm (WMD= -0.32, P <0.001). Total sexual function (WMD= -2.47, P <0.001) and sexual satisfaction (WMD= -0.42, P <0.001) were also significantly lower. Selection on fertility status did not change the direction of results. VAS results were not pooled in meta-analysis due to using the same control group across the included studies. However, effects within individual VAS studies demonstrated the negative impact of excess body hair on sexuality, lower sexual attractiveness and lower sexual satisfaction in women with PCOS compared with controls, with no differences in the perceived importance of a satisfying sex life. Certainty of evidence was graded low for all included studies.

Conclusion: Women with PCOS place similar importance on sexual satisfaction as women without PCOS, yet have lower sexual function and sexual satisfaction. Distress was not assessed. Sexual function assessment, and, psychosexual counseling should be considered as part of standard care in women with PCOS.

Limitations, reasons for caution: Sexual distress was not assessed. Hence, prevalence of sexual dysfunction which requires both lower sexual function and sexual distress, remains unkown.

Certainty of evidence was graded low for all included studies.

Wider implications of the findings: Sexual function assessment, and, psychosexual counseling should be considered as part of standard care in women with PCOS.

Findings from this review have directly informed the international PCOS guideline on sexual function assessment and management.

Trial registration number: NA

Abstract citation ID: dead093.927
P-598 Analysis of the oocyte yield and Ovarian Sensitivity Index with the use of two gonadotropins (FSHr/FSHu) and two pituitary suppression protocols (PPOS/GnRH-ant)

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Study question: Is there a difference, in terms of ovarian yield and sensitivity, comparing a progestin-primed protocols (PPOS) to a GnRH antagonist (GnRH-ant) and using different follitropin?

Summary answer: PPOS is an effective choice, with both follitropis. Nevertheless, could improve ovarian sensitivity to FSHu increasing the number of oocyte yield without compromising oocyte quality.

What is known already: The use of new ovarian stimulation protocols, starting during the luteal phase or randomly shows that endogenous progesterone alone is sufficient to block the LH surge and does not compromises oocyte competence and pregnancy outcomes. For these reasons, exogenous progesterone was utilized as an innovative model of pituitary suppression. Most studies have shown the effectiveness of the use of PPOS compared to the GnRH (antagonist or agonist) in IVF cycles. For oocyte donation only

three studies were carried out analyzing the effect of PPOS with a recombinant FSH (FSHr)

Study design, size, duration: It is a retrospective, cohort, paired study. We analyzed egg donation cycles carried out in Next Fertility Valencia from January 2020 to May 2021. In total 93 donors were selected as eligible, each one undergoing both the PPOS and GnRH antagonist cycles for a total of 186 cycles, 54 received FSHr and 39 HP-FSHu. To attempt to eliminate possible bias due to interpersonal differences, every patient was her own control.

Participants/materials, setting, methods: All donors underwent two controlled ovarian stimulation cycles within 6 months, one using flexible GnRH antagonist (Ganirelix) and the other using medroxyprogesterone acetate (MPA), with FSHr or FSHu monotherapy and a GnRH-agonist triggering. The oocyte yield was analyzed focusing on: the number of oocytes retrieved, mature (MII) oocytes and useful oocytes (mature oocytes without morphological alterations). We also evaluated the Ovarian sensitivity index (OSI) calculating the oocyte number for each 1000 IU of gonadotropin used

Main results and the role of chance: Total gonadotropin consumption and duration of ovarian stimulation were similar in both models of pituitary suppression regardless the type of FSH. One less control is required with MPA than the GnRH-ant in both FSH groups. There were no premature ovulations in MPA or GnRH antagonist cycles regardless of the gonadotropin used. In the FSHr group no difference was observed in the oocyte yield and OSI between MPA and GnRHa. On the other hand, in the FSHu group a statistically significant improvement in the number of total oocytes retrieved (20,2 ± 8,5 vs 23,8 ± 10,9), total MII (18,0 ± 7,2 vs 21,2 ± 10,4) and OSI (6,4 ± 3,5 vs 7,7 ± 4,6) was observed in favor of the use of MPA. Also, the number of useful MII was higher with MPA (15,7 ± 6,5 vs 18,6 ± 10,4). No significant differences were detected in fertilization and blastocyst rate regardless the type of pituitary suppression and FSH used

Limitations, reasons for caution: The main limitation lies in the retrospective nature of the study. However, since each patient is her own control (Paired study), it gives an added value

Wider implications of the findings: The efficacy and efficiency of the MPA does not seem to be affected by the type of gonadotropin used, this further reassures about the possibility of using it with patients in case of fertility preservation cycles, PGT-A/M, "DuoStim" cycles

Trial registration number: Not applicable

Abstract citation ID: dead093.928

P-599 The use of serum test to detect the LH surge in ultrasound-monitored intrauterine insemination (IUI) significantly increases pregnancy rates.

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Study question: Could a serum test to detect LH surge in ultrasound-monitored IUI cycle improve pregnancy rates?

Summary answer: Serum test to detect LH surges with ultrasound monitoring during IUI cycle significantly increased pregnancy rates.

What is known already: IUI is known as the first line of treatment for infertile couples and is usually administered with human chorionic gonadotropin (hCG) before ovulation with clomiphene citrate (CC) or/and human menopausal gonadotropin (hMG) to ovarian known to stimulate. One of the key factor for IUI is that insemination should be performed close to ovulation time, as there are many reports that sperm injection at the ovulation time has a high pregnancy rates. However, there are still few reports on the optimal timing of ovulation for performing IUI.

Study design, size, duration: The IUI performed from January 2016 through November 2022 (n = 3187) was included in this study. A total of 875 patients underwent the IUI by inferring LH surge with procedure with serum test, while 2312 patients underwent the procedure IUI without the serum test (control).

Participants/materials, setting, methods: In this study included 3187 IUI cycle were analyzed. Controlled ovarian hyperstimulation was conducted with CC (100 mg/day) and hMG. Patients were divided into two groups with serum test or not test (control). The serum test group was monitored LH surge by inferring LH peak during IUI cycle. When the sufficient follicular size and endometrial thickness had been reached, hCG was administrated and insemination was performed. Pregnancy rates were compared by Chi-squared test.

Main results and the role of chance: In each group, there were no significantly difference in age (34.4 ± 3.7 vs. 34.7 ± 3.7 years old) and endometrial thickness (8.8 ± 2.2 vs. 8.9 ± 2.2 mm) for IUI cycle condition. The serum test group was significantly higher clinical pregnancy rates compared to control group (19.0 vs. 15.7 % respectively) during IUI cycle ($P < 0.05$). In addition, patients were classified according to the history of at least one or more failed IUI cycle. The clinical pregnancy rates was significantly higher in the serum test group than control group (21.6 vs. 10.8 % respectively) who history of at least one or more failed IUI cycle ($P < 0.001$).

Limitations, reasons for caution: A limitation of this study has a too small size of the intervention group compared with the control group.

Wider implications of the findings: In our results, the serum test group had a significantly higher clinical pregnancy rates than the control group. Therefore, detecting a surge in LH with a serum test during the IUI cycle could predict the optimal timing of ovulation and thus increase the pregnancy rates.

Trial registration number: not applicable

Abstract citation ID: dead093.929

P-600 cannabinoids exposure during pregnancy affects female offsprings reproduction

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Study question: Could in-utero exposure to cannabinoids alter the reproductive health of female offspring?

Summary answer: Prenatal exposure to cannabinoids causes a reduction of the ovarian reserve in F1 with compromised reproductive lifespan and low birth weight in F1/ F2 generations.

What is known already: Cannabis use during pregnancy is associated with adverse neonatal outcomes: preterm birth, intrauterine growth retardation, low birth weight, and neurological developmental changes. However, the impact on the germ line is still little known. Cannabis/cannabinoids exert their biological effect by activating two main cannabinoid receptors: CB1 and CB2. We previously demonstrated that fetal oocytes express CB2 receptors at a higher level than CB1. *In vitro* treatment of fetal oocytes with JWH-133, a selective CB2 agonist, induces an acceleration of meiosis and an increase in the percentage of γ-H2AX-positive cells and in TUNEL-positive cells (apoptotic cells).

Study design, size, duration: Five pregnant CD1 female mice were intraperitoneally injected with a single dose of JWH-133, at 1.5 mg/kg for 5 days between embryonic period (E) 12.5 to E16.5, while control pregnant females received a vehicle containing saline. At delivery, on post-natal day (PND) 2, 30 and 365, F1 pups were counted, weighted and tissues from females were collected. At adult age, F1 females were mated with control mice and F2 generation was morphologically analysed.

Participants/materials, setting, methods: Ovaries from control and in utero exposed mice were fixed in paraformaldehyde and stained with Haematoxylin and Eosin (H&E). Gonads were embedded in paraplast and sectioned at 5μm Leica-RM 2035 Microtome. Follicles in each ovary were counted serially in every third section through the entire ovary. Only healthy, non-atretic follicles with visible oocyte nuclei were scored.

Main results and the role of chance: We demonstrate that *in vivo* exposure to the cannabinoid JWH-133 during fetal life, through the administration of the drug to pregnant females, causes a significant reduction in the offspring body weight at birth (LBW). Histological analysis of the ovaries isolated from

newborn females (PND2) shows a significant reduction of the primordial and primary follicles. This reduced ovarian reserve is observed also at PND30 and at PND365. At old adult age, these females, crossed with control males, show a drastic decrease in their litter size (F2) across the life course in comparison with control females. This suggests that the pool of primordial follicles becomes prematurely depleted in in-utero exposed F1 females, compromising their reproductive lifespan. Moreover, we find that F2 pups from prenatally exposed mothers show LBW but have an unaltered number of ovarian follicles in females. Since F2 pups have never been exposed to the drug, our results suggest that LBW is transmitted from the mother intergenerationally, while the reduced ovarian reserve is a consequence of direct exposure to the drug during fetal life of F1 females and it is not transmitted to the F2 generation.

Limitations, reasons for caution: The effects observed in mice with JWH-133 cannot be directly translated to cannabis effects since the active principle of cannabis (THC) activates both the cannabinoid receptors. Moreover, this study lacks molecular analysis of gene expression implicated in intergenerational transmission.

Wider implications of the findings: This is the first direct demonstration that prenatal exposure to cannabinoids could have long-term critical consequences for females' reproductive health. In humans, diminished ovarian reserve is characterized by poor fertility outcomes and it represents a major challenge in reproductive medicine.

Trial registration number: not applicable

Abstract citation ID: dead093.930
P-601 Comparison of adverse obstetric outcomes in women with the hyperandrogenic syndromes, polycystic ovary syndrome and Cushing's syndrome: an evaluation of a population database

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Study question: How does the risk for adverse obstetric outcomes differ among women with polycystic ovary syndrome (PCOS) and women with Cushing's syndrome (CUS)?

Summary answer: PCOS increased the risk of gestational diabetes and cesarean section relative to CUS, whereas CUS increased the prevalence of operative vaginal delivery and blood transfusions.

What is known already: PCOS and CUS are hyperandrogenic disorders that have previously been associated with unique adverse obstetric outcomes. Despite there being similarities in the hyperandrogenism and insulin resistance of these disorders, there is a lack of knowledge when comparing their specific risks of pregnancy complications.

Study design, size, duration: A retrospective population-based study utilizing data from the Healthcare Cost and Utilization Project—Nationwide Inpatient Sample (HCUP-NIS), was performed. A dataset of all deliveries between 2004 and 2014 inclusively, was created. 14 881 deliveries to women with PCOS and 134 deliveries to women with CUS were identified. The HCUP-NIS presents information on approximately 20% of admissions to US hospitals. Data was not continued into 2015 when ICD-10 codes were used, which are not comparable.

Participants/materials, setting, methods: Descriptive analyses were performed to compare the demographic features among both groups using chi-squared tests. Multivariate logistic regression analysis was performed to calculate unadjusted and adjusted odds ratios (aORs) and corresponding 95% confidence intervals (CI). According to Tri-Council Policy statement (2018), IRB approval was not required, given data was anonymous and publicly available.

Main results and the role of chance: At baseline, CUS was associated with a higher risk of chronic hypertension ($P < 0.001$), pregestational diabetes mellitus ($P = 0.01$), thyroid disease ($P = 0.004$), and higher rates of smoking during pregnancy ($P = 0.02$) whereas PCOS was associated with higher rates of obesity ($P = 0.01$). In terms of obstetric outcomes, PCOS increased the prevalence of gestational diabetes mellitus ($P = 0.002$, adjusted[a] OR 2.73; 95% CI 1.46 to 5.12), and cesarean section ($P < 0.001$, aOR 2.63; 95% CI 1.81-3.83) in comparison to CUS. CUS increased the prevalence of operative vaginal delivery ($P < 0.001$, aOR 0.10; 95% CI 0.06-0.14), and transfusion ($P = 0.002$, aOR 0.25; 95% CI 0.11-0.59) in comparison to deliveries to women with PCOS. No significant differences were found in terms of pregnancy-induced hypertension ($P = 0.78$), gestational hypertension ($P = 0.86$), preeclampsia ($P = 0.25$), preeclampsia or eclampsia superimposed on pre-existing hypertension ($P = 0.13$), premature rupture of membranes ($P = 0.99$), preterm delivery ($P = 0.17$), placental abruption ($P = 0.82$), chorioamnionitis ($P = 0.16$), spontaneous vaginal delivery ($P = 0.35$), postpartum hemorrhage ($P = 0.29$), and maternal infection ($P = 0.11$). In terms of neonatal outcomes, both deliveries to women with PCOS and women with CUS had similar outcomes for small for gestational age infants ($P = 0.52$), intrauterine fetal demise ($P = 0.94$), and congenital anomalies ($P = 0.53$).

Limitations, reasons for caution: The data within this retrospective cohort study relies on the accuracy of the individuals collecting the data. Data on medication use and compliance was unavailable.

Wider implications of the findings: Pregnant women with PCOS and CUS are at risk for certain specific obstetric complications, with most risks being similar. The magnitude of the insulin resistance in PCOS may be greater than in CUS due to the increased risk of gestational diabetes in PCOS when controlling for obesity and confounding effects.

Trial registration number: Not applicable

Abstract citation ID: dead093.931
P-602 Ovarian stimulation without LH suppression in the freeze-all era

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Study question: Can controlled ovarian stimulation be performed without LH suppression in the freeze-all era?

Summary answer: The oocyte and embryo quality in patients who were treated with freeze-all cycles is not affected without LH suppression.

What is known already: The LH suppression is a crucial part of the controlled ovarian stimulation in fresh IVF cycles. It is generally accepted that prolonged or premature exposure to LH can cause early progesterone secretion and abnormal oocyte maturation. Several agents such as GnRH agonists, antagonists and progestins have been used for LH suppression. Recently, different protocols such as duostim, luteal phase stimulation, random start stimulation have been developed in parallel with the widespread application of the freeze-all strategy. No decrease was observed in the quality, maturation and fertilization of oocytes obtained in these protocols.

Study design, size, duration: This study was retrospectively performed, with cycles extracted from freeze-all-IVF treatments performed between May 2022 and January 2023, to compare the efficacy of ovarian stimulations with or without LH suppression. LH suppression cycles were matched 1:1 with only gonadotropin cycles using age, BMI and antral follicle count, resulting in 160 matched followed by IVF or ICSI with the freeze-all strategy. The primary outcome of the trial was the number of oocytes and mature oocytes retrieved.

Participants/materials, setting, methods: The patients were evaluated in 2 groups as the only gonadotropin (rec FSH- study group) or antagonist protocol (rec FSH+cetrorelix- control group). The rec-FSH was administered at the second or third day of menstrual cycle in both groups. The cetrorelix was started when the leading follicle was 13mm or more in the control group. Oocyte maturation was triggered by hcg or with GnRH agonist (triptorelin). All viable embryos were cryopreserved for frozen embryo transfer.

Main results and the role of chance: Basic characteristics in both groups were similar. There was no significant difference in the number of oocytes ($11.87 \pm 6.22.3$ (mean $\pm$ SD) for the study group versus 13.47 ± 7.69 for the control group, ($P = 0.14$)) or the mature oocytes retrieved (8.46 ± 4.8 for the study group versus 9.78 ± 6.3 for the control group, ($P = 0.13$)) between the groups. The number of fertilized oocytes did not differ between the two groups (6.67 ± 4.6 for the study group vs 7.56 ± 5 for the control group). The mean progesterone LeveL at trigger day in the only rec FSH

group was higher than that in the rec FSH+ Cetrorelix group (1.71 ± 1.37 for the study group vs 1.35 ± 0.5 for the control group P = 0.03). No patients experienced ovarian hyperstimulation syndrome.

Limitations, reasons for caution: Main limitation of this study is, that it is retrospective. Another limitation is that this study includes number of oocytes, mature oocytes and fertilization rate solely. The clinical pregnancy rates and live birth rates were not determinated in the freeze-all cycles due to the short duration of the study.

Wider implications of the findings: LH surge and elevation of progesterone do not have negative effects on oocyte quality and maturation. Ovulation induction can be performed without LH suppression in freeze-all cycles. Undoubtedly, there is a need for new studies investigating the effects of only gonadotropin administration on live birth rates in frozen-thawed embryo transfers.

Trial registration number: Not applicable

Abstract citation ID: dead093.932

P-603 Viability of home monitoring of estrone-3-glucuronide (E1-3G) urine levels in controlled ovarian stimulation: A pilot study

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Study question: Can the regular determination of E1-3G levels in the patient's urine be used for successful monitoring of progestin-primed ovarian stimulation protocol (PPOS)?

Summary answer: Regular determination of E1-3G levels in urine can be successfully used in ovarian stimulation (OS), significantly reducing patient visits to the clinic.

What is known already: To reduce direct and indirect costs and to optimize the workload in IVF clinics, innovative approaches are being sought. It has only recently become possible to implement elements of telemedicine in OS monitoring. Patients have begun performing vaginal sonograms on their own.

Studies have further shown that there is a close correlation in the growth of E2 in serum and E1-3G in urine during the menstrual cycle and ovarian stimulation. Thus, theoretical models for the use of urinary E1-3G in ovarian stimulation were developed, which are subject to experimental verification and analysis.

Study design, size, duration: This is a pilot study carried out in a clinic, which aims to establish the growth rate of E1-3G and to determine its upper and lower limits of growth on each consecutive day at which stimulation is adequate and safe. Inclusion criteria were patients aged < 40 years and AMH ≧ 1.1 ng/mL. From September to December 2022, in total 28 first ovarian stimulation cycles from 28 infertility patients were included.

Participants/materials, setting, methods: A PPOS protocol with fixed doses of gonadotropins was used. From the second day of stimulation, the patients determined the E1-3G levels in their urine every day at home with a portable analyzer. In parallel, a standard ultrasound follow-up protocol was applied, accompanied by determination of E2, LH and P levels, in order to optimally control stimulation. Patients' convenience of this new approach was assessed using a questionnaire developed for the purpose.

Main results and the role of chance: The average female age was 32,1 years (± 4.4), BMI 22,9 kg/m2 (± 4,3), AMH 3,9 ng/ml (± 2,7), stimulation days 10,1 (± 1,2), collected oocytes 12,6 (± 8,5), MII oocytes 10,8 (± 7,9), fertilization rate 83,4% (± 22,7), blastocyst formation 66,9% (± 28,6), good quality blastocysts 31,1% (± 16,6).

The log-linear mixed effect model (LLMM) estimation produced reasonable estimates of 49% average day-to-day growth rates (slope fixed effect), with one standard deviation (SD) range of 25% to 77% across patients (SD of the slope random effect).

Moreover, there was a comparatively high correlation of 0.76 between the individual growth rates of E1G estimated over days 3-6 (the slope random effects of the LLMM model) and the E1-3G levels at day 10. In this way, the estimated slope random effects appear to have a prognostic value and may potentially have therapeutic implications, for example, adjustment of the stimulation dose. Moreover, the Spearman correlation between Estradiol and E1-3G was 0.83

After analyzing interviews and questionnaires, patients evaluated the applied method as easy and convenient, with 97% of them preferring OS monitoring to be performed in this manner compared to the standard method, which includes regular ultrasound examinations and determination of serum hormone levels.

Limitations, reasons for caution: The small statistical sample size is a limitation of this study, which was carried out in a single clinic. The results have to be confirmed in other clinics and with more diverse populations.

Wider implications of the findings: This is the first study to analyze the dynamics of E1-3G in the PPOS protocol and the limits of its increase during the entire OS. The results confirm our theoretical model for the successful use of urinary E1-3G in OS. The method is easy to apply and well-accepted by patients.

Trial registration number: N/A

Abstract citation ID: dead093.933

P-604 Inhibin B in Low Ovarian Reserve Patients: Correlation With Ovarian Reserve Markers and Oocyte Yield

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Study question: Is there a correlation between Inhibin B (InhB) serum levels and oocyte yield after ovarian stimulation (OS) in women with low ovarian reserve?

Summary answer: InhB presents a positive relationship with oocyte yield during OS in women with AMH<1.1ng/mL.

What is known already: InhB is produced by granulosa cells in the ovary with the goal of suppressing FSH production. Assay and intercycle variability have been the main limitations affecting specificity and reproducibility of InhB as a marker of ovarian reserve or oocyte yield after OS. This fact is attributed to low specific antibodies for the molecule in previous assays, exhibiting cross-reactivity with other glycoproteins in the transforming growth factor beta family. High specific ELISA assay,measuring bioavailable InhB (bio-InhB), may provide more accurate results, specially important in poor responders, as results may involve clinical decisions on whether or not to proceed with OS.

Study design, size, duration: Prospective observational study measuring bio-InhB in 72 women with low ovarian reserve, performing OS for IVF/ICSI, at a tertiary referral fertility center, from February 2019 to December 2021. Low ovarian reserve was defined as AMH serum levels <1.1ng/ml (Elecsys, Roche), following Bologna criteria. All patients performed OS for IVF/ICSI in a GnRH-antagonist protocol.

Participants/materials, setting, methods: On day 2/3 of cycle, before starting OS, AFC, FSH, LH, estradiol, progesterone and AMH (Elecsys, Roche) were measured. Extra serum samples were frozen at -20C for subsequent bio-InhB analysis (AnshLabs, Texas). Ethical approval was obtained (Research Ethics Committee-REFA033c) and informed consent was signed for all participants.

Main results and the role of chance: Patient characteristics were [Median(IQR)]: age: 39(36-42)years, BMI: 28.51(26.1-30.1) Kg/m2, AFC: 5.5(4-7), FSH:8.23(6.4-12.5) mIU/mL, LH: 6.54(4.51-8.91) mIU/mL, Estradiol: 41.18(28.47-56.44) pg/mL, AMH: 0.64(0.29-0.81)ng/mL, InhB: 62.69(33.78-97.92) pg/mL. Stimulation outcomes were: follicle number on day of trigger (Fdot): 5(3-7), cumulus-oocyte-complexes (COC's): 3(2-4.5)

andmetaphase II oocytes (MII): 3(1-4). Inh-B showed significant positive correlation (Spearman correlation) with AMH ($rs = 0.35$, $p = 0.003$), although was better with AFC ($rs = 0.43$, $p < 0.001$). Moreover, Inh-B serum levels presented positive association with oocyte yield: Fdot ($rs = 0.41$, $p < 0.001$), COC's ($rs = 0.36$, $p = 0.002$) and MII ($rs = 0.37$, $p = 0.002$).

AUROC analysis was performed to investigate which model predicted better a bad outcome (defined as <=3COC's after OS) in low ovarian reserve women. No significant benefit was observed when Inh-B was added to AMH+AFC model (AUROC AMH+AFC: 0.757; AUROC AMH+AFC+Inh-B: 0.759, $p = 0.843$).

Limitations, reasons for caution: Although clinical assessment was performed in the same centre following the same methodology, inter-observer variability for AFC is a limitation. Besides, fresh AMH serum samples were analysed using Elecsys assay, yet frozen serum samples were shipped to Ansh Lab (Texas) for batched Inh-B analysis.

Wider implications of the findings: Bio-InhB is correlated to oocyte yield during OS. Although no additional benefit for prediction in women with AMH<1.1ng/mL was observed when added to other currently used markers, future research should explore the utility of bio-inhB on other outcomes associated to ovarian reserve, as embryo quality and ploidy.

Trial registration number: Not applicable

Abstract citation ID: dead093.934

P-605 Hypoxia leads to diminished ovarian reserve in an age dependent manner

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Study question: To determine whether hypoxic ovarian damage is due to increased growth and follicles "burnout" or due increased apoptosis, and whether this damage is age dependent.

Summary answer: Direct tissue hypoxia causes activation and recruitment of dormant follicles without apoptotic changes. This effect is age dependent, more pronounced in adult- versus newborn ovaries.

What is known already: In a previous study we have shown that perinatal hypoxia causes premature activation and growth initiation of dormant follicles leading to diminished ovarian reserve. Other reports have also linked intrauterine deprivation conditions, premature delivery and small for gestational age newborns with diminished ovarian reserve due to premature ovarian follicular recruitment and exhaustion. Nevertheless, an indirect mechanism influencing ovarian follicle recruitment under hypoxic conditions, such as the release of stress related hormones cannot be ruled out.

Study design, size, duration: Animal studies were carried out using adult 6-week-old ($n = 8$) and one-day-old newborn ($n = 20$) ICR (CD-1) female mice. Throughout the study, animals were housed with an alternating 12-h light/dark cycle with access to food and water ad libitum, under constant room humidity and temperature. Animals were sacrificed and ovaries harvested and immediately cultured in Leibovitz media with L-Glutamine and 10% Fetal Bovine Serum.

Participants/materials, setting, methods: Ovarian tissue from dams and pups was cultured for 3 hours at 37°C to hypoxia (1% O_2 and 99% N_2) or normoxia (21% O_2 and 5% CO_2). Afterwards tissue was embedded in 4% formaldehyde for further processing and analyses. Sections were stained with H&E for follicular counts. For immunohistochemistry, sections were stained with Ki-67 (proliferation marker), anti-Caspase 3 and anti-FOXOp (apoptosis markers).

Main results and the role of chance: Hypoxia led to a significant decrease in the percentage of primordial follicles out of total follicles as compared to normoxia, both among dams and among pups ($3.17 \pm 2.75\%$ vs. $17.89 \pm 4.4\%$; $p = 0.004$ and 40.59 ± 14.88 vs. $81.92 \pm 31.56\%$, $p = 0.001$ respectively). This was accompanied by a concomitant increases in the percentages of growing (primary and secondary) follicles. Strikingly, this effect was more pronounced in adult dams as compared to that in newborn pups (6 fold versus 2 fold respectively). Ki67 staining indicated increased cell proliferation scores in follicular granulosa cells following hypoxia as compared to normoxia . Both Caspase 3 and FoxoP staining did not detect any changes in both markers of apoptosis either in oocytes, granulosa cells, theca cells or in stromal cells when ovaries were exposed to hypoxia as compared to normoxia.

Limitations, reasons for caution: This model was performed in mice and generalization to human ovarian physiology remains to be determined.

Wider implications of the findings: We hereby show that direct tissue hypoxia causes premature activation and growth initiation of dormant follicles without concomitant changes in apoptosis. This effect is age dependent being more pronounced in adult ovaries. Taken together it supports follicular "burn out" as a potential mechanism for hypoxia induced loss of ovarian reserve.

Trial registration number: IL-117-08-2017

Abstract citation ID: dead093.935

P-606 Does trigger timing make a difference in oocyte donation?

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Study question: Is there optimal trigger timing in controlled ovarian stimulation (COS) for egg donors for better clinical outcome?

Summary answer: Different trigger timings may not affect oocyte maturity rate. 35 hour's trigger may create optional timing for embryology to complete fertilization into a natural concept.

What is known already: Trigger exposure time is still a debatable clinical issue. Ovulation is triggered by administration of hCG or GnRH agonist, with retrieval taking place between 35th and 38th hour post triggering. There was a tendency to decrease post maturity risk for MII oocytes also early triggering will create optional timing for embryology laboratory for denudation, oocyte vitrification and fertilization process under oocyte cytoplasmic maturity (38 hours post trigger) and natural concept fertilization (till 40 hours post trigger) for better clinical outcome.

Study design, size, duration: This single center retrospective study included oocyte donors ($n = 1289$) with different timing of eggs retrieving. They were divided into two groups: group A, with exposure of the medicine trigger for 35 h and group B with timing which was 36 h before egg retrieval.

We evaluated the concordance between the follicle-to-oocyte index (FOI), antral follicle count (AFC), number of MII oocytes, maturation rate (MR), utilization rate (UR) and the timing of triggering.

Participants/materials, setting, methods: The number of donors was 784 and 505 in A and B groups respectively. The mean age of the patients in this study was (SD) 26,2 (3,2) and 26,7 (3,3) years. Cycle characteristics were compared. Data was assessed using the Shapiro-Wilk test for the normality of the distribution of the variables. The association between COS cycle parameters and the differences in COS output was assessed by T-Student (parametric) or U-Mann-Whitney (non-parametric).

Main results and the role of chance: Donors in groups A and B had a similar mean number of oocytes retrieved ($33,50 \pm 14,12$ vs $30,44 \pm 13,00$), in groups A and B respectively. The same tendency with no statistical difference was mentioned for values number of MII oocytes (SD): 25,16 (11,73) and 20,50 (10,19), for MR - 81% vs 80% and for AFC: 33,50 (14,18) and 30,44 (13,00) in groups A and B, respectively.

Unexpectedly, values for UR and FOI (SD) were statistically significant different ($p = <0.05$): 94,1% (17,2) vs 89,1% (20,2) and 92,51 (11,43) vs 83,43 (15,63), accordingly, in group A and B respectively. Group A donor oocytes vitrified on 38 hours post trigger and fertilized on 40 hours from sibling oocytes. Group B donor oocytes vitrified on 39 hours and fertilized on 42 hours from sibling oocytes. Values for oocyte survival rate post warming were statistically non-significant different ($p > 0.05$): 96,64% (10,95) vs 95,08% (10,03). Cleavage arrest rate were statistically significant different; 8,01% vs 28,06% and used blastocysts rate were statistically significant

different; 54,67% (5,02) vs 42,36% (4,08). Values for fertilization were statistically non-significant different; ($p > 0.05$): 98,64% (12,24) vs 97,08% (11.96) and used blastocysts rate were statistically significant different; 67,22% (6,18) vs 51,88%. (5,07)

Limitations, reasons for caution: This study is limited by its retrospective nature and the established strict time parameters of the study can be considered a limitation.

Wider implications of the findings: As we can see, the issue of the optimal period for oocyte retrieval after the administration of human chorionic gonadotropin should be considered very individually and in further studies it is possible to take into account wider time intervals in the design of trials.

Trial registration number: not applicable

Abstract citation ID: dead093.936

P-607 Interindividual variation of progesterone elevation post LH rise: Implications for natural cycle frozen embryo transfers in the individualized medicine era.

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Study question: To what extent does the period between LH-rise and P4-rise vary in ovulatory natural menstrual cycles?

Summary answer: Three distinct ovulatory patterns were noted defined as the period between LH-rise and P4-rise and varied by up to two days.

What is known already: The key to success of FET in a NC is the accurate diagnosis of ovulation to determine the optimal timing of embryo transfer. The most common method used to determine time of ovulation in NC is the detection of luteinizing hormone (LH) surge. It is well recognized that the endocrine profile of menstrual cycles varies not only amongst women but also from cycle to cycle for any given female. Most hormone trajectories in individual women differ considerably from the mean hormonal curves and LH surges culminating in ovulation appear to be highly variable in timing, amplitude and duration.

Study design, size, duration: Retrospective, observational study including 102 women who underwent endocrine monitoring during a frozen embryo transfer in a natural cycle between 1st January 2017 and 31st August 2021 in a tertiary IVF centre.

Participants/materials, setting, methods: Ultrasound scans were performed to monitor follicular growth. Serial measurements of serum LH, FSH, estradiol and P4 were commenced once a dominant follicle measuring $\geq$ 14mm was identified and on three consecutive days until (including) the day of ovulation. The LH surge was considered to have begun when the concentration rose by 180% above the most recent serum value. Progesterone concentrations of 1.0ng/ml and above were regarded as confirmation of ovulation to schedule FET.

Main results and the role of chance: Patients' characteristics presented as median and (range): age 35 years (23-43), BMI 25.24kg/m^2 (17.32-38.21), AMH 2.07 ng/ml (0.06-8.12). Twenty-one (20.6%) women had the LH-rise 2 days prior to P4 -rise, 71 (69.6%) had on the day immediately preceding and 10 (9.8%) on the same day of P4-rise. If FET was scheduled based on LH-rise, 30.4% of the patients would have their FET scheduled on a different day than FET scheduled according to P4-rise. The period between LH-rise and P4-rise would be approximately one day longer in 20.6% of the participants and one day shorter in 9.8% than the anticipated 24 hour period. There was a significant difference in body mass index between those women who had LH-rise 2 days prior to P4-rise (BMI 26.91 kg/m^2 (range 23.74-30.75) and the women who had LH-rise on the same day (BMI 22.39 kg/m^2 (range 20.91-24.96) ($p = 0.03$). The AMH levels were also found to be significantly lower in those women who had LH rise 2 days prior to P4-rise (AMH 1.59 ng/ml (range: 0.82-2.09) as compared to those who had LH-rise on the same day (AMH 2.67ng/ml (range: 1.83—6.63) ($p = 0.04$). LH may not serve as the best benchmark to schedule FET.

Limitations, reasons for caution: Frozen embryo transfers were scheduled according to serum progesterone levels therefore our study does not provide a direct answer to the question whether FET scheduled according to serum progesterone levels provides higher live birth rates than FET scheduled based on urinary or serum LH levels in a natural cycle.

Wider implications of the findings: Variation in the period between LH rise and progesterone rise in ovulatory cycles has implications for the choice of marker for the start of secretory transformation in frozen embryo transfer cycles. The study participants are representative of the relevant population of women undergoing frozen embryo transfer in a natural cycle.

Trial registration number: Not Applicable

Abstract citation ID: dead093.937

P-608 Intra-ovarian platelet-rich plasma administration plus successive accumulated embryo transfer could be a promising strategy for poor ovarian response management: a retrospective cohort study

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Study question: Does intra-ovarian injection of platelet-rich plasma (PRP) plus successive accumulated embryo transfer improve the outcome of Patients with Poor Ovarian Response (POR)?

Summary answer: Intra-ovarian injection of PRP plus successive embryo accumulation following mild stimulation and accumulated embryo transfer appears to be a optimal strategy for POR management.

What is known already: The treatment of poor responders is a challenge for both patients and clinicians. Various protocols have been adopted in attempts to increase the pregnancy rates in patients with POR; however, the outcomes of these strategies are disappointing. Alternatives are needed. A handful of clinical datas have reported promising results of the use of PRP injections into the ovary. In addition, some clinical evidence proved that embryo accumulation following mild stimulation appears to be an optimal strategy for POR management. However, limited knowledge is available on the effect of the combination of intra-ovarian injection of PRP and accumulated embryo transfer strategy.

Study design, size, duration: This single-center retrospective study has been performed from May 2021 to May 2022. Using the POSEIDON criteria, 13 group 3 and 36 group 4 POR women undergone intra-ovarian PRP injection combined with accumulation of embryos through 3 successive mild stimulation IVF (ICSI) cycles before transfer.

Participants/materials, setting, methods: POR Women (AMH<1.2ng/ml) who have undergone intra-ovarian PRP injection and followed by accumulation of embryos through 3 successive mild stimulation IVF (ICSI) cycles before transfer were eligible. According to the POSEIDON criteria, 49 women were included. The primary outcomes were Clinical pregnancy and cancelled cycles. The helpful variables (LH, FSH, AMH, estradiol and AFC) and embryology outcomes of IVF (ICSI) cycles were also collected.

Main results and the role of chance: The mean age of all participants was 37.67 $\pm$ 4.15 years and their mean body mass index was 21.52 $\pm$ 2.80 kg/m2. Autologous intraovarian PRP therapy significantly increased AMH levels (0.41 $\pm$ 0.05ng/mL vs 0.65 $\pm$ 0.13ng/mL, $P = 0.035$), AFC (2.02 $\pm$ 0.19 vs 2.94 $\pm$ 0.21, $P = 0.0013$) and decreased FSH (14.57 $\pm$ 1.141U/L vs 11.42 $\pm$ 0.81IU/L, $P = 0.03$). Autologous intraovarian PRP therapy accompanied with 3 successive cumulated cycles, significantly increased No. of accumulated embryos (0.46 $\pm$ 0.08 vs 1.61 $\pm$ 0.17, $P < 0.0001$) and blastocysts (0.04 $\pm$ 0.03 vs 0.92 $\pm$ 0.14, $P < 0.0001$). Also, intra-ovarian injection of PRP plus successive embryo accumulation strategy significantly reduced the rate of cancelled cycle (57.13% (28/49) vs 10.2% (5/49), $P < 0.0001$). Following this stategy, of 44 cases with accumulated embryos/blastocysts transfer, 20 (45.45%) got clinical pregnant.

Limitations, reasons for caution: The study was small samples and retrospective, without randomization. In addition, the ongoing pregnancy or live birth may reflect the effect much better.

Wider implications of the findings: Intra-ovarian injection of PRP plus successive embryo accumulation strategy significantly reduced the risk of cancelled cycle in POR; the clinical pregnant rate is about 45%. It could be the optimal strategy for POR management. Further studies are needed to verify these conclusions and the exact mechanisms of PRP.

Trial registration number: not applicable

Abstract citation ID: dead093.938

P-609 Progesterone monitoring in fresh and frozen in assisted reproductive technique (ART) cycles - a Systematic review and Meta-analysis.

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Study question: What is the impact of serum progesterone monitoring in fresh and frozen ART cycles on live birth rates (LBR)?

Summary answer: There is no evidence of benefit that monitoring progesterone impacts LBR in fresh and FET cycles.

What is known already: Elevated progesterone (EP) during the follicular phase is postulated to cause detrimental effects on pregnancy outcomes by prematurely advancing the endometrium and affecting endometrial receptivity. In the luteal phase, optimisation of the supplementation of progesterone is in question. However, there has not been a consensus on the timing of monitoring or a threshold level to define an optimal progesterone level that leads to improved pregnancy outcomes.

Study design, size, duration: A systematic search of the following electronic databases: EMBASE, MEDLINE, CINAHL and PubMed identified non-randomised comparative cohort studies between the year 2000 to 2022. The following keywords progesterone, assisted reproductive technique and pregnancy outcomes and their respective variants were used. Study selection was performed following initial screen of the titles and abstracts. The full manuscripts were examined for compliance with the inclusion criteria and studies eligible for inclusion in the review were selected.

Participants/materials, setting, methods: PICOS study protocol was used. We included studies reporting per woman data of women undergoing fresh IVF/ICSI cycles (with controlled ovarian stimulation) and FET cycles (natural or medicated), with extractable data on pregnancy outcomes and using serum progesterone monitoring. We excluded interventional studies and studies involving oocyte or gamete donation. Comparisons were made between the population with EP versus non-elevated progesterone in fresh and FET cycles.

Main results and the role of chance: We included 63 studies (N = 57,586 women) for the meta-analysis. EP at baseline did not reveal any difference in LBR (odds ratio (OR) 0.76, 95% confidence interval (CI) 0.39 to 1.49; I^2 = 0%; 2 studies, 309 women; very low-quality evidence); EP on the day of trigger is associated with a reduction in LBR and CPR in various threshold, (LBR: P > 1.0ng/ml, P > 1.1ng/ml, P > 2.0ng/ml, CPR: P > 1.0ng/ml, P > 1.1ng/ml, P > 1.5ng/ml, P > 2.0ng/ml) in studies which examined both D3 and D5 embryos. In studies including only D5 embryos, there was no difference in pregnancy outcomes between EP and NEP groups; LBR (P > 1.5ng/ml, OR 0.96, 95% CI 0.81 to 1.14; I^2 = 55%; 3 studies, 5,174 women; very low-quality evidence); CPR (P > 1.5ng/ml, OR 0.90, 95% CI 0.78 to 1.04; I^2 = 50%; 6 studies, 5,705 women; very low-quality evidence). We were unable to meaningfully meta-analyse studies examining EP at oocyte retrieval or during luteal phase in fresh cycle and EP at day before ovulation or during luteal phase in FET cycle.

Limitations, reasons for caution: Observational studies were included in this meta-analysis with wide variation of progesterone thresholds and timing that increases the clinical heterogeneity. The incidence of EP and low progesterone varies among the included studies. The immunoassays used for progesterone measurement were not standardised with questionable accuracy at low levels.

Wider implications of the findings: There is no evidence that EP at baseline and at the time of ovulation trigger with D5 embryos impacts LBR. There is insufficient evidence that progesterone monitoring during day of oocyte retrieval, luteal phase in both fresh and frozen cycles impact LBR.

Trial registration number: PROSPERO (registration ID CRD42022382423)

Abstract citation ID: dead093.939

P-610 Lipid metabolism mediated the effect of glucose homeostasis and insulin resistance on IVF/ICSI outcomes in PCOS women

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Study question: To investigate the potential mediation role of lipid metabolism on the effect of glucose metabolism on IVF/ICSI outcomes in PCOS women.

Summary answer: Lipid metabolism indicators were possible mediators of the effect of glucose metabolism indicators on IVF/ICSI early reproductive outcomes in PCOS women.

What is known already: Women with PCOS have high incidences of dyslipidemia, obesity, impaired glucose tolerance (IGT), diabetes, and insulin resistance (IR) and are fragile to female infertility. The causality between dyslipidemia, glucose homeostasis dysregulation, and IR has been widely discussed. Dyslipidemia may be an intermediate biological mechanism for the associations between abnormal glucose metabolism and potential disorders of oocytogenesis and embryogenesis in PCOS women.

Study design, size, duration: We retrospectively analyzed 917 PCOS women aged between 20 – 45 years old who underwent their first fresh IVF/ICSI cycle with autologous oocytes during 2018 – 2020 at a reproductive center.

Participants/materials, setting, methods: Data on baseline reproductive and cycle characteristics were collected from the internal database derived from the electronic medical records. The pregnancy outcomes were collected by well-trained follow-up staff from telephone interviews. Associations between glucose and lipid metabolism indicators and IVF/ICSI outcomes were explored using multivariable generalized linear regressions. Mediation analyses were conducted to examine the role of lipid metabolism on the association between glucose metabolism and IVF/ICSI outcomes.

Main results and the role of chance: Significant dose-dependent relationships were found between glucose metabolism indicators and IVF/ICSI early reproductive outcomes, as well as between glucose metabolism indicators and lipid metabolism indicators (all $P < 0.05$). Also, we found significant dose-dependent relationships between lipid metabolism indicators and IVF/ICSI early reproductive outcomes (all $P < 0.05$). The mediation analysis indicated that elevated FPG, 2hPG, FPI, 2hPI, HbA1c, and HOMA2-IR were significantly associated with decreased retrieved oocyte count, MII oocyte count, normal fertilization count, normal cleavage count, high-quality embryo count, or blastocyst formation count after controlling for lipid metabolism indicators. Serum TG mediated 6.0 – 31.0% of the associations; serum TC mediated 6.1 – 10.8% of the associations; serum HDL-C mediated 9.4 – 43.6% of the associations; serum LDL-C mediated 4.2 – 18.2% of the associations; and BMI mediated 26.7 – 97.7% of the associations.

Limitations, reasons for caution: Given the retrospective nature, bias in data collection cannot be excluded. Besides, we did not consider any adjunctive therapy, including diet, exercise, or medication (i.e., metformin) before or during IVF treatment. The possible random errors in single measurements may also lead to certain imprecise estimates of association.

Wider implications of the findings: Our study emphasizes a mediation role of lipid metabolism in the impact of glucose metabolism on oocytogenesis and embryogenesis in PCOS women, suggesting that the monitoring and management of preconception lipid metabolism, glucose homeostasis, and IR are essential for improving the IVF/ICSI outcomes of PCOS women.

Trial registration number: Not Applicable

Abstract citation ID: dead093.940

P-611 Progestin-Primed Ovarian Stimulation is a non-inferior alternative to the GnRH Antagonist Protocol in patients undergoing ovarian stimulation: a propensity score weighting analysis

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Study question: To evaluate the effectiveness of the progestin-primed ovarian stimulation (PPOS) protocol versus the gonadotropin-releasing hormone antagonist (GnRH-ant) protocol in ovarian stimulation.

Summary answer: The PPOS protocol with dydrogesterone provided similar embryo outcomes to the GnRH-ant protocol. The serum LH concentration during ovarian stimulation using PPOS was well-controlled.

What is known already: Since Yanping Kuang's proposal PPOS in 2015, several studies on the effectiveness of this treatment regimen have been published. Although it has been suggested that outcomes of the PPOS regimen were comparable to those of GnRH-ant regimen, the findings were limited due to differences in study design, patient characteristics, dosage and type of progestins. While medroxyprogesterone acetate is often used in PPOS studies, dydrogesterone has seen far less popularity. Additionally, dydrogesterone is a relatively weak LH inhibitor compared to MPA, leading to concern about the increased incidence of unexpected LH surge

Study design, size, duration: We conducted a retrospective study among women who underwent In Vitro Fertilization at the Assisted Reproduction Center, Tam Anh General Hospital, Vietnam from January 1st, 2022 to October 10th, 2022.

Participants/materials, setting, methods: We included 804 patients who underwent ovarian stimulation, among which 208 used the PPOS protocol with dydrogesterone (30 mg dydrogesteron per day from the beginning of stimulation until the trigger-day) and 598 women used the GnRH-ant protocol (cetrorelix 0.25 daily, from day 6 of ovarian stimulation until the trigger-day). In addition to unadjusted analysis, we used propensity score weighting to account for confounding that might possibly remain.

Main results and the role of chance: Baseline characteristics were comparable in both groups. In both unadjusted and adjusted analysis, the mean number of good cleavage embryos in PPOS (6.33) was non-inferior to GnRH-ant (6.44; unadjusted ratio of two means 1.02, 95%CI 0.92, 1.13). The trigger-day estradiol level in patients with PPOS was higher (4,420 vs 3,830 pmol/L in GnRH-ant) despite similar total follicle stimulating hormone dose and fewer days of ovarian stimulation. The overall number of oocytes retrieved, the number of MII oocytes, the number of cleavage embryos, the number of blastocysts, and the number of good blastocysts were comparable between the two protocols. None of the PPOS patients had an unexpected LH surge, and serum LH levels decreased slightly during ovarian stimulation.

Limitations, reasons for caution: Due to the nature of the retrospective study design, we did not record the progesterone level on the trigger-day in the PPOS regimen; therefore, identifying LH surge based on LH and progesterone levels is not feasible. Furthermore, we did not study the outcomes of embryo transfer.

Wider implications of the findings: These results supported the use of PPOS with dydrogesterone as a cheap and effective alternative for clinicians in clinical practice. In patients with an indication for freeze-all embryo, the PPOS protocol could be recommended.

Trial registration number: Not Applicable

Abstract citation ID: dead093.941

P-612 Comparison the effectiveness and safety of letrozole-stimulated and hormone replacement treatment in endometrial preparation for frozen embryo transfer in patients with polycystic ovary syndrome

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Study question: Whether letrozole-stimulated protocol has advantages over hormone replacement therapy (HRT) in frozen embryo transfer in patients with polycystic ovary syndrome (PCOS)?

Summary answer: Letrozole-stimulated protocol is more suitable for endometrial preparation than HRT in patients with PCOS, which can increase the live birth rate and reduce pregnancy complications.

What is known already: The current routine endometrial preparation protocol for women with PCOS is HRT. However, HRT is expensive, and recent studies have found HRT may increase the risk of adverse maternal and neonatal complications such as hypertensive disorders of pregnancy. Letrozole induces mono-ovulatory and has no antiestrogenic effect, and has been found to increase live birth rate compared with clomiphene in fresh embryo cycles. However, Letrozole is rarely used in frozen embryo cycles. Whether letrozole-stimulated protocol is suitable for frozen embryo transfer in patients with PCOS and for whom is suitable is still lack of evidence.

Study design, size, duration: This is a retrospective cohort study involving all frozen embryo transfer cycles with letrozole-stimulated and HRT for PCOS during the period from August 2018 to December 2020 at a tertiary care center.

Participants/materials, setting, methods: A total of 2011 letrozole-stimulated cycles and 2211 HRT cycles were included in the analysis. Multivariate Logistic regression was used to analyze the differences in clinical pregnancy rate, live birth rate, miscarriage rate, the incidence of other pregnancy and obstetric outcomes between letrozole-stimulated protocol and HRT after adjusting for possible confounding factors. Subgroup analysis was used to explore the population for which letrozole-stimulated protocol was suitable.

Main results and the role of chance: After adjusting for confounding, letrozole-stimulated protocol increased the clinical pregnancy rate (OR,1.44; 95%CI,1.21-1.70), live birth rate (OR,1.49; 95%CI,1.27-1.74) and reduced the incidence of miscarriage (OR, 0.71; 95%CI,0.55-0.92), hypertensive disorders of pregnancy (OR, 0.66; 95%CI,0.45,0.98), gestational diabetes mellitus (OR,0.75; 95%CI,0.57,0.98) and cesarean section (OR,0.78; 95%CI,0.61,0.99) than HRT. There were no significant differences in other outcomes such as preterm birth, small for gestational age, and large for gestational age between the two endometrial preparation protocols. Subgroup analysis according to maternal age, BMI, insulin resistance and PCOS classification showed that the live birth rate of letrozole-stimulated protocol was significantly higher than that of HRT in all subgroups.

Limitations, reasons for caution: This study is a retrospective study, the population characteristics of the two endometrial preparation protocols existed differences. Although multivariate logistic regression was used to adjust some factors, the interference of known and unknown factors on the outcomes cannot be completely avoided.

Wider implications of the findings: This retrospective study found letrozole-stimulated protocol has more advantages than HRT, which can improve the live birth rate and reduce the incidence of hypertensive disorders of pregnancy, gestational diabetes mellitus in patients with PCOS. High-quality well-powered randomised clinical trials and possible mechanistic studies are needed to further validate this conclusion.

Trial registration number: NA

Abstract citation ID: dead093.942
P-613 Deletion of the CLPP gene in oocytes exacerbates chemotherapy-induced ovarian damage

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Study question: We attempted to investigate the role of the *Clpp* in chemotherapy-induced ovarian damage and the decline of oocyte function

Summary answer: We clarified that the deletion of *Clpp* gene in oocytes will aggravate the ovarian damage and the decline of oocyte function caused by chemotherapeutic drugs.

What is known already: Chemotherapy is extensively used to treat cancers and is often associated with ovarian damage which leads to premature ovarian insufficiency and infertility. Mitochondria have the critical role of producing energy and maintaining normal cellular activities for oocyte maturation and folliculogenesis.

Study design, size, duration: This study used a mouse model with oocyte-specific deletion of mitochondrial stress response gene Caseinolytic peptidase P (*Clpp*) to investigate mitochondrial homeostasis in oocytes from mice receiving a chemotherapeutic drug cyclophosphamide (CTX).

Participants/materials, setting, methods: First, analyze the reproductive phenotype and fertility test of mice, observe the ovarian development, the proportion of follicles at all levels, the spindle of oocytes and the aneuploidy rate of chromosomes at the MII stage, and using multiple mitochondrial function indicator kit to detect mitochondrial function. Then, we modeled 8-week-old mice with a single injection of 100ul 75uM cyclophosphamide. After 14 days, check the above indicators again. the impact of cyclophosphamide modeling will be analyzed.

Main results and the role of chance: We found the specific deletion of *Clpp* in oocytes reduced fecundity of the mice at advanced age. The *Clpp* deletion led to meiotic defects and impaired mitochondrial distribution and function in the MII oocytes. CTX induction further affected oocyte competence in *Clpp* cKO mice and lead to an exhaustive disruption of blastocytes formation due to the severe effects on mitochondrial distributions and functions.

Limitations, reasons for caution: We only clarified that the deletion of *Clpp* gene in oocytes will through affecting the mitochondrial function aggravate the ovarian damage and the decline of oocyte function caused by chemotherapeutic drugs.But the underlying mechanism has not been able to explore further.

Wider implications of the findings: This is first study specifically on the impact of oocyte specific deletion of *Clpp* on oocyte competence and early embryo development. At the same time, the role of *Clpp* gene in the ovary damaged by chemotherapy drugs was further clarified.

Trial registration number: not applicable

Abstract citation ID: dead093.943
P-614 Endometrial preparation: effect of estrogen levels before the embryo transfer on the live birth rate from 14825 freezing-all cycles

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Study question: Is there a difference in live birth rate during discrepant estrogen levels before the embryo transfer following either hormone replacement therapy (HRT) or natural cycle(NC)?

Summary answer: The higher estrogen level on the day before embryo transfer may reduce the live birth rate in either HRT or NC.

What is known already: The endometrial preparation of frozen embryo transfer cycle is to synchronize of endometrium and embryo under the condition of exogenous estrogen and progesterone drugs in HRT or follicle monitoring in NC. Estrogen is crucial and different estrogen levels could imply variations on endometrial preparation. Information on the effect of discrepant estrogen levels before the embryo transfer on reproductive outcomes of women undergoing freezing-all cycles is scarce.

Study design, size, duration: Retrospective cohort study including 14825 first frozen embryo transfers following IVF/ICSI undergoing freezing-all cycles from January 2016 to December 2020. A total of 12305 (83.0%) patients received NC while 2520 (17.0%) received HRT.

Participants/materials, setting, methods: Effect of estrogen levels on live birth rates, stratififfied by deciles and ROC to determine the cutoff values for estrogen level on the day before embryo transfer.Multivariable logistic regression analysis was performed to adjust for confounders.

Main results and the role of chance: The live birth rate was 44.7% and 52.4% in HRT and NC respectively. The live birth rate of HRT began to decline when the estrogen level was P60 in the deciles which the cut-off values of ROC was closed with the estrogen level was 891.6 pg/ml. The lower level of estrogen on the day before embryo transfer in HRT corresponds to a higher live birth rate(51.4% vs 32.9%).The cut-off values of ROC in NC was statistically different with the estrogen level was 194.4 pg/ml. The lower level of estrogen on the day before embryo transfer in NC corresponds to a higher live rate(53.0% vs 48.6%).After adjusting the possible confounding factors by multivariate logistic regression, it was found that the estrogen level on the day before embryo transfer in HRT and NC was the independent influencing factor of live birth rate with the OR of 0.57 (0.47,0.68) and 0.82 (0.74,0.90), respectively.

Limitations, reasons for caution: The greatest limitation of this study is its retrospective study. On the other hand, this study was performed using non-PGT cycle, although this is unlikely to affect the results,we cannot exclude the possibility that euploid embryo responds differently to endometrial state in comparison to aneuploid embryo.

Wider implications of the findings: In freezing-all cycles, the higher estrogen level on the day before embryo transfer may reduce the live birth rate in either HRT or NC. Clinicians should pay attention to the control of estrogen level in luteal phase.

Trial registration number: Nona

Abstract citation ID: dead093.944
P-615 Ovarian rejuvenation with human placenta hydrolyzate

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Study question: Is it possible to rejuvenate the ovaries with human placenta hydrolyzate?

Summary answer: Injecting human placenta hydrolyzate into the ovaries can activate dormant follicles and produce mature oocytes in patients with ovarian insufficiency.

What is known already: Patients with low ovarian reserve and/or ovarian failure are a difficult category for reproductive specialists. Attempts to rejuvenate the ovaries have been made for several years. The use of adjuvant therapy such as PRP can activate dormant follicles. Human placenta hydrolyzate contains growth factors, hormones, trace elements, vitamins. Administration of human placenta hydrolyzate directly to the ovaries can also activate folliculogenesis and oogenesis.

Study design, size, duration: A cohort study was conducted, which involved 20 women with low ovarian reserve who underwent IVF / ICSI at the Person ICCR in 2022. AMH, FSH, the number of antral follicles, the number of received oocytes were determined before the injection of the human placenta hydrolyzate (JAPAN BIO PRODUCTS Co, LTD, Japan) into the ovaries and 1-3 months after procedures.

Participants/materials, setting, methods: All patients had previously undergone IVF/ICSI. 20 women with infertility, mean age was 39 ± 6 years. In September-October 2022, we received a human placenta hydrolyzate intraovarially. The human placenta hydrolyzate was injected into the ovaries as in a transvaginal puncture with a 20 G needle, 1 ml into each ovary. All participants gave their voluntary informed consent to the procedure.

Main results and the role of chance: On the 2-3rd day of the cycle before the procedure, the average level of AMH was 0.11 ± 0.09 ng/ml, FSH 38.11 ± 10.86 mIU/ml, the number of antral follicles 1.18 ± 0, 38, the number of mature oocytes obtained on TVP, 0.68 ± 0.19. After the introduction of the human placenta hydrolyzate into the ovaries, measurements were repeated 1-3 months later: AMH was 0.46 ± 0.18 ng/ml, p = 0.09; FSH was 13.54 ± 7.86 mIU/ml, p = 0.074; CAF 1.96 ± 0.17, p = 0.068; oocytes M2 1.51 ± 0.38, p = 0.058. Due to small cohort size and short follow-up time, we believe that ovarian activation by placental hydrolyzate takes place, this is especially noticeable in the number of obtained mature oocytes that went for freezing or fertilization: 4 patients received embryos suitable for PGT-A, one the embryo (mosaic) was transferred into the uterine cavity and pregnancy occurred. There was no any side effect after the injection.

Limitations, reasons for caution: Limitations relate to study design. More patients and longer follow-up are also needed.

Wider implications of the findings: Ovarian rejuvenation is currently an adjuvant therapy, high-level evidence is not yet available. However, for women with POI and POR, any opportunity to improve the chances of obtaining their own oocytes is valuable.

Trial registration number: NO APPLICABLE

Abstract citation ID: dead093.945
P-616 Evaluation of salivary ELISA for Oestradiol and Progesterone monitoring in IVF patients

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Study question: How do salivary E2 and P correlate with serum determinations across ovarian stimulation (OS) for IVF and artificial endometrial preparation cycles for embryo transfer?

Summary answer: Saliva-blood correlations were higher for E2 than for P. E2 saliva-blood ratios were patient-dependent. High serum P cases on trigger day could be identified.

What is known already: Steroid hormone concentrations in saliva are lower than in serum, even if the hormone is predominantly free. Therefore, quantitative assays need to be as sensitive as possible. Saliva-based assessments are a reliable and proven method for quantifying female hormone levels in premenopausal women during the natural cycle while being very precise and painless for patients. However, the cumulative experience in ovarian stimulation is relatively low. A recent study including 652 pairs of saliva-serum E2 samples reported correlations between 0.68 and 0.91, while 237 pairs of samples for P showed correlations ranging from -0.02 to 0.22 (Sakkas et al, 2020).

Study design, size, duration: Prospective cohort study including 300 saliva-blood pair samples from 120 patients undergoing OS for IVF (n = 90) or artificial endometrial preparation for embryo transfer (n = 30) between February and October 2022. Data were collected at each of 3 points of OS: early (days 1-5), mid (days 6-9), and trigger. Patients following endometrial preparation collected just one sample on the day of embryo transfer

Participants/materials, setting, methods: Thirty expected poor responders, 30 normal, and 30 expected high responders according to ovarian reserve evaluation were included. Two saliva samples per blood sample were collected: immediately after waking up, and at the clinic just before or after the blood sample was taken. Saliva samples were stored at -20°C and shipped to Mint Diagnostics (Kent, UK) for analysis of salivary E2 and P4

Main results and the role of chance: Saliva-blood E2 correlations (Spearman) were 0.68 (fasting) and 0.69 (clinic). 72% of individuals showed saliva-serum correlations > 0.8. Correlations improved when controlling for subgroup (low, normal, high responders). P4 saliva-blood correlations (Spearman) were 0.44 (fasting) and 0.29 (clinic). 41% of individuals showed correlations >0.8 on fasting samples, and 33% on clinic samples. There was a high variance of individual correlations (0-1), which were dependent on ovarian response subgroups ($p < 0.05$). A multivariable approach was developed to identify patients with a serum P4>1.5 ng/mL the day of trigger (n = 11) with clinic samples showing 81% sensitivity and 91% specificity and fasting samples showing 83% and 91% respectively demonstrating the possibility of shifting hormone analysis to the home rather than the clinic. Eighteen of 30 (60%) saliva samples collected on the day of ET on patients under hormonal replacement therapy showed values out of the assay range (>5000 pg/mL). Therefore, no correlations could be estimated.

Limitations, reasons for caution: Detection of patients with high P4 on the day of trigger was limited due to a short number of cases. No conclusions could be drawn in HRT patients for embryo transfer due to high salivary P4 values.

Wider implications of the findings: The current study shows that salivary monitoring of E2 and P4 is feasible. This more friendly non-invasive method could allow frequent hormone monitoring without the need for appointments at the clinic and for phlebotomy, thus resulting in more convenience for both the clinics and the patients.

Trial registration number: NCT05184777

Abstract citation ID: dead093.946
P-617 Combined oral contraceptive pill (COCP) pre-treatment and progestin-primed ovarian stimulation (PPOS) protocol: a detrimental combination for the oocyte yield in egg donation programs

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Study question: Could the use of combined oral contraceptive pill (COCP) pre-treatment before controlled ovarian hyperstimulation (COH) have a negative effect on oocyte yield in normo-responders patients?

Summary answer: When COH with progestin-primed ovarian stimulation (PPOS) protocol in normo-responders patients is performed, the COCP pre-treatment could have a detrimental effect on oocyte yield.

What is known already: COCP pre-treatment is widely used to schedule the start of COH and to organize the working week, but its usefulness and efficiency are still not clear. Most of studies on IVF outcomes have shown no effect of COCP on oocyte yield. For oocyte donation only few studies were carried out showing controversial effects on oocyte yield, but all considered short antagonist or long agonist protocols and no one PPOS protocol. COCP leads to long lasting pituitary suppression and lower LH level in follicular phase that could reduce the ability to effectively respond to GnRH-agonist trigger leading to poor oocyte yield.

Study design, size, duration: The egg donation cycles carried out from January to September 2022 in Next Fertility Valencia were retrospectively analyzed. Two analyses were conducted. Analysis A, comparative retrospective cohort study of 397 COH cycles: 290 with COCP and 107 without COCP. Analysis B, comparative retrospective paired study of 90 COH cycles: 45 with COCP and 45 without COCP, in which, to attempt to eliminate possible bias due to interpersonal differences, every patient was her own control.

Participants/materials, setting, methods: All donors underwent to COH with FSH monotherapy, PPOS protocol for pituitary suppression and a GnRH-agonist triggering. The oocyte yield was analyzed focusing on: the number of oocytes retrieved, mature (MII) oocytes and useful oocytes (good mature oocytes used for ICSI), the appropriate response to the GnRH-agonist trigger, the stimulation days, the total gonadotropins dose used and the cancellation rate (for low response or low LH level in follicular phase the first day of COH).

Main results and the role of chance: In both analyses, the data showed no differences in terms of stimulation days, total FSH dose, number of follicles $\geq$ 15 mm on the trigger day regardless of use of COCP. After conducting the analysis based on the administration or not of COCP, in the Analysis A a higher number of total oocytes retrieved, mature oocytes and useful oocytes were found in the group that did not used COCP. A significant difference in a nominal variable (Pearson's chi-square test) was observed, consisting of a higher proportion of cancellations due to low LH level (< 2,5 mUI/ml) in the group that used COCP (COCP no: 0.7% vs. COCP yes: 3.7%). While, in the Analysis B (analysis of variance of one factor, Anova test), the variables total oocytes retrieved, total MII oocytes and total useful oocytes showed significant differences consisting of a higher number of oocytes in the group that did not received COCP. These results have statistical significance both before removing the cases in which the values were 0 due to a cancellation ($p < 0.01$), and after removing the cancelled cases ($p < 0.05$).

Limitations, reasons for caution: The main limitation lies in the retrospective nature of the study. However, since each patient is her own control (Paired study), it gives an added value.

Wider implications of the findings: COCP before COH in PPOS cycles could have detrimental effects on oocyte yield, reducing the efficacy and efficiency of egg donation programs. This could be extended to social freezing programs in normo-responders patients, in which the probability of future pregnancy is closely linked to the number of good oocytes vitrified.

Trial registration number: Not Applicable

Abstract citation ID: dead093.947

P-618 Morning or evening start of exogenous progesterone five days before blastocyst transfer does not have an impact on ongoing pregnancy rates in artificial cycles.

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Study question: Does morning or evening start of exogenous progesterone five days before a blastocyst transfer have an impact on pregnancy rates in artificial cycles?

Summary answer: Ongoing pregnancy rate is comparable when exogenous progesterone is started either in the morning or evening on the first day of progesterone exposure before ET.

What is known already: Luteal phase support (LPS) with exogenous progesterone (P) is mandatory in artificial cycles due to the absence of corpus luteum. The time of P exposure needed to optimize the outcome remain uncertain. Some endometrial receptivity tests claim for individualization of duration of LPS before ET to optimize the implantation potential of the embryo. As routine practice, 5 days of P exposure are needed for a blastocyst transfer in general population, but it is not clear yet if exogenous P has to be started in the morning or evening of the first day of P administration.

Study design, size, duration: Single-centre retrospective cohort study of 6493 artificial cycles for blastocyst transfer between December 2018 and July 2022. Endometrial preparation was performed with oestrogens and LPS with micronized vaginal progesterone (400 mg/12h MVP). LPS was given from five days before blastocyst transfer. Ongoing pregnancy rate (>12w) was analysed according to the moment of starting progesterone (morning or evening) and hours of P exposure.

Cases in which LPS duration was different to 5 days were not included.

Participants/materials, setting, methods: Infertile patients undergoing ET in artificial cycles. Until March 2021, exogenous P was started in the evening of day 0 of P exposure ("evening start"); since April 2021, P was started in the morning of day 0 ("morning start"), before blastocyst ET. ET are performed between 12 and 18pm.

Serum P was measured at the time of ET (+2h). Routinely, patients with suboptimal P levels were added subcutaneous P (25mg/day) from the day of ET.

Main results and the role of chance: LPS was started in the evening in 3993 patients and in the morning in 2500 patients, all started five days before ET. Of note, there were no differences in these baseline/cycle characteristics: age, BMI, serum P in the proliferative phase, mid-luteal serum P, oocyte origin, EMT, number of embryos transferred. Patients with morning start had a significantly higher estradiol level (320.4 pg/mL vs 282.2pg/mL, $p = 0.001$).

Mean number of hours of P exposure until ET was 113.8 + 2.1h after evening start of MVP and 125.5 + 2.1h after morning start, $p = 0.000$. The minimum was 108.2 hours and maximum of 132.9 hours of P exposure.

Quartiles of P exposure (hours) before ET were: Q1:113.2h, Q2:115.8h, Q3:124.7h. No differences in OPR were observed according to these quartiles (<Q1:47.4%; Q1-Q3:45.7%; >Q3:47.1%, $p = 0.46$).

Likewise, evening or morning start of LPS did not exert any impact either in OPR (46.2% vs 46.8%, $p = 0.65$) or clinical miscarriage rate (17.2% vs 18.1%, $p = 0.53$). Binary logistic regression showed no differences in OPR (aOR (95%CI): 0.96 (0.85-1.09; $p = 0.52$) after adjusting for potential confounding variables.

Suboptimal mid-luteal P levels were observed in 23% (evening) vs 19.4% (morning start) of patients ($p = 0.01$). Having low P didńt impact on OPR (45.7%vs46.7%, $p = 0.55$) as rescue treatment was performed.

Limitations, reasons for caution: The limitation is the retrospective study design, although sample size is large enough to draw conclusions and control for potential confounding factors. Moreover, study period was different in both groups, although protocols and general results were comparable. If these results are similar in other ways of P administration, remains unknown.

Wider implications of the findings: These findings suggest that a difference of 12 hours in the initiation of exogenous P does not impact on the cycle outcome in artificial cycles as far as it is started 5 days before ET. Thus, LPS can be started either in the morning or evening without hampering the results.

Trial registration number: not applicable

Abstract citation ID: dead093.948

P-619 Is the algorithm derived, maximum daily dose of 12μg follitropin delta indeed sufficient, even for women considered at risk for underexposure?

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Study question: Does the follitropin delta (FD) dosing algorithm potentially underexpose high body weight (BW) women, especially those with a low ovarian reserve?

Summary answer: Our data indicate no decrease of ovarian response with increasing BW in women with serum AMH <15pmol/L treated with daily FD 12μg.

What is known already: The FD dosing algorithm stipulates a 1st cycle dose of daily 12μg FD (equivalent to approximately daily 188 IU rFSH) for patients with serum AMH <15pmol/L (2.1ng/ml), irrespective of BW. In trial and real-word study populations (ESTHER-1, 2017; Blockeel, 2022), respectively, nearly 40-50% of patients are thereby exposed to the same 12μg FD dose. In the phase III trial (ESTHER-1), however, only 5% of patients had a

BW > 85kg. It has so-far been unclear, if women with high BW, e.g. large volume-of-distribution, are potentially underexposed by the algorithm dose of 12µg daily FD, especially when in the AMH 7-15pmol/L strata.

Study design, size, duration: A single, university center, retrospective analysis of all (n = 337) stimulation cycles with FD treatment performed from 12-June-2016 to 10-Dec-2021. FD was prescribed according to the summary of product characteristics in all patients. BW was assessed in the center on cycle day two or three. Primary outcome is the cumulus-oocyte-complex (COC) number retrieved. A target response is defined as 8-14 COCs, in line with the ESTHER-1 study.

Participants/materials, setting, methods: Women with an indication for IVF or ICSI, treatment naïve (e.g., 1st cycle), undergoing controlled ovarian stimulation in a GnRH-antagonist protocol with hCG or GnRH-agonist triggering for fresh or frozen-thawed transfer. There was no restriction on AMH levels, BW, cycle regularity or presence of PCOS. Data are shown as mean standard deviation and/or median and range, as appropriate, or proportion.

Main results and the role of chance: Inclusion of 182 first cycles with mean age 33 ± 4.2 years (22-44 years), AMH 25.4 ± 16.7 pmol/L (<0.07-97.86) and BW 72.6 ± 16.3 kg (47-135). 37 out of 182 (20%) women had a BW > 85kg. Median COC number was 7 (0-19) in women with BW < 85kg and 7 (2-20) COCs with BW ≥ 85kg receiving 12µg FD in the AMH <15pmol/L stratum (p > 0.05). A separate comparison by BW strata and AMH strata 0-7 and 7-15 pmol/L, respectively, also indicated no decrease of ovarian response with higher BW. Overall, in all maximally dosed 12 µg cycles (75/182, 41%), a target response was achieved in 32.7% of patient cycles in women with BW < 85kg and 35.7% in women with BW ≥ 85kg.

In patients with AMH ≥15pmol/L, 21/70 patients (30%) had <8 COCs. While no difference in age, BMI, and mean FD dose was observed for those, they were on average taller (1.71 ± 0.06m vs. 1.66 ± 0.06m; p = 0.01), showed a tendency for an increased BW (73kg (48-110kg) vs. 63kg (47-119kg), p = 0.08) and body surface (1.87 ± 0.21 vs. 1.76 ± 0.2m^2, p = 0.05) vs. patients with target response. In all subgroups, the prevalence of cycle irregularity was not different between target and hypo- or hyperresponding patients.

Limitations, reasons for caution: The cohort is uncontrolled and the analysis is of retrospective nature. Women with high BW are relatively rare limiting the sample size and power. High BW may be associated with alterations of follicular recruitment and ovarian physiology.

Wider implications of the findings: The maximum FSH dose for low ovarian reserve patients is debated (ESHRE COS guideline 2020). The FD algorithm predicts a maximum response at 12µg FD, e.g. equivalent to only approximately 188 IU FSH/day, in all women with AMH <15pmol/L. Our analysis supports this assumption.

Trial registration number: not applicable

Abstract citation ID: dead093.949

P-620 First step for personalized luteal support in frozen-thaw embryo transfer: Understanding the factors associated with serum progesterone concentrations on embryo transfer day

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Study question: What factors determine serum progesterone (P) concentration on embryo transfer (ET) day after artificially prepared (AP) cycles receiving intramuscular progesterone (IM-P)?

Summary answer: Lower serum P concentrations on ET day in AP frozen-thaw cycles are associated with high body mass index(BMI) and low estradiol(E_2) levels on ET day.

What is known already: Large sample sized studies reported low serum P levels on ET day in AP cycles related to low success rates. There are limited studies evaluating the pregnancy rates in patients with low serum P on ET day after the rescue doses. These studies included the patients who received vaginal P. There is no study evaluating rescue doses with low serum P concentration after IM-P. Common sense is that personalization of luteal phase support may increase the success rates. To adjust the P dosages, patient and cycle characteristics need to be analyzed for prediction of low serum P concentration.

Study design, size, duration: This retrospective cohort study conducted at Bahçeci Ankara IVF Center between November 2019 and February 2022. The study included 637 single or double frozen-thawed blastocyst transfers undergoing AP endometrium receiving 100 mg IM-P after incremental oestrogen treatment. Serum P concentrations were evaluated from the blood samples which were obtained between 117-119 hours after the first IM-P administration and 21 ± 2 hours after last IM-P administration.

Participants/materials, setting, methods: A total of 637 patients, first frozen-thawed blastocyst ET cycles were analyzed. The mean age, BMI, duration of oestrogen treatment, ET day E_2 and ET day P concentration of the study population were 31.9 ± 5.1 years, 26.7 ± 4.7 kg/m^2, 13.9 ± 1.3 days, 266.1 ± 90.9 pg/ml and 30.2 ± 9.3 ng/ml respectively. Correlation analysis for patient and cycle characteristics and serum P concentrations were evaluated. Multivariate linear regression analysis was performed to determine the independent factors.

Main results and the role of chance: The overall live birth rate in total study group was 50.7% (323/637). Female age, serum P concentrations on ET day, and number of embryos transferred were significantly associated with live birth. Bivariate correlation analysis revealed that ET day P concentrations were negatively correlated to BMI (*r*:-0.12, p: 0.002) and positively correlated to ET day E_2 concentrations (*r*:0.14, p:0.001). However, no correlation to female age, causes of infertility, E_2, P and LH concentrations on P administration day, duration of oestrogen treatment and presence or absence of GnRH agonist suppression. When a multivariate linear regression was performed to correct potential confounders, increasing BMI presented a negative correlation to ET day serum P concentrations (p = 0.003; 95% CI -0.384 to -0.079). On the contrary, significant positive correlations to ET day serum P concentrations were shown with ET day E_2 levels (p = 0.01; 95% CI 0.003 to 0.018). Other patient and cycle characteristics were not found to be an independent factor for ET day P concentration.

Limitations, reasons for caution: The retrospective nature of the study is the main limitation. Only patients with IM-P were included therefore the results cannot be extrapolated to other P administration forms and needs to be validated. After determining the low serum P threshold in large sample size, the parameters should be re-evaluated.

Wider implications of the findings: Monitoring P levels and adjusting the doses of P administration (via personalization) may have a positive impact on outcome. Not only patient and cycle characteristics, also pharmacokinetic and pharmacogenetic studies are warranted to optimize luteal phase support. Personalization of the luteal phase can be achieved by this way.

Trial registration number: NA

Abstract citation ID: dead093.950

P-621 Machine learning software significantly increase clinical pregnancy rates in natural frozen-thawed embryo transfer cycles

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Study question: To study the effect of physician support software for determining ovulation time on pregnancy outcomes in natural cycle frozen embryo transfers (NC-FET)

Summary answer: Match between the physician and the algorithm regarding the time of ovulation in NC-FET cycles resulted in significant 14.4% increase in clinical pregnancy rates.

What is known already: Today the preferred method for frozen embryo transfer is natural cycle based on ovulation detection. As of today, an algorithm for predicting ovulation has not been developed and its use and its effect on success rates have not been tested. The aim of this study was to test a physician support artificial intelligence (AI) software for determining ovulation time and to study the effect of its use on pregnancy outcomes in frozen-thawed embryo transfers (NC-FET).

Study design, size, duration: NC-FET cycles from September 2018 to June 2021 were used to develop ovulation detection algorithm. Next, the effect of the selection of the ovulation date by the algorithm on pregnancy rates was tested by retrospective analysis on NC-FET cycles performed between July 2021 to June 2022. Cycles were divided into two groups: The Matched group in which there was a match between the ovulation date set by the physician and the algorithm and Mismatched group.

Participants/materials, setting, methods: Study group included 349 single embryo transfer patients after applying strict inclusion criteria. The Matched group included 198 patients and 151 patients in the Mismatched group. Patient's age, BMI, smoking, number of past retrievals and past embryo transfers, endometrium thickness, embryos age and quality, number of tests per cycle and pregnancy rates were compared. Statistical analysis included multivariable logistic regression to study the association between Match/Mismatch and clinical pregnancy rates while controlling for potential confounders

Main results and the role of chance: Matched group and Mismatched group had similar characteristics. Mean patients age at oocyte retrieval were 34.5 + 5.5 vs. 34.7 + 5.5 respectively (p=0.72). Clinical pregnancy rate in the Matched group were 38.9% compared to 24.4% in the Mismatched group (p =0.006). The difference was particularly noticeable when day 3 embryos were transferred (39.8% vs. 17.6% respectively p=0.009), and was also observed on day 5 embryos transfer, although non-significant (39.1% vs. 30.0% respectively, p=0.197). In the Matched group the chance of pregnancy actually almost doubled (OR = 1.97, CI 95% 1.23-3.17, p=0.005) while controlling potential confounders. Further analysis of the Mismatch group found that in 120 cycles (%) the doctor set the ovulation date one day or more before the date set by the algorithm and in 31(%) cases the date was a day or more after the ovulation date according to the algorithm. Logistic regression showed that the chance of pregnancy was significantly affected when the doctor determines the day of ovulation earlier than the algorithm (OR = 0.42, CI 95% 0.29-1.43, p=0.005)

Limitations, reasons for caution: Although cycles selected for comparison were controlled for key factors that affect pregnancy outcomes, The main drawback is the retrospective analysis. Further prospective randomized trials are needed to study the effectiveness of the algorithm and its effect on treatment results.

Wider implications of the findings: This is the first AI algorithm designed to predict ovulation. Using AI algorithms in NC-FET can help in identifying ovulation accurately and reliably and may increase pregnancy rate

Trial registration number: Not applicable

Abstract citation ID: dead093.951
P-622 Age-specific reference intervals for anti-müllerian hormone in Mexico: A population-based study. implications for Latin America

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Study question: To determine the age-specific normal range of antimullerian hormone (AMH) levels in a female Latin American population.

Summary answer: Age-related nomograms for general population for the 3rd-97th percentiles were produced. These nomograms could provide a reference guide for clinicians to consult women with infertility.

What is known already: The variation of AMH levels among different races and ethnicities has been well documented (Seifer et al., 2009). Despite the wide use of AMH measurements in clinical assessment of ovarian reserve, a population-based estimate for its reference range in Latin America is not available.

Study design, size, duration: A longitudinal, observational, retrospective, real-world study was carried out, including all AMH measurements performed from January 2017 to December 2018 at a large clinical laboratory network in the Mexico City area, encompassing 35 locations. A total of 6444 AMH measurements were included.

Participants/materials, setting, methods: AMH was assayed using either the cobas or ELISA methodologies. All AMH measurements from women 25-45 years were included. The Kolmogorov-Smirnov test was used to confirm that AMH levels between patients assayed with cobas were comparable to those assayed with ELISA at every age.

Main results and the role of chance: In total, 6444 measurements were identified and available for analysis. Age-related normograms for the 3rd, 10th, 25th, 50th, 75th, 90th, and 97th percentiles of AMH were produced. The overall rate of AMH decline accelerated after 40 years of age. In women 25-30 years, 11.4% (63/554) had an AMH <1.0ng/mL. In women 30-35 years, 18.6% (276/1484) had an AMH <1.0ng/mL. In women 35-40 yeras, 35.8% (830/2319) had an AMH <1.0ng/mL. In women 40-45 years, 58.0% (1120/1932) had an AMH <1.0ng/mL.

Limitations, reasons for caution: The lack of medical history for patients precluded identification and exclusión of patients with iatrogenically abnormal AMH levels (e.g. due to oophorectomy). Patients undergoing measurement of AMH were primarily referred through fertility clinics, limiting the potential for extrapolation to the general population.

Wider implications of the findings: The study reports age-specific AMH levels drawn from a large sample of women around Mexico City. The study includes the largest dataset to date. Latin American patients considering fertility preservation should be aware of the high prevalence of diminished ovarian reserve in this large cohort, including among women 35-39.

Trial registration number: Not Applicable

Abstract citation ID: dead093.952
P-623 Using machine learning to determine follicle sizes on the day of trigger most likely to yield oocytes

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Study question: Which follicle sizes on the day of trigger (DoT) are most likely to yield oocytes after different IVF treatment protocols and trigger types?

Summary answer: Follicles sized 11-19mm on DoT are most likely to yield oocytes in both 'long' and 'short' protocols after using either hCG or GnRH agonist triggers.

What is known already: On the DoT, both follicles that are too small, or too large, are less likely to yield oocytes, but the precise range of follicle sizes that are most contributory to oocyte yield remains uncertain. Knowledge of this optimal follicle size range can aid in selecting the DoT and in quantifying the efficacy of the trigger by benchmarking the expected number of oocytes to be retrieved. Machine learning can aid in the analysis of large complex datasets and thus could be used to determine the follicle sizes on the DoT that are most predictive of the number of oocytes retrieved.

Study design, size, duration: We applied machine learning techniques to data from 8030 patients aged under 35 years who underwent autologous fresh IVF and ICSI cycles between 2011-2021 in a single IVF clinic. The DoT was determined by 2-3 leading follicles reaching ≥ 18mm in size. Follicle sizes from ultrasound scans performed on the DoT (n = 3056), a day prior to DoT (n = 2839), or two days prior to DoT (n = 2135), were evaluated in relation to the number of oocytes retrieved.

Participants/materials, setting, methods: A two-stage random forest pipeline was developed, with the number of follicles of a certain size on DoT as input, and the number of oocytes retrieved as output. First, a variable preselection model to determine the most contributory follicle sizes. Second, a model to identify the optimal range of follicle sizes to yield oocytes. Both models were trained and cross-validated with fixed hyperparameters. The pipeline was run for each protocol and trigger type independently.

Main results and the role of chance: The machine learning pipeline identified follicles sized 11-19mm on the DoT as most contributory in IVF/ICSI cycles when using an hCG trigger. After a GnRH agonist trigger, follicles sized 10-19mm were most predictive of the number of oocytes retrieved. To mitigate the role of chance, the statistical methods were further validated by utilizing scans prior to the DoT to rerun the pipelines, as well as a comparison against the true number of retrieved oocytes with linear regression. In 'short' protocol cycles triggered with hCG (n = 1581), follicles sized 11-19mm on the DoT were more closely associated with the number of oocytes retrieved (r^2=0.58) than either smaller (r^2=0.031), or larger (r^2=0.051), follicle size ranges ($p < 0.0001$). The most predictive follicles sizes on the day prior to DoT were 10-18 mm (n = 1421), and 6-17 mm for two days prior to the DoT (n = 1103), consistent with expected median follicle growth rates of 1-2 mm per day. Using fivefold cross-validation, the mean absolute error was 3.47 oocytes for hCG-triggered 'short' protocol patients. Similarly, significant trends were seen across all protocols and trigger types.

Limitations, reasons for caution: This was a single-center retrospective study and thus the analysis would benefit from further validation by extension to multiple centers using varying clinical practices to ensure model generalizability.

Wider implications of the findings: This data-driven target could enable greater personalization of treatment by guiding selection of the DoT to optimize oocyte yield. Prospective studies to assess whether this proposed target for follicle size range is preferable to standard methods based on lead follicle size are needed to confirm the implication of this data.

Trial registration number: not applicable

Abstract citation ID: dead093.953

P-625 Artificial intelligence-enabled analysis of endometrial CD138 positive plasma cells in infertility-associated conditions; Polycystic ovary syndrome (PCOS) and recurrent implantation failure (RIF)

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Study question: Can artificial intelligence (AI) algorithm identify CD138+ cells in the endometrial stroma, and is the expression altered in PCOS and RIF?

Summary answer: CD138+ cell detection by AI shows high accuracy. The cell count in controls was comparable to that in PCOS cases but differed in RIF patients.

What is known already: Immunohistochemical (IHC) staining for CD138+ plasma cells has been applied to detect endometrial inflammation; however, due to a lack of cost-effective and reliable tools, there is no golden standard for CD138+ analysis. PCOS is a common endocrine disorder characterized by irregular menstrual cycles and hyperandrogenism. The women are also affected by systemic low-grade inflammation, and the PCOS endometrium shows dysregulated immune profile. RIF is one of the main causes of a low IVF success rate, and chronic endometrial inflammation has been detected in women with RIF. To date, AI technology has been applied in the diagnostics of various clinical conditions.

Study design, size, duration: In this study, AI analysis was done for CD138+ cells in a total of 193 endometrial biopsy samples: 73 were obtained from healthy and fertile controls, 91 from women with PCOS (ovulatory samples; n = 78, anovulatory samples; n = 13, Rotterdam criteria), and 29 samples from RIF patients on day five after the initiation of P4 administration.

Participants/materials, setting, methods: The biopsy samples were collected by suction curette (Pipelle), and endometrial receptivity was assessed by gene expression profiling (beREADY test). The tissues were stained with CD138 antibody, and the AI algorithm, AITAH, was trained with 150 whole slide images (WSIs) to segment epithelium and stroma and segregate CD138- and CD138+ cells in the stroma. AITAH was validated by three external pathologists. The cell counts were compared according to cycle phases, ovulatory status, and endometrial receptivity.

Main results and the role of chance: The performance of AITHA was excellent, as the final training error for CD138+ cells was 3.23%, and the decisions for the cells between pathologists and AITAH were in complete agreement. The CD138+ cell percentages were higher in the proliferative phase (PE) endometrial tissue than in the ones obtained in the secretory phase (PCOS, $p < 0.001$ or control, $p < 0.001$), regardless of PCOS diagnosis. Similar CD138+ cell percentages were observed between PE endometrium and anovulatory endometrium in PCOS cases ($p < 0.001$). The count of CD138+ plasma cells was negatively correlated with the endometrial thickness (PCOS, $p = 0.001$ or control, $p = 0.009$) and P4 (PCOS, $p = 0.001$ or control, $p = 0.003$), and positive correlations between the cell counts and gonadotropin levels were observed in ovulatory PCOS samples (FSH, $p = 0.001$ and LH, $p = 0.031$). More CD138+ cells were seen in RIF patients compared to the controls at the receptive stage ($p = 0.046$); however, there was no difference between RIF samples of three different receptivity statuses ($p = 0.426$).

Limitations, reasons for caution: Further validation for AITAH using confirmed chronic endometritis cases as positive controls is required. And further analysis using control samples obtained during the hormone replacement treatment is required. The small number of anovulatory PCOS subjects may explain the absence of correlations between the CD138+ plasma cell counts and clinical characteristics.

Wider implications of the findings: AI-enabled analysis tools could improve histological examination of endometrial immune cells by analyzing high amounts of whole tissue slides within a short time with a high pixel resolution, thus overcoming many current impediments in manual microscopic evaluation.

Trial registration number: NA

Abstract citation ID: dead093.954

P-626 Time interval between ovulation triggering and oocyte retrieval and ART outcomes: an updated meta-analysis

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Study question: To explore whether prolonged hCG-ovum pickup(hCG-opu) interval improves assisted reproductive technology outcomes.

Summary answer: The clinical pregnancy rates can be increased by prolonging the hCG-opu interval, which would help develop more reasonable time schedules for fertility centers and patients.

What is known already: hCG-OPU interval greatly affects ART success, which allows to obtain the maximum number of competent mature oocytes while avoiding spontaneous ovulation. There is also no consensus regarding the optimal time between hCG-OPU, which some studies had shown that ideal ART outcomes could be obtained when oocyte retrieval was done more than 36 h -39 h after hCG priming, while others have concluded that an interval of more than 36 hours does not improve ART outcomes.

Study design, size, duration: CENTRAL, CNKI, Cochrane Systematic Reviews, EMBASE, MEDLINE, PUBMED, and Web of Science up to February 7, 2022 were searched for studies reporting associations between hCG-ovum pickup intervals and assisted reproductive technology outcomes. We searched for prospective or retrospective cohort studies measuring the hCG-OPU interval, using 36 hours as the cutoff value between short and long intervals. A combination of the following key search terms was used: "oocyte retrieval," "human chorionic gonadotropin," and "interval."

Participants/materials, setting, methods: Studies analyzing the relationship between the hCG-OPU interval and ART outcomes were considered eligible for abstract screening. Intervention types included short (≤36 h) and long (>36 h) hCG-ovum pickup intervals in assisted reproductive technology cycles. All outcomes were based upon only fresh embryo transfers. Data were pooled using random-effects models. Heterogeneity was assessed using the *I* 2 statistic.

Main results and the role of chance: 12 studies were included in the meta-analysis, including five retrospective cohort studies, one prospective cohort study, and six randomized or quasi-randomized controlled trials. The short and long interval groups had similar oocyte maturation rates, fertilization rate and high-quality embryo rate (OR, 0.69; 95% CI, 0.45–1.06; $I^2 = 91.1\%$, OR, 0.88; 95% CI, 0.77–1.0; $I^2 = 44.4\%$ and OR, 1.05; 95% CI, 0.95–1.17; $I^2 = 8.6\%$, respectively). The clinical pregnancy rates in the long retrieval group were significantly higher than in the short retrieval group (OR, 0.66; 95% CI, 0.45–0.95; $I^2 = 35.4\%$). The groups had similar miscarriage and live birth rates (OR, 1.92; 95% CI, 0.66–5.60; $I^2 = 0.0\%$ and OR, 0.50; 95% CI, 0.24–1.04; $I^2 = 0.0\%$, respectively).

Limitations, reasons for caution: Using studies without patient data represents a potential source of bias. The hCG-OPU intervals were selected based on clinical relevance, resulting in statistical heterogeneity. Despite sensitivity analysis, such heterogeneity sources call for caution when interpreting the results.

Wider implications of the findings: The clinical pregnancy rates can be increased by prolonging the hCG-OPU (>36h), which would support the search for better clinical outcomes. The finding should be validated. And if validated by multicenter, randomized, controlled trials, long interval could be implemented in ART programs, resulting in better clinical pregnancy outcomes.

Trial registration number: not applicable

Abstract citation ID: dead093.955
P-627 Effects of ntraovarian Injection of Autologous Platelet-Rich Plasma on Ovarian Reserve in Poor Responders undergoing IVF Treatment

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Study question: Does intracortical injections of autologous platelet-rich plasma (PRP) improve the outcomes in patients with poor ovarian response (POR) undergoing the IVF treatment?

Summary answer: PRP injections could improve the markers of low ovarian reserve, especially the first month after the injection.

What is known already: Poor ovarian responders (PORs) with poor reproductive outcomes during the IVF treatment is considered the main challenges of reproductive science. PORs are presented by low ovarian reserve, poor oocyte quality and low response to ovarian stimulation protocols. Despite several approaches that have been investigated to improve the ovulation outcome in PORs, the pregnancy rate remains low.

Study design, size, duration: The pilot study was designed as prospective observational studies. 39 PORs based on Bologna criteria were included and followed by 3 months. Couples presenting with any other infertility etiology other than POR were excluded. All participants provided written informed consent prior to treatment.

Participants/materials, setting, methods: The patients in the study were received at least two previous failed Intracytoplasmic Sperm Injection (ICSI)-Embryo Transfer (ET) fresh cycle. PRP intraovarian infusion treatment was performed following the last failed ICSI-ET cycle. The patients were followed 3 months after the injection. Primary outcome measures were levels of anti-müllerian hormone (AMH), antral follicle count (AFC), follicle stimulating hormone (FSH), and oocyte yield after the PRP treatment.

Main results and the role of chance: The level of AMH was significantly elevated from 0.52 ± 0.43ng/ml before the PRP treatment to 0.82 ± 0.61ng/ml after the treatment. The AFC showed the same trend from 2.52 ± 1.50 to 5.83 ± 4.25 and the FSH level was decreased by 31.98%. But the number of retrieved oocytes and embryo available were not significantly increased.

Limitations, reasons for caution: The design of the observational studies and the small sample size.

Wider implications of the findings: PRP injection may be a potiental treatment for PORs in IVF.

Trial registration number: not applicable

Abstract citation ID: dead093.956
P-628 Prenatally androgenized PCOS-like mice present with dysregulated endometrium during window of implantation (WOI) and decidualization

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Study question: How do prenatal androgenization and excessive body weight impact endometrial preparation for implantation?

Summary answer: Prenatal androgenization rather than excessive body weight causes abnormal uterine function and gene expression during artificially induced window of implantation and decidualization events *in vivo*.

What is known already: Polycystic ovary syndrome (PCOS) is a common endocrine disorder in women characterized by reproductive defects including irregular menstruation and pregnancy complications, but also by metabolic derangements such as obesity, insulin resistance and cardiovascular diseases. It is hypothesized that PCOS originates from prenatal androgenization (PNA), hence PNA mice models mimicking PCOS features have been generated. PNA has been shown to disrupt reproductive cycles, ovarian functioning and normal embryonic development; however, endometrial function in PNA mice is currently understudied. Therefore, this project aimed to study endometrial function during endometrial preparation for implantation in a PNA PCOS-like mouse model.

Study design, size, duration: Female PNA mice received a high-fat high-sugar (HFHS) diet to capture both reproductive and metabolic phenotypes of PCOS. Uteri were collected for histological and transcriptome analysis from 12-week-old ovariectomized PNA mice undergoing hormonal treatments to induce window of implantation (WOI) or decidualization states. Sample collection was done from minimum 6 animals per group from 4 different study

groups depending on PNA status (PNA versus control) and diet (HFHS versus normal diet).

Participants/materials, setting, methods: PNA mice were generated by administrating 250 µg dihydrotestosterone daily to pregnant C57BL/6J dams during E16.5-E18.5. The female pups received a HFHS diet to induce obesity. Estrous cyclicity and anogenital distance were assessed to confirm PCOS phenotype. After ovariectomy, estrogen and progesterone injections were used to artificially induce WOI or decidualization states (intra-uterine injection of 25 µl oil was used for decidual trauma). The collected uteri were analyzed using RNA sequencing (RNAseq) and immunohistochemistry.

Main results and the role of chance: PNA mice showed increased anogenital distance and disrupted estrous cycles, confirming the PCOS-like phenotype. PNA, but not HFHS diet caused defective uterine luminal closure. PNA mice had altered gene expression during the WOI, including genes important for endometrial receptivity, genes associated with human PCOS, and genes related to reproduction failure. The HFHS diet induced a milder phenotype, i.e., enriched pathways related to mitochondrial functions, cell cycle and DNA damage response. Analysis of artificially decidualized uteri showed a severe morphological decidualization defect in PNA groups, as demonstrated by the reduced weights of the decidualized uterine horns, whereas HFHS diet did not have a significant additive effect. RNA sequencing data showed that PNA decidualized horns clustered together with non-decidualized control and PNA horns, irrespective of diet. Pathways enriched in PNA were associated with changes in extracellular matrix and ion homeostasis. HFHS diet with PNA further aggravated endometrial dysfunction, as shown by reduced prolactin expression, a classical marker for endometrial decidualization. Role of chance: while drastic effects of PNA on endometrial function are detectable, the sample size might be too small to detect more subtle morphological effects caused by the HFHS diet.

Limitations, reasons for caution: PNA mouse models display selective features of PCOS but cannot mirror the comprehensive PCOS phenotype due to the complexity and heterogeneity of the disorder. Nevertheless, this model provides insights into the impact of PNA and excessive body weight on endometrial physiology.

Wider implications of the findings: This in-dept investigation of PNA and overweight mice demonstrates the utility of the model for studying PCOS-related endometrial dysfunction and provides cues towards its pathophysiology. This report supports the hypothesis of prenatal androgen exposure as the major factor in the aetiology of PCOS-related subfertility, rather than metabolic status.

Trial registration number: not applicable

Abstract citation ID: dead093.957

P-629 Dietary and/or physical activity interventions in women with overweight or obesity prior to fertility treatment – an individual participant data meta-analysis (Venus-IPD)

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Study question: Do dietary and/or physical activity interventions prior to fertility treatment improve live birth rates in women with overweight or obesity?

Summary answer: Dietary and/or physical activity interventions prior to fertility treatment do not significantly increase live birth rates in women with overweight or obesity.

What is known already: Existing guidelines recommend lifestyle interventions based on dietary and/or physical activity targeting at a 5 to 10% reduction in body weight as an initial step prior to fertility treatment for women with infertility and overweight or obesity. However, the evidence underlying this recommendation is limited and findings from recent randomized controlled trials (RCTs) are inconsistent. Individual participant data meta-analysis (IPDMA) has been considered the "gold standard" for evidence synthesis.

Study design, size, duration: We performed an IPDMA of RCTs comparing dietary and/or physical activity interventions as core interventions prior to fertility treatment versus standard advice concerning a healthy diet and physical activity, routine care or no intervention. We searched MEDLINE, EMBASE and Cochrane Central Register of Controlled Trials up to September 2022 to identify potential eligible RCTs.

Participants/materials, setting, methods: After identifying eligible RCTs in women with infertility and overweight or obesity (BMI $\geq$ 25 kg/m^2), we contacted the investigators of eligible RCTs to share the deidentified IPD and established the Venus-IPD Collaboration. The primary outcome was live birth. Secondary outcomes included weight loss and other fertility outcomes. We performed a two-stage random-effects IPDMA as the primary analysis.

Main results and the role of chance: Of 14 eligible RCTs, as of January 2023 we obtained IPD of 9 RCTs with 1715 participants (916 in the intervention group and 799 in the control group). The mean female age was 30.7 years (standard deviation 4.5) and the median baseline BMI was 34.8 kg/m^2 (Interquartile range 32.4 to 38.5). The duration of the diet/physical activity interventions ranged from 2.5 to 6 months. Physical activity and/or dietary interventions prior to fertility treatment resulted in more weight reduction compared to those in the control group (9 RCTs, 1715 participants, mean difference –5.71 kg, 95%CI –7.76 to –3.66). The intervention group did not have a significantly higher rate of live birth (8 RCTs, 1697 participants, OR 1.30, 95%CI 0.74 to 2.27, I^2=60%) or clinical pregnancy (8 RCTs, 1697 participants, OR 1.22, 95%CI 0.81 to 1.83, I^2=28%). The asymmetrical contour-enhanced funnel plot indicates possible small-study effects.

Limitations, reasons for caution: Blinding was not possible due to the nature of the intervention. IPD of three small RCTs are not obtained at this time. The heterogeneity in the type and period for lifestyle interventions and in the follow-up duration was moderate to high.

Wider implications of the findings: Dietary and/or physical activity interventions are effective in reducing body weight in women with overweight or obesity, but such a benefit in weight loss may not translate to improved live birth rates.

Trial registration number: CRD42021266201

Abstract citation ID: dead093.958

P-630 Comparison of follitropin delta versus follitropin alfa in progestin-primed ovarian stimulation in IVF

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Study question: Is there a difference in a clinical outcome between using follitropin delta and follitropin alfa undergoing a progestin primed ovarian stimulation (PPOS) protocol?

Summary answer: There was no difference between follitropin delta and follitropin alfa in terms of pregnancy rates.

What is known already: Follitropin delta (REKOVELLE, Ferring Pharmaceuticals, Switzerland) is the first recombinant human follicle-stimulating hormone (FSH) using the algorithm-based individualized dosing regimen while reducing the risk of ovarian hyperstimulation syndrome (OHSS) as compared with conventional dosing strategies. The efficacy and safety of follitropin delta has been demonstrated in randomized controlled trials (RCTs).

Study design, size, duration: We performed a retrospective analysis of 645 patients who underwent ovarian stimulation by a PPOS protocol using follitropin delta or follitropin alfa between April 2022 and August 2022. (325 patients using follitropin delta and 320 patients using follitropin alfa, respectively.)

Participants/materials, setting, methods: 325 cycles of women who used follitropin delta were compared to 320 cycles of women who used follitropin alfa. The following outcomes were analysed; fertilized rate, 2 pronuclear embryo rate, good quality embryo rate, blastocyst rate, good quality blastocyst rate. A total of 499 women who underwent embryo transfer in cryo cycles using natural cycle or hormone replacement cycle were analysed about biochemical pregnancy rate, clinical pregnancy rate.

Main results and the role of chance: Baseline demographics for 325 women in follitropin delta group and 320 women in follitropin alfa were: age, 35.1 ± 4.3 years and 35.2 ± 4.3 years; number of previous IVF cycles, 1.6 ± 1.6 and 1.7 ± 1.7, respectively. The laboratory data were: fertilization rate, 81.4% and 80.1%, 2 pronuclear embryo rate, 73.9% and 71.5%, good quality cleavage stage embryo rate, 59.4% and 55.9%, blastocyst rate, 58.9% and 55.7%, good quality blastocyst rate, 41.3% and 38.7%, respectively. Only good quality cleavage stage embryo rate and good quality blastocyst rate were significantly higher ($P < 0.05$) in follitropin delta group. A vitrified-warmed embryo transfer was performed for 257/325 women (79.1%) and 242/320 women (75.6%) and subsequent positive β-human chorionic gonadotropin (βhCG) reported for 218/621 cycles (35.1%) and 194/570 cycles (34.0%), respectively. Ongoing pregnancy rate after embryo transfer were 19.2% (n = 119) and 19.6% (n = 112) and cumulative ongoing pregnancy rate were both 46.3%. Mild OHSS was reported for 131 women (40.3%) and 170 women (53.1%), respectively ($P = 0.07$).

Limitations, reasons for caution: This is a non-controlled, retrospective study.

Wider implications of the findings: The present study shows that in addition to reducing the OHSS risk, follitropin delta in its individualised fixed-dose regimen has the potential to improve the quality of embryos although there was no significant difference in clinical outcomes.

Trial registration number: not applicable

Abstract citation ID: dead093.959

P-631 Alleviating effects of EGCG on ovarian hyperstimulation syndrome and the potential modulating mechanism through the VEGF pathway

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Study question: What are the underlying mechanisms of EGCG to alleviate Ovary hyperstimulation syndrome (OHSS)?

Summary answer: EGCG inhibited VEGF via TGF-β1 classical-SMAD pathway and 67LR-mediated CREB pathway and could reduce the ovarian inflammatory effect and attenuated OHSS progress in rat model.

What is known already: Ovarian hyperstimulation syndrome (OHSS) is one of the most severe complications of COH during IVF treatment. The pathophysiology of OHSS is characterized by increased capillary permeability, VEGF is an important mediator in OHSS, and serum VEGF levels have been shown to correlate with OHSS severity. (-)-epigallocatechin-3-gallate (EGCG) is the most abundant and biologically active polyphenolic catechin in green tea and has been reported to have multiple effects in humans. Many research indicated that in many pathological processes, EGCG could inhibit VEGF and its receptor expression and have an angiogenesis effect. Herein, EGCG might have a therapeutic effect on OHSS.

Study design, size, duration: We investigated the role of EGCG in OHSS in vitro and in vivo. The primary human granulosa-lutein(hGL) cells and human granulosa-like tumor (KGN) cell line were cultured and treated with different concentrations of EGCG for 24 hours. Animal OHSS model was established in SD rats by injection of pregnant mare serum gonadotropin (PMSG), and randomly assigned to receive vehicle only or EGCG for 3 days. All experiments were performed 3-8 times for comparisons.

Participants/materials, setting, methods: The effect of EGCG on KGN and hGL cells was determined by MTT assay. The body weight of rats was measured every day, and the ovary size was measured after removal, the permeability was determined by Evans-blue. The serum estrogen and VEGF level in rats were detected by ELISA. RNA and protein expressions of VEGF, VEGFR-2, TGF-β, and TβR II were detected by qPCR and Western-blotting and immunostaining in vitro and in vivo experiments.

Main results and the role of chance: Our study demonstrated that administration of EGCG attenuated the development of OHSS in rats, as shown by histological examination and ovarian weight and morphology. The ovary weight was significantly decreased in the EGCG treatment group compared with the OHSS group (147.9 vs 206.5 mg). Additionally, compared to OHSS rats, EGCG treated rats exhibit downregulated ovarian VEGF expression determined by IHC and RT-qPCR. VEGF and E2 protein levels significantly decrease in the serum of the EGCG treatment group. EGCG exerted inhibitory effects on cell growth only in high dose (50uM) and longtime (48h) treatment in KGN and hGL cells. In KGN cells and hGL cells, EGCG significantly reduces the expression of VEGF and TGF-β at the RNA and protein levels. Furthermore, EGCG inhibits TGF-β1-induced VEGF production and secretion in KGN cells by suppressing TGF-β expression and its traditional Smad signaling pathway. EGCG also downregulates VEGF expression through the 67-kDa laminin receptor-mediated PKA-CREB pathway.

Limitations, reasons for caution: EGCG has been reported to employ a wide range of biological effects. Therefore, its suppressive effects on VEGF and TGF-β signaling pathway might be part of the pharmacological mechanisms to alleviate Ovary hyperstimulation syndrome. Further studies are also needed to explore the therapeutic mechanisms of melatonin from other perspectives.

Wider implications of the findings: Our findings add mechanistic insight in support of using EGCG as an adjuvant therapy in the management of OHSS. EGCG shows the potential to act as a novel alternative therapeutic drug to treat OHSS. Daily intake of green tea might be beneficial for women under COH to prevent OHSS.

Trial registration number: Not applicable

Abstract citation ID: dead093.960

P-632 Micronized progesterone is better than GnRH antagonist to prevent LH surge in oocyte donation cycles

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Study question: Micronized progesterone or GnRH antagonist: which is better to control the LH surge in the egg donation process?

Summary answer: Micronized progesterone is better. Is just as effective as GnRH antagonist and offers three additional advantages: cheaper, more convenient, and saves intermediate controls.

What is known already: GnRH antagonists have been widely considered to prevent LH surge. When fresh embryo transfer is not advised, micronized progesterone has been shown to be an effective alternative with some other very interesting advantages. If efficacy would prove to be similar, there will be three obvious advantages for the preferential use of micronized progesterone over the antagonist protocol: oral or vaginal administration is preferred over subcutaneous injection, cost would be much lower, and progesterone can be used from day one and avoid inconvenient intermediate controls. This would be particularly interesting in egg preservation, preimplantation genetic screening, and oocyte donation programs.

Study design, size, duration: We retrospectively analyzed our oocyte donation program from 2018 to 2021. All 358 cycles under short cycle protocol were studied. 67 donors were under the Antagonist cycle; age 24,7 years (18 – 32) with normal Body Max Index (22,2 Kg/m2 range 17-29) 291 donors were under the micronized progesterone cycle; age 24,5 years (18-31) with normal Body Max Index (22,3 Kg/m2 range 18-29)

Participants/materials, setting, methods: 67 donors were under the Antagonist cycle: LH suppression was accomplished by subcutaneous injections of Ganirelix 0.25 mg starting when follicles >14mm or E2 levels >400 pg/ml and continued until GnRH triggering. No patients were cancelled.

291 donors were under the micronized progesterone cycle: endogenous LH suppression was accomplished by vaginal administration of micronized progesterone (200 mg) once a day at bedtime, from stimulation day 1 and continuing until GnRH triggering. Four patients were cancelled.

Main results and the role of chance: 67 donors were under the GnRH antagonist cycle. 1115 oocytes were retrieved (16,6 range 2-39). All but two donors had egg vitrification: 65 patients, 916 oocytes (14,1 range 1-30) so 82% could be preserved. (916 / 1115). From 67 cycles, 916 vitrified oocytes were obtained (average 13,7).

291 patients were under the micronized progesterone cycle. Four patients were cancelled (no response in two, follicular asynchrony in one and personal reasons in one. No LH surges were observed). From 287 egg collections, 4683 oocytes were retrieved (16,3 range 2-49). All but two patients had vitrification: 285 patients, 3784 oocytes (13,3 range 2-37) so 81% could be preserved. (3784 / 4683). From 291 cycles, 3784 vitrified oocytes were obtained (average 13,2)

Both groups were similar: total oocytes recovered per patient (16,3 vs 16,6) egg maturation (81 vs 82%) and global results (13,2 vs 13,7)

Micronized progesterone offers three additional advantages: is obviously cheaper than GnRH antagonist. Also, vaginal route is commonly preferred to subcutaneous injections. And allows to offer a more flexible donor program making unnecessary to set some intermediate follicular controls.

CONCLUSION: Micronized progesterone offers similar results than GnRH Antagonist with three additional advantages: is cheaper, is more convenient and intermediate controls can be avoided.

Limitations, reasons for caution: Although they are most likely similar, fertilization and pregnancy rates after both protocols should be deeply analyzed.

Wider implications of the findings: Micronized progesterone would also be particularly useful in egg preservation cycles and preimplantation genetic screening programs.

Trial registration number: not applicable

Abstract citation ID: dead093.961

P-633 Progesterone elevation on the day of trigger in the presence of low ovarian response signifies abnormal follicular environment and poorer oocyte health

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Study question: Is progesterone elevation (PE) in the presence of low number of follicles (unexpected PE) associated with oocyte or embryo quality?

Summary answer: PE on day of trigger in cycles with ≤4 follicles is associated with significantly reduced fertilisation rates and fewer top-quality blastocysts

What is known already: The negative effect of PE on pregnancy rates in fresh IVF cycles has been demonstrated by multiple studies and meta-analyses, and most studies support no negative effect on oocyte and embryo quality. However, as it is thought that PE is the product of cumulative secretion of low levels of progesterone from multiple developing follicles, PE in the presence of a low number of follicles (unexpected PE) is considered a different pathophysiological entity. It has been hypothesized, but never proven, that in these cases, PE is the result of follicular dysregulation and that it might indicate poorer follicular and oocyte health.

Study design, size, duration: This is a multi-centre retrospective analysis of a large cohort (n = 1,393) of IVF/ICSI cycles with a low response to ovarian stimulation (1-4 follicles ≥14mm) performed from January 2018 to April 2022. Only cycles of ovarian stimulation using gonadotrophins and GnRH analogues with a measurement of serum progesterone on the day of triggering final oocyte maturation were included. Natural cycles and cycles involving the administration of clomiphene were excluded.

Participants/materials, setting, methods: Unexpected PE was defined as serum progesterone levels greater than 4.77 nmol/L (1.5 ng/mL) on day of trigger. The primary outcome was the number of usable blastocysts. Secondary outcomes included the number of oocytes retrieved, two pronuclei (2PN) oocytes, number of top-quality embryos, fertilisation rate and blastocyst formation rate. Generalised estimating equations were used to account for the clustered nature of data. Multivariable regression analysis was performed to control for the effect of multiple confounders.

Main results and the role of chance: In the univariate regression analysis, the number of oocytes retrieved was not significantly different between cycles with and without PE (mean 2.3 vs. 2.1 respectively; p = 0.550). The number of 2PN oocytes was significantly (p = 0.021) lower in the PE group (mean 0.72, 95%CI 0.31-1.14) compared to the non-PE group (mean 1.21, 95%CI 1.12-1.31). The number of top-quality blastocysts was also significantly (p = 0.003) lower in the PE group (mean 0.03, 95%CI -0.03-0.10) compared to the non-PE group (mean 0.14, 95%CI 0.11-0.17). Fertilisation rates per oocyte retrieved, fertilisation rates per oocyte inseminated and the top-quality blastocyst rate per oocyte retrieved were consistently lower in the PE group at 33.3% (95% CI 19.0-47.6), 26.9% (95% CI 14.8-39.0) and 0.7% (95% CI -0.8-2.1) compared to 58.7% (95% CI 55.8-61.7), 52.3% (95% CI 49.3-55.2) and 5.5% (95% CI 4.3-6.8) in the non-PE group.

The negative association of PE with the primary outcome measure of this study was confirmed in the multivariable regression analysis where the number of usable blastocysts was significantly lower in the PE group (adjusted mean 0.14, 95%CI 0.03-0.25) compared to the non-PE group (adjusted mean 0.27, 95%CI 0.23-0.31) after adjusting for confounders.

Limitations, reasons for caution: This is a retrospective observational study, thus not all confounders may be accounted for. Despite being one of the largest studies conducted in poor responders, the sample size of unexpected PE cycles is relatively small due to its rarity. Therefore, this study may be underpowered to detect smaller effect sizes.

Wider implications of the findings: This study demonstrates for the first time that unexpected PE on the day of trigger (i.e. in cycles with low ovarian response) is associated with poorer oocyte health as reflected in the reduced fertilisation rates and fewer blastocysts available for transfer. This information can aid in better understanding this phenomenon.

Trial registration number: Not applicable

Abstract citation ID: dead093.962

P-634 Is duo stim a better approach than non-consecutive cycles in patients undergoing PGTA?

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Study question: Are there differences in the results of cycles and in the time to achieve an euploid embryo in cycles with DuoStim compared to non-consecutive cycles?

Summary answer: A DuoStim strategy does not compromise ovarian response, oocyte competence or euploidy rate, and reduces time to pregnancy.

What is known already: In patients with advanced maternal age undergoing PGT-A, the risk of not reaching embryo transfer is relatively high. Increasing the number of oocytes available should increase the chances of finding a healthy euploid embryo and reach embryo transfer. Recently, DuoStim has been proposed as an alternative to accumulate oocytes prior to the final cycle in which oocytes will be fertilized, cultured, and biopsied Duostim strategy allows to reduce the time between ovarian stimulations, which may reduce the stress that waiting for assisted reproduction treatments entails for patients

Study design, size, duration: Retrospective study carried out at our institution during 2015-2020 with 451 patients > 38 years undergoing PGT-A and whose previous cycles were distributed as follows: n = 101 performed DuoStim (first OS with oocyte vitrification, second was reinitiating OS five days after OPU) and a control group of 350 patients who underwent two standard non-consecutive cycles, the first to accumulate oocytes and from the second cycle, and then performed PGT-A with all oocytes, fresh and vitrified.

Participants/materials, setting, methods: All patients undergoing PGT-A performed 1 or 2 ovarian stimulations (OS) cycles to accumulate oocytes. OS cycles were re-initiated after a 5-day interval. Trophectoderm biopsies were studied by NGS. The variables were expressed as mean ± SD and comparison among groups by using ANOVA.

Main results and the role of chance: Mean age was comparable in both groups, DuoStim vs control 39.3 ± 3.1 vs 39.2 ± 3.0; p:0.76

AMH was lower in DuoStim group compared with control group (1.07 ± 0.56 vs 2.64 ± 4.1 ng/mL; p:0.006, and AFC were 4.3 ± 2 vs 7.1 ± 3; p:0.013 respectively.

Gonadotropin doses were higher in DuoStim group compared control group 4576.8 ± 1847 vs 4045 ± 1857 IU; p:0.01, and total stimulation days were significantly higher in DuoStim compared with control group (22.7 ± 4.85 vs 18.5 ± 10,74; p < 0.001.

The mean of total eggs retrieved in both OS were similar between DuoStim and control group (12.9 vs 13.1; p = 0.78)

The ratio of euploid embryos found was similar 35.99 % vs 38.6%

When we compared pregnancy rates per embryo transfer, were similar between both groups 66.6% (36/54) vs 65.3% (132/211); p value=0.98.

We confirmed differences in the time to achieve the first euploid embryo in favour of DuoStim group 30.2 ± 6 vs 36 ± 9 with a p value: 0.001.

Limitations, reasons for caution: The retrospective nature is the major limitation of this study. The study population reflects the indication of PGT-A in a particular setting, mainly advanced maternal age and in DuoStim group a poor prognosis profile

Wider implications of the findings: Accumulating oocytes through vitrification prior to PGT-A with DuoStim offers similar clinical outcomes in both groups, it may reduce time to reach an euploid embryo, which is relevant for advanced maternal age group of patients.

Trial registration number: Not applicable

Abstract citation ID: dead093.963

P-635 RELATION BETWEEN THE TIME OF CONTROLLED OVARIAN STIMULATION IN PROGESTIN AND GnRH ANTAGONIST PROTOCOLS AND THE OUTCOMES OF IVF – A RETROSPECTIVE OBSERVATIONAL STUDY

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Study question: Comparison between IVF outcomes of two controlled ovarian stimulation (COS) protocols (the antagonist protocol and the progestin protocol) and their duration.

Summary answer: This data suggests that the protocols have comparable efficiency on the ovarian stimulation, leading to similar outcomes on IVF treatments.

What is known already: Both protocols aim to prevent early ovulation by blocking the LH surge but have slightly different mechanisms: the antagonist protocol uses a GnRH antagonist to block LH whereas in the progestin protocol a progestogen is administered. The aim of COS is to stimulate follicular growth for oocyte punction, avoiding an early ovulation. In this sense, some studies have shown that the use of oral dydrogesterone can offer a more stable maintenance of LH levels, with less occurrence of an early peak of this hormone, leading to fewer cases of premature ovulation and cycle cancellation.

Study design, size, duration: In this retrospective observational study data were collected from patients undergoing in vitro fertilization (IVF) treatment at Instituto Ideia Fértil between September 2019 and September 2020. The study was submitted to the evaluation of the Ethics and Research Committee (CEP) of the Faculdade de Medicina do ABC (CAAE: 54150621.8.0000.002) and approved. A total of 447 patients were included in the study, with 205 undergoing the antagonist protocol and 242 the progestin protocol.

Participants/materials, setting, methods: The participants were women of reproductive age that have undergone IVF at Instituto Ideia Fértil between September 2019 and 2020. They were divided between two groups: L-COS, which refers to cycles lasting ≥ 9 days, and S-COS, with cycles lasting ≤ 8 days. The variables included in the statistical analysis were: age, basal antral follicle count, and numbers of days of COS, collected and MII oocytes, zygotes, cleaved embryos and blastocysts.

Main results and the role of chance: Of the 447 patients, 392 underwent L-COS and 55 S-COS. Mean age was 38.5. When comparing groups, there was no statistical difference in the basal AFC (antral follicle count), average of recovered oocytes, mature oocytes, cleaved embryos, and blastocysts. There was a difference in the mean number of zygotes, which was greater in the L-COS group (2.77 ± 2.38 - 2.05 ± 2.16; p < 0.05). However, the final outcome did not show superiority, since the number of cleaved embryos and blastocysts did not show a significant difference. There were no significant differences between the L-COS and S-COS groups of women submitted to the progestin protocol for any outcomes. When comparing the outcomes of the L-COS and S-COS groups of women submitted to the antagonist protocol, a

significant difference in the basal AFC was observed, which was higher in the L-COS group (3.87 ± 1.69 - 3.0 ± 1.57; $p < 0.05$), which may justify the need for more days of ovulation induction for oocyte growth and maturation. No other outcomes showed a significant difference between groups. Therefore, the duration of the COS does not appear to have an impact on outcomes in either the progestin or antagonist protocols.

Limitations, reasons for caution: The limitations of this study are the lack of data on BMI (body mass index), pregnancy rate and live birth rate, which does not allow us to analyze the future success of each of the ovarian stimulation protocols or trace the relationship between BMI and IVF.

Wider implications of the findings: This study showed no relevant differences between protocols. As the progestin protocol is cheaper and easier to administer and seems to have the same quality as the antagonist protocol it might be an interesting alternative, despite another step on the treatment, the freeze all.

Trial registration number: CAAE: 54150621.8.0000.002

Abstract citation ID: dead093.964
P-636 Euploid blastocysts obtained in advanced maternal age women either in donor or autologous egg cycles showed similar live birth rates: a retrospective cohort study

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Study question: Do euploid blastocysts have the same implantation and live birth rate in autologous and donor egg cycles in patients with advanced maternal age (AMA)?

Summary answer: Euploid blastocysts transferred in AMA patients showed that implantation and live birth rates (LBR) were not significantly different between reproductively younger and older women.

What is known already: Aneuploidy rates increase steadily with age, reaching >80% in women >42 years old. The high prevalence of aneuploidies may provide an explanation for the low LBR achieved by assisted reproductive treatments, especially for women with AMA. Similarly, among oocyte donors, age is critical to achieving reproductive success independently of the recipients' age. In donor cycles, recipients using oocytes from older donors had a statistically significantly lower LBR than younger donors. Currently, it still remains unclear whether the LBR differs between young donors versus old infertile patients in the context of euploid blastocyst transfers.

Study design, size, duration: Multicenter retrospective study with the preimplantation genetic test for aneuploidies (PGT-A) in both autologous and donor oocytes cycles between January 2018 and January 2021 was conducted at three different institutions (IVI-RMA, GeneraLife and Humanitas Fertility Center). 556 patients were included and matched 1:1 for the age of recipients, euploid blastocyst morphological quality, and day of development. In the frozen embryo transfers, endometrial preparation was done using hormonal replacement therapy (HRT) or natural cycles (NC).

Participants/materials, setting, methods: AMA patients older than 39 years underwent IVF. 556 patients who underwent autologous or donor oocyte cycles with at least one euploid blastocyst transferred were matched for oocyte age, blastocyst morphological quality and day of development. Blastocyst ploidy was assessed by aCGH or NGS analysis. Patients with uterine pathologies reported in the dataset were excluded. The primary outcome was LBR per first euploid blastocyst transfer. Outcomes were adjusted for confounders via logistic regression analyses.

Main results and the role of chance: 278 single euploid blastocysts tested by PGT-A were transferred in both autologous and donor oocyte cycles. No statistical differences were reported in the maternal age at transfer in the two groups (41.91 ± 2.03 and 41.75 ± 1.98, $p = 0.55$), endometrial thickness [(8.67, 95%CI: 8.45-8.88) and (8.36, 95%CI: 8.05-8.67)] and type of endometrial preparation (HRT or NC). Implantation rate and pregnancy rate were similar without statical difference in autologous or donor cycles, respectively [57.91%, (95%CI: 51.87-63.78) versus 56.83%, (95%CI: 50.79-62.74); $p = 0.99$)] and N = 158/278 (56.83%, 95%CI: 50.79-62.74) versus N = 161/278 (57.91%, 95%CI: 51.87-63.78), $p = 0.86$. The rate of ectopic pregnancy was not statistically different in both arms. Even the miscarriage rate was comparable in autologous N = 46/158, 16.55% (95%CI:12.38-21.45) versus donor group N = 45/161, 16.19%, (95%CI:12.06-21.05); $p = 0.98$. The LBR was not statistically different in autologous N = 111/278 (39.93%, 95%CI:34.13-45.95) and donor group 114/278 (41.01%, 95%CI:35.17-47.04), $p = 0.86$. No statistical difference was reported for gestational age and birthweight at delivery. The logistic regression analyzed as putative confounders: oocyte age, number of inseminated MII-oocytes, zygotes and euploid blastocysts, sperm parameters, endometrial preparation and endometrial thickness showed no statistical differences.

Limitations, reasons for caution: Despite the matched design, the retrospective nature is the major limitation of this study. Also, patients received PGT-A using NGS and CGH arrays technology. The aCGH analysis is less sensitive than more recent NGS technology. Lastly, the prevalence of adenomyosis could be underestimated due to retrospective data collection.

Wider implications of the findings: The dramatic decline in IVF treatment success is primarily caused by aneuploidies. Euploid blastocysts show LBR largely independent of oocyte age. Our study suggests no uterine effect of aging. The reproductive decline in women is mainly related to meiotic failure, therefore future insights should develop strategies to overcome chromosomal aneuploidies.

Trial registration number: NA

Abstract citation ID: dead093.965
P-637 pseudo-targeted metabolomics of follicular fluid reveals ovarian GPD1L-mediated glycerophospholipid metabolism dysfunction in patients with biochemical premature ovarian insufficiency

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Study question: Does the metabolic profile of follicular fluid from patients with biochemical premature ovarian insufficiency (bPOI) differ from that of healthy women?

Summary answer: The metabolic signature of follicular fluid reveals a significant decrease of phosphatidic acid (PA), phosphatidylcholine (PC), lysophosphatidylcholine (lysoPC) and lysophosphatidylethanolamine (lysoPE) in bPOI patients.

What is known already: Metabolites in follicular fluid have attracted extensive attention as they are important components of the microenvironment during follicular development and oocyte maturation. Studies on follicular fluid metabolomics may enhance the understanding of the pathogenesis of ovarian aging, particularly premature ovarian insufficiency. Although a variety of metabolites have been suggested to be related to cell senescence, their specific roles in regulating follicular atresia remain unclear.

Study design, size, duration: An observational study of follicular fluid samples collected from 120 women undergoing in vitro fertilization and embryo transfer was conducted. All enrolled women were classified into two groups: one with 60 bPOI patients and the other with 60 healthy controls. Each group was further subdivided into two subgroups (young and aged group, 30 women for each section).

Participants/materials, setting, methods: The follicular fluids of mature follicles (diameter over 17 millimeters) were isolated and collected for pseudo-targeted metabolic analysis. The intensities of captured metabolites were utilized for further bioinformatic analysis. The correlation between the significant differential metabolites screened and their clinical outcomes were

analyzed. *In vitro* experiments were used to demonstrate the cellular function of mainly regulated signaling pathway.

Main results and the role of chance: A total of 608 metabolites were identified in 120 follicular fluid samples. There were significant differences in 143 metabolites between the bPOI and healthy controls. As revealed by metabolic pathway analysis, glycerophospholipid metabolism was the most enriched pathway in bPOI follicular fluids. The intensities of several glycerophospholipid-associated metabolites PA, PC, lysoPC and lysoPE were positively correlated with the ovarian reserve indicator anti-Müllerian hormone (AMH), female age, as well as the expression level of glycerol-3-phosphatedehydrogenase 1-like (GPD1L), a key enzyme of this pathway, in granulosa cells. Furthermore, knockdown of GPD1L induced mitochondria dysfunction and granulosa cell apoptosis via GPD1L/PKCε/ATF2/NEAT1 pathway, as well as led to follicular atresia and impaired the quality of oocytes.

Limitations, reasons for caution: The validity of differential intensified metabolites we detected as biomarkers for clinical diagnosis needs to be confirmed in a large-scale study. And the multiple effects of glycerophospholipid metabolic pathway posed on follicular development need to be study in-depth.

Wider implications of the findings: Our results suggested that a highly coordinated follicular metabolism exhibits a strong relationship with the quality of follicles. An understanding of the mechanisms underlying ovarian aging will provide a theoretical basis, as well as new ideas for POI diagnosis, treatment and intervention.

Trial registration number: Not applicable.

Abstract citation ID: dead093.966

P-638 Impact of amphiregulin on oocyte maturation and embryo quality in patients with the polycystic ovarian syndrome.

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Study question: Does the concentration of amphiregulin in the follicular fluid affect oocyte maturation and embryo quality in patients with polycystic ovarian syndrome?

Summary answer: The concentration of amphiregulin affects oocyte maturity and embryo quality in patients with polycystic ovarian syndrome and can be used as a predictive biomarker.

What is known already: Polycystic ovary syndrome (PCOS) is a common metabolic dysfunction and heterogeneous endocrine disorder in women of reproductive age. Epidermal growth factor receptors and their ligands, such as amphiregulin, which are expressed in female reproductive tissues, have been shown to regulate various important reproductive functions. Amphiregulin is reportedly involved in oocyte maturation through autocrine and paracrine mechanisms, however, the impact of amphiregulin on oocyte maturity end embryo quality in patients diagnosed with POCS which goes under controlled ovarian stimulation remains unknown. Determination of potential biomarkers might be the key to successful IVF outcomes.

Study design, size, duration: The study consisted of a total of 87 oocytes obtained from 30 patients who underwent ICSI at UKS Homburg (Germany) between October 2021 and March 2022. Fifteen patients were diagnosed with polycystic ovary syndrome while the other fifteen were fertile without any diagnosis. For the study purpose, follicles were aspirated separately, and the follicular fluid was centrifuged for 5 min at 1,500×g. After the preparation, the supernatant was removed and stored at -20 °C until analysis.

Participants/materials, setting, methods: Concentrations of amphiregulin in follicular fluid were determined by using a commercially available sandwich ELISAkit (Duo Set; R&D Systems Inc., Minneapolis) in accordance with the manufacturer's instructions. Good embryo quality (GQE) on day 3 was defined as a 7-9 cell stage embryo with less than 25% of fragmentation and with equally sized blastomeres. Between-group comparisons were conducted with a Mann-Whitney test. Logistic regression analysis was used to determine the influence of amphiregulin on studied parameters.

Main results and the role of chance: The mean age of all patients was 33.5 (± 5.2) years. Patients included in the study were stimulated according to a gonadotropin-releasing hormone (GnRH) antagonist protocol. Out of the 87 oocytes, 59 (67.8%) were at MII stadium, 13 (14.9%) were at MI and 8 (9.1%) were at GV stadium. ICSI was done only with MII and MI oocytes and the fertilization rate was 70.1% (61 oocytes were fertilized out of 72).

Obtained results showed that concentrations of amphiregulin were significantly lower in follicular fluid of PCOS patients [(107.00(51.95-131.00) ng/mL vs 115.27(43.19-209.51) ng/mL) respectively; $p = 0.024$].

Data comparisons between mature and immature oocytes indicated that amphiregulin concentration was significantly higher in follicles where mature oocytes were developed [(117.50 (65.99-209.51) ng/mL vs 81.75(43.19-128.20) ng/mL) respectively; $p < 0.0001$]. Additionally, logistic regression analysis confirmed the positive effect of amphiregulin concentration on oocyte maturity ($p < 0.001$).

Out of the 61 fertilized oocytes, 36 (59.0%) formed good-quality embryos. Additional analysis indicated that oocytes that developed into good-quality embryos came from follicles where amphiregulin concentrations were significantly higher compared to those that developed into poor-quality embryos [(124.05 (91.88-209.51) ng/mL. vs 86.72(43.19-109.40) ng/mL) respectively; $p < 0.001$]. Logistic regression analysis confirmed the positive effect of amphiregulin concentration on embryo quality ($p < 0.01$).

Limitations, reasons for caution: The limitation of the presented study was the relatively small patient cohort. Although the pregnancy rate in the presented study was 27.8 % , single embryo transfer was not performed, therefore further research should link amphiregulin concentrations with the pregnancy rate as well.

Wider implications of the findings: Results obtained in the study might be useful for further handling of PCOS patients since the literature already confirmed that PCOS patients produce an increased number of mature oocytes, so culture

medium-containing amphiregulin might be a future solution for those patients.

Trial registration number: 146/19

Abstract citation ID: dead093.967

P-639 Are our advanced maternal age population being affected by the shortage of HP-hMG?

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Study question: Could we stimulate patients of advanced maternal age in the face of a shortage of HP-hMG without impacting outcomes?

Summary answer: Given the current low availability of HP-hMG, it is safe to stimulate only with recombinant FSH in women of advanced maternal age without affecting results.

What is known already: . Studies on the role of luteinizing hormone (LH) supplementation in patients undergoing assisted reproductive treatments use different sources of LH bioactivity. It is well known that the addition of LH supplementation was associated with a tendency towards improved IVF outcome in specific profiles of patients undergoing Assisted Reproductive treatment. However, during the last quarter of 2022, we are facing a temporary fall in the global supply of HP-hMG, which has hCG-driven LH activity, and may impact ovarian stimulation outcomes in women of advanced maternal age undergoing a preimplantation genetic testing treatment (PGT).

Study design, size, duration: Retrospective and observational study during November-December 2022 in any of the 11 clinics belonging to the IVIRMA group in Spain. Study population included women ≥38 years undergoing PGT treatment who received monotherapy with recombinant FSH (n = 73), a combination of FSH + HMG containing an FSH: LH activity in a ratio 1:1 (n = 103), or a fertility drug with recombinant FSH + recombinant LH in a 2:1 ratio (n = 112). The study was approved by an IRB (2003-MAD-020-AR)

Participants/materials, setting, methods: All women underwent short GnRH antagonist protocol. Initial doses were adjusted based on weight and body mass index according to the clinician's experience. Daily doses of

0.25 mg GnRH antagonist were started on day 6 of stimulation. Finally, a single dose of 0.2 mg GnRH agonist was administered to trigger final oocyte maturation. Embryo biopsy was performed on day 5 with a subsequent freeze-all. A frozen single embryo transfer was accomplished in a subsequent cycle.

Main results and the role of chance: Assuming the limitations of the study design, we observed significant differences in gonadotropin doses but not in retrieved oocytes, metaphase II oocytes or euploidy rate: the results were as follows for recombinant FSH, FHS + hMG and FSH + LH respectively: gonadotropin doses [2254 (692) vs. 2033 (680) vs. 2000 (660), p = 0.0035]; retrieved oocytes [10.1 (5.4) vs. 11.1 (8.0) vs. 9.2 (6.0), p = 0.131], metaphase II oocytes [8.7 (4.7) vs. 9.8 (7.1) vs. 8.3 (5.1), p = 0.147]; and euploidy rate (38.7% vs. 36.3% vs. 31.5%, p = 0.182). Moreover, recombinant FSH in monotherapy in this specific population could guarantee the efficiency of the cycle since there are no significant differences in the usable blastocyst rate. Finally, clinical results were analyzed in those cases in which a frozen single embryo had been performed in a subsequent artificial cycle, in which the effect of LH on the endometrium is obviated; once again, no significant differences were observed between the study groups neither for the implantation rate (50% vs. 56% vs. 45%, p = 0.780) or the clinical pregnancy rate (50% vs. 56% vs. 45%, p = 0.772).

Limitations, reasons for caution: This is a retrospective and observational study and thus possible confounders cannot be completely excluded. More data are needed to draw firm conclusions and it will be critical to increase the sample size to check if the results observed in this work remains in the general population.

Wider implications of the findings: In view of tailoring assisted reproductive protocols to meet patient needs and improve the likelihood of positive outcomes, these data suggest that it is optimal to perform an ovarian stimulation protocol only with recombinant FSH in a frozen embryo program in advanced maternal age context without clinical outcomes being affected.

Trial registration number: Not applicable

Abstract citation ID: dead093.968
P-640 No impact of Progestin Primed Ovarian Stimulation (PPOS) on euploid blastocyst rate per cohort of metaphase-II oocytes during PGT-A cycles: a case-control study

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Study question: Can progestins be used to prevent spontaneous LH surge during ovarian stimulation (OS) without affecting the euploid blastocyst rate (EBR) per metaphase-II (MII) oocytes?

Summary answer: Progestin Primed Ovarian Stimulation (PPOS) (conducted with norethisterone acetate) and conventional-OS with GnRH-antagonist show similar EBR per MII-oocytes

What is known already: PPOS is a novel OS protocol based exogenous progesterone to inhibit LH surge and spontaneous ovulation as an alternative to GnRH analogues. The rationale of its use is that multiple follicular waves arise during the ovarian cycle and no spontaneous ovulation occurs during the luteal phase. PPOS effectiveness and safety have been supported lately in the literature. A recent study reported similar number of euploid blastocysts after PPOS (conducted with medroxyprogesterone acetate) versus conventional-OS with GnRH-antagonist.

Study design, size, duration: Case-control study involving advanced-maternal-age women undergoing ICSI with PGT-A at two private IVF centers (May-2017 to February-2020). 80 PPOS women were matched with 160 control based on maternal-age and ovarian reserve markers. Patients with poor-ovarian-reserve (AFC<3), premature-ovarian-failure, III-IV stage endometriosis, ovarian cyst, severe-male-factor, PGT-SR or PGT-M were excluded. EBR per MII-oocytes was the primary outcome. All other embryological and clinical outcomes were reported.

Participants/materials, setting, methods: Both groups underwent recombinant-FSH OS with GnRH-agonist ovulation trigger; the only difference was that norethisterone acetate 10mg/day was administered orally to PPOS patients starting from the second day of the menstrual cycle until trigger. The control group, instead, underwent conventional antagonist protocol. The laboratory procedures were similar in both groups: ICSI, blastocyst culture, trophectoderm biopsy, comprehensive-chromosome-testing to report non-mosaic aneuploidies and vitrified-warmed euploid single-embryo-transfers (SETs).

Main results and the role of chance: The mean fertilization rate per MII-oocytes and blastulation rate per 2PN-zygotes were similar among PPOS and control (75.5% ± 21.8% versus 71.7 ± 20.7%, p = 0.1; and 56.5% ± 27.7% versus 53.2% ± 27.5%, p = 0.4, respectively). No difference was reported for the EBR per MII-oocytes among PPOS and control (15.4% ± 19.8% versus 14.8% ± 18.2%, p = 0.9). No difference was reported among the biopsied blastocysts in terms of morphological quality and day of development. A total of 47 and 86 SETs were conducted in the two groups. There was no difference in the live-birth-rates (LBR) per SET in the two groups (N = 19/47, 40.4%, 95%CI 26.7-55.7 versus N = 35/86, 40.7%, 95%CI 30.4-51.8, p = 0.9), nor in the miscarriage rate per clinical pregnancy (N = 1/20, 5%, 95%CI 0.3-26.9 versus N = 3/36, 8.3%, 95%CI 2.2-25.6, p = 0.9). At last, the cumulative-LBR among concluded cycles (i.e., LB achieved or no transferable/all transferred euploid blastocysts) was also similar (N = 19/68, 27.9%, 95%CI 18.1-40.3 versus N = 31/132, 22.0%, 95%CI 15.4-30.2, p = 0.4).

Limitations, reasons for caution: The main limitation is the retrospective and non-randomized design. More data are required, especially for follicle recruitment, oocyte yield, gestational and perinatal outcomes.

Wider implications of the findings: Norethisterone acetate-based PPOS protocol has no impact on oocyte competence when compared to conventional GnRH-antagonist OS. These promising data support further investigation on PPOS, especially in view of its reduced cost, and putative increase in patient compliance and decrease in discomfort.

Trial registration number: none

Abstract citation ID: dead093.969
P-641 Clinical significance of differential expression of miRNA642-a/miRNA5739/miRNA3648 in the follicular microenvironment in patients with polycystic ovary syndrome

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Study question: What is the relationship between differential expression of miRNA in the follicular microenvironment,follicular fluid hormone and oocyte quality in polycystic ovary syndrome (PCOS) patients.

Summary answer: In PCOS patients, differential expression of miRNA in the follicular microenvironment is associated with abnormal hormone levels in the follicular fluid and oocyte quality.

What is known already: PCOS has abnormalities in local ovarian morphology and function, and studies have shown that the expression of local miRNA in the ovaries will be accompanied by dynamic changes in follicular recruitment, oocyte growth, follicular microenvironment changes, etc., which may affect the quality of oocytes.

Study design, size, duration: 19 patients with PCOS (PCOS group) and 17 patients with infertility due to male factors alone (control group) aged 22-35 years was included in the study. The main results included differential expression of miRNA642-a/miRNA5739/miRNA3648, MII egg rate, high-quality embryo rate, testosterone and insulin levels in follicular fluid.

Participants/materials, setting, methods: From April 2017 to January 2019, 19 PCOS patients (PCOS group) and 17 patients (control group) who underwent Intracytoplasmic sperm injection-embryo transfer at the Reproductive Center of the Affiliated Hospital of Shandong University of Traditional Chinese Medicine were collected. RT-PCR was used to detect the differential expression levels of miRNA642-a/miRNA5739/miRNA3648 in follicular fluid and granule cells in PCOS group and control group, and Chemiluminescence was used to detect follicular fluid T and insulin levels.

Main results and the role of chance: The levels of testosterone and insulin in follicular fluid in PCOS group were significantly higher than those in the control group, and the difference was statistically significant ($P < 0.05$). The MII egg rate and optimal embryo rate in the PCOS group were both lower than those in the control group, and the difference was significant ($P < 0.05$). The expression level of miR-642a-3p in follicular fluid and granule cells was consistent, and the PCOS group was lower than that in the control group. The difference was statistically significant ($P < 0.05$). The expression levels of miRNA-5739 and miRNA-3648 in follicular fluid were higher than those in the control group, but the expression levels in granule cells were lower than those in the control group, and the difference was significant ($P < 0.05$).

Correlation analysis showed that the expression levels of miRNA-3648 and miRNA-5739 in follicular fluid were closely related to the normal fertilization rate of PCOS patients, and the expression levels of miRNA-5739 were closely related to the optimal embryo rate of PCOS patients. The expression levels of miRNA-642a-3p, miRNA-5739 and miRNA-3648 in granule cells were closely related to the MII egg rate and 2PN cleavage rate of PCOS patients.

Limitations, reasons for caution: MiRNAs participate in follicle development and maturation and hormone secretion through multiple signaling pathways. We only examined miRNA-642a-3p, miRNA-5739, miRNA-3648 in follicular fluid and granule cells, and did not detect the exosomal miRNAs. Dynamic changes in miRNA in the follicular microenvironment are not fully revealed.

Wider implications of the findings: Our results further demonstrate that the abnormal expression of miRNA in follicular fluid and granule cells in women with polycystic ovary syndrome (PCOS) is closely related to the local abnormal hormone secretion and follicle development of PCOS ovaries.

Trial registration number: not applicable

Abstract citation ID: dead093.970

P-642 Estrogen deprivation and related risks in primary ovarian insufficiency with various hormone therapy initiation time points - evaluating 122,785 Asian women

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Study question: Does the initiation time point and/or duration of hormone therapy affect the long-term health consequences in primary ovarian insufficiency (POI)?

Summary answer: POI women with immediate hormone therapy following their diagnosis showed significantly reduced risk of metabolic syndrome and dyslipidemia, and such effect magnified with aging.

What is known already: POI women are exposed to extended duration of estrogen deficiency and premature aging, consequently associated with higher risks of cardiovascular, metabolic, osteoporotic and neurodegenerative diseases. The first line of treatment is hormone therapy (HT), preferably until the age of natural menopause. "Timing hypothesis" in terms of hormone replacement therapy (HRT) in menopause has globally changed the treatment protocol, but its evidence in POI is scarce.

Study design, size, duration: This population-based retrospective cohort study included 122,785 women registered to the National Health Insurance in South Korea from 2009 to 2015. The study group with POI diagnosis contained 24,557 women, determined by the International Classification of Disease, tenth revision (ICD-10) code E283 in the database. Women aged more than 40 years with the E283 code were considered being previously-diagnosed POI. The 1:4 age-matching control group with the systematic random-sampling method included 98,228 women.

Participants/materials, setting, methods: The participants' demographic data, diagnosis codes, use of inpatient and outpatient services, pharmacy dispensing claims and mortality data were retrieved and statistically analyzed. According to their use of HT, the study group was further divided into two groups: with (n = 13,240) or without HT (n = 11,317); their routine health check-up data collected in the database were then compared. In all analysis, a *p*-value <0.05 was considered statistically significant.

Main results and the role of chance: Compared to the control group, POI women had significantly lower body mass index (BMI), blood pressure, serum total cholesterol and triglyceride (TG), but the incidence rate ratio (IRR) was significantly higher in POI regarding thyroid diseases, type 2 diabetes, dyslipidemia, hypertension, osteoporosis and cardiovascular diseases (IRR with 95% confidence interval (CI) of 1.731[1.691; 1.772], 1.300[1.260; 1.342], 1.499[1.470; 1.529], 1.100[1.071; 1.129], 2.449[2.388;2.513] and 1.468[1.392; 1.547], respectively). Among POI women overall, the HT users had significantly lower BMI, lower systolic blood pressure but similar diastolic blood pressure, and lower serum total cholesterol and TG, compared to the non-users. In terms of IRRs for chronic diseases, the HT users under the age of 40 years did not show any statistically significant difference in comparison to the non-users; however, the HT users aged more than 40 years had significantly lower IRRs for type 2 diabetes, dyslipidemia and hypertension compared to the non-users (0.876[0.822; 0.933], 0.873[0.840; 0.907], 0.849[0.805; 0.896], respectively). Such effect became more statistically vivid when the participants grew older (IRR[95% CI] of 0.849[0.749; 0.963] for thyroid diseases; 0.806[0.708; 0.917] for type 2 diabetes; 0.652[0.592; 0.718] for dyslipidemia; 0.739[0.662; 0.825] for hypertension; 0.835[0.752; 0.928] for osteoporosis; and 0.890[0.728; 1.089] for cardiovascular disease, respectively).

Limitations, reasons for caution: The current study relied on the health insurance claim data which did not include detailed patient medical record including menstruation pattern and obstetrical/gynecological histories. Also, critical biochemical assay results such as anti-mullerian hormone or pituitary hormones were not available because they were not covered by the national health insurance program.

Wider implications of the findings: Agreeing with the previous literature, the immediate use of HT is associated with the reduced incidence of chronic diseases in POI women, and its extended use exerts more significant results with aging. If acknowledged early and initiated with HT, previously-inevitable health risks with POI could be conceivably compensated.

Trial registration number: The current study was supported by Biomedical Research Institute Grant (#202201970001), Pusan National University Hospital.

Abstract citation ID: dead093.971

P-643 The effect of hyperinsulinemia on FSH-mediated signalling pathways in granulosa cells - implications for follicle growth in women with PCOS

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Study question: Does *in vitro* exposure of granulosa cells (GC) to an equivalent hyperinsulinemic (HI) dose of insulin alter key signalling pathways, potentially slowing follicle growth?

Summary answer: Exposure of GC to HI dose of insulin down-regulated FSH-mediated phosphorylation of AKT and ERK proteins, which was reversed by metformin but not myoinositol treatment

What is known already: Antral follicles utilise glycolytic methods of energy production releasing the metabolites pyruvate and lactate, which are then secreted into the follicular fluid or passed directly to the oocyte via gap junctions. Insulin increases GC glucose uptake and cross-talks with FSH-mediated intracellular pathways that determine follicle growth and glucose utilisation. We and others have shown that there is significant perturbation in glucose metabolism (as measured by reduced lactate production) in cultured luteinised GC (GLC) from women with anovulatory PCOS, compared to those with normal ovaries or polycystic ovarian morphology (PCOM), that represents both attenuated glucose uptake and reduced glycolytic activity.

Study design, size, duration: KGN-GC were exposed to 1000ng/ml insulin for 48h (mimicking prolonged HI *in vivo*) with/without metformin [10^{-7}M] or myo-inositol [25mM]; followed by acute stimulation with FSH (10ng/ml at 15 and 30 mins) ($n = 4$-5). GLCs obtained from women with normal ovaries ($n = 5$), PCOM ($n = 7$) and PCOS ($n = 2$) were serum-starved and exposed acutely to insulin (10ng/ml for 30mins), glucose starved (20mins) and treated with a radiolabelled 2-deoxy-glucose mix. Fasting serum glucose and insulin were measured in PCOS women.

Participants/materials, setting, methods: Western blotting on proteins from KGN-GC was performed using antibodies against total and phosphorylated forms of AKT and ERK. Densitometry measurements of phospho:total forms were taken from individual treatments, normalised to loading controls (either β-actin/Vinculin) and then untreated controls. GLCs were lysed and uptake of ^{14}C-2DG (non-metabolised) measured and expressed as percentage uptake of ^{14}C-2DG in the insulin-treated normalised to untreated/control cells (100%). Insulin resistance (IR) was calculated from fasting glucose and insulin using HOMA2.

Main results and the role of chance: Chronic exposure of KGNs to high dose insulin significantly down-regulated acute FSH-mediated phosphorylation of AKT and ERK *(ANOVA *p=0.03, multiple post-hoc t-tests $p < 0.05$, two-tailed)*, without altering insulin receptor levels. Addition of metformin to FSH treatment, significantly reversed the HI-induced reduction in pAKT levels and enhanced it further *(*p=0.02)* but had no effect on pERK levels. Myoinositol had no effect on either pAKT or pERK levels. These findings demonstrated that HI directly inhibited FSH signalling events downstream of the insulin receptor in GC, but that metformin could counteract some of these detrimental effects. The 2-deoxy form of glucose is taken up into the cell but not metabolised further. Measurement of ^{14}C-2DG showed that acute stimulation with post-prandial levels of insulin of GLCs taken from normal and PCOM women significantly increased glucose uptake *(*p<0.05, ratio of paired t-test, 2-tailed)*; whereas GLCs from women with PCOS and insulin resistance were unable to respond to insulin and take up glucose. Therefore, GLCs from insulin resistant women with PCOS retain their phenotype *in vitro* and this is not an artefact, since GLCs from normal and PCOM also subjected to the same stimulation regime were able to take up glucose in response to insulin stimulation.

Limitations, reasons for caution: A clear limitation is the small number of insulin resistant women with PCOS. In addition, human GLCs were collected after hormonal stimulation.

Wider implications of the findings: Women with IR, HI and PCOS undergoing ART may require extra gonadotrophin stimulation to grow and mature sufficient eggs. Additionally, the oocyte quality maybe compromised, as indicated by reduced glucose uptake. Treatment of women with metformin but not myoinositol may improve the dysregulated FSH-signalling pathways brought about by HI.

Trial registration number: not applicable

Abstract citation ID: dead093.972

P-644 The mitochondrial DNA copy number in cumulus cells associate with age and AMH level but do not predict implantation potential of embryos

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Study question: is there correlation between the mtDNA copy number in cumulus cells and anti-Mullerian hormone level,female age, oocyte quality, embryo morphology, ploidy and blastocyst implantation rate?

Summary answer: A positive correlation of mtDNA quantity in CCs with the AMH,female age was revealed.There was no correlation between mtDNA quantity and embryo morphology,ploidy,implantation rate

What is known already: Recent studies have suggested that age-related decreased competence of oocytes may be due to low quantity of mtDNA copy number. Moreover, quantification of mtDNA in CCs may serve as an predictor of blastocysts viability. The purpose of this study is to investigate relative levels of mtDNA in the OCCCs in association with female age, ovarian reserve, embryo morphology, ploidy and blastocyst implantation rate.

Study design, size, duration: Prospective clinical study performed on 470 CCs retrieved from 72 advanced reproductive age patients undergoing ART treatment with intracytoplasmic sperm injection (ICSI) and preimplantation genetic testing for aneuploidy (PGT-A). Out of the 130 obtained blastocysts 56 embryos were diagnosed as aneuploid, and 74 as euploid. Presently, 51 frozen euploid embryos were transferred (FET). All transferred euploid blastocysts (n = 51) divided into 2 groups: 1 group (n = 21) - implanted embryos, 2 group (n = 30) - non-implanted.

Participants/materials, setting, methods: Inclusion criteria: age 35-45years;BMI: 18 – 24,9kg/m^2; FSH ≤15IU/ml; normal female/male karyotype; non-smokers. Exclusion criteria: genital endometriosis III-IV;severe extragenital pathology; polycystic ovary syndrome; chronic endometritis; > 96% of sperm with abnormal morphology (WHO criteria). MtDNA was assessed by using a quantitative real-time polymerase chain reaction technique. DNA from the trophectoderm samples were amplified and subjected to aneuploidy analysis using array comparative genomic hybridization. In statistic analysis was used Pearson's correlation coefficients and Fisher's exact test; $p < 0.05$ was considered significant.

Main results and the role of chance: The median age was 37.8 years old (range, 35–45), the mean level of anti-Mullerian hormone (AMH) was 2.66 ± 1.09, and the mean body mass index was 22.3 ± 1.5. A positive correlation of the relative level of mtDNA in the CCs with the patients' age ($p = 0.008$) and AMH levels ($p = 0.003$) was revealed. There was no statistically significant correlation between mtDNA copy number and embryo morphology on day 5 ($p = 0.7$). There was a tendency to increase mtDNA copy number in group 1 vs. group 2, 390 and 299, respectively ($p > 0.05$). In this study we didn't find relationship between median mtDNA content of CCs and embryos ploidy (356 vs. 325, in euploid (n = 74) and aneuploidy (n = 56) blastocyst, respectively, $p > 0.05$).

Limitations, reasons for caution: Our study was carried out in a relatively small subset of participants and obtained embryos. Correspondingly, the results obtained cannot be readily extrapolated on other groups of patients and need to be confirmed in larger trials.

Wider implications of the findings: This study showed that mtDNA quantification in CCs isn't a useful biomarker for prediction embryos implantation potential or ploidy. MtDNA content in CCs correlated only with female age and AMH level.

Trial registration number: *

Abstract citation ID: dead093.973

P-645 standard- and low-dose dual trigger compared with human chorionic gonadotropin trigger in preimplantation genetic testing cycles

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Study question: Standard-dose dual trigger (SDT) and low-dose dual trigger (LDT) could improve the number of oocytes, blastocysts and euploid blastocysts or not compared with hCG trigger.

Summary answer: LDT increased the number of oocytes and blastocysts but not euploid blastocysts among high ovarian responders, while SDT cannot help poor to normal ovarian responders.

What is known already: For low and normal responders, hCG trigger was recommended by the ESHRE guidelines for ovarian stimulation for in vitro fertilization (IVF) /intracytoplasmic sperm injection (ICSI) in 2020. For high responders, this guideline recommended gonadotropin-releasing hormone agonist (GnRH-a) as the first-choice treatment.

While recent studies showed that SDT could increase the number of oocytes and blastocysts to achieve equivalent or better pregnancy outcomes among poor and normal ovarian responders. And LDT was designed to prevent severe ovarian hyperstimulation syndrome (OHSS) while guaranteeing embryo quality among high responders by previous studies.

Study design, size, duration: This retrospective cohort study was performed at the Center for Reproductive Medicine, Department of Obstetrics and Gynecology, Peking University Third Hospital from July 2018 to December 2021. A total of 2,649 IVF-PGT cycles involving 2,275 patients were analyzed, of which 2,270 cycles had complete baseline data. A total of 1,131, 715, and 424 cycles were included in the hCG trigger, SDT, and LDT groups, respectively.

Participants/materials, setting, methods: Patients who were triggered by SDT(triptorelin acetate 0.2 mg and rhCG 250 μg), LDT(triptorelin acetate 0.2 mg and rhCG 125 μg or hCG 2,000 IU), and hCG (rhCG 250 μg) with GnRH-antagonist stimulation protocol in IVF-PGT cycles were enrolled. The number of oocytes retrieved, blastocysts, and euploid blastocysts were compared as primary endpoints. Propensity score matching (PSM) was used to control for confounding factors of retrospective study.

Main results and the role of chance: The SDT and hCG groups had a comparable number of oocytes retrieved (11.0 [7.0, 16.0] vs. 11.0 [7.0, 15.0], $p = 0.580$), blastocysts (2.0 [1.0, 3.0] vs. 2.0 [1.0, 3.0], $p = 0.517$), and euploid blastocysts (1.0 [0.0, 2.0] vs. 1.0 [0.0, 2.0], $p = 0.383$) after PSM. LDT increased the number of oocytes retrieved (19.0 [14.0, 25.0] vs. 16.0 [12.0, 21.0], $p < 0.001$) and blastocysts (3.0 [2.0, 6.0] vs. 3.0 [2.0, 5.0], $p = 0.001$), but not euploid blastocysts (2.0 [1.0, 3.0] vs. 1.0 [1.0, 3.0], $p = 0.111$), compared with the hCG trigger after PSM.

Limitations, reasons for caution: This was a single-center retrospective cohort study. The baseline characteristics of SDT and LDT differed from those of the hCG group because of clinical decision preferences. PSM was used to control for these effects and generate a balanced cohort. Pregnancy outcomes after embryo transfer has not been assessed yet.

Wider implications of the findings: The hormone change curve induced by LDT is more similar to the natural cycle. Probably LDT could also benefit poor to normal ovarian responders in IVF/ICSI cycles. And LDT might help in fresh embryo transfer cycles because of its advantage on endometrial receptivity.

Trial registration number: the National Natural Science Foundation of China (Grant No.82171626)

Abstract citation ID: dead093.974

P-646 The effect of profound LH suppression in GnRH antagonist cycles on IVF/ICSI outcomes-A retrospective study of 400 cycles

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Study question: Does low pretrigger LH in GnRH antagonist cycles affect the blastulation rates and cumulative live birth rates?

Summary answer: Low pretrigger LH in GnRH antagonist cycles does not affect the blastulation rates and cumulative live birth rates in frozen embryo transfer cycles.

What is known already:

• There is conflicting evidence that in GnRH antagonist cycles stimulated with recombinant FSH, low serum LH may effect negatively the pregnancy outcomes in fresh embryo transfer.(Benmachiche et al 2019).

• Whether the observed effect of low LH is on the embryo or on the endometrial receptivity is not clear.

Study design, size, duration: • Retrospective observational single centre study with a study population of 400 undergoing IVF/ICSI in between December 2018- December 2021.

• Controlled ovarian stimulation in GnRH Antagonist protocol in unselected population > 21yrs old undergoing IVF/ICSI.

• Exclusion Criteria-

• Long protocol

• Flare protocol

• Oocyte cryopreservation cycle

• Donor oocyte cycle

Participants/materials, setting, methods: • Controlled ovarian stimulation is done according to the hospital's standard operations of practice in flexible antagonist cycle with human menopausal gonadotropins and recombinant/urinary follicle stimulating hormone.

• When 3 or more follicles >/=17mm, triggered with GnRH Agonist or dual trigger.

• Oocyte pick up scheduled at 35-36hrs following trigger.

• Serum LH measured at baseline and at trigger.

• Clinical and embryological outcomes are assessed in between low serum LH i.e., <1.5MIU/ML and normal serum LH >/= 1.5 MIU/ML.

Main results and the role of chance: Demographic variables were comparable between both the arms, except that baseline LH is low in subjects with low pretrigger LH.

Mean dose of gonadotropins although not statistically significant, is slightly higher in the low pretrigger LH group.

Mean duration of stimulation is higher in low pretrigger LH group and is statistically significant.

Mean number of oocytes retrieved, mature oocytes, fertilisation rate, number of blastocysts, day5 and day6 blastocyts, good quality blastocysts and cumulative live birth rates are similar between low pretrigger LH and normal pretrigger LH group.

Outcomes between low pretrigger LH and normal pretrigger LH-

Mean number of blastocysts- 5.02 +/-3.84 vs 4.72 +/-3.82 ($p = 0.44$)

Mean number of day5 blastocysts- 4 (0-14) vs 3.5(0-20) ($p = 0.86$)

Mean number of day6 blastocysts- 1 (0-8) vs 1 (0-9) ($p = 0.4$)

Mean number of good quality blastocysts- 2(0-19) vs 2 (0-17) ($p = 0.68$)

Number of cycles with no blastocysts- 11.1 vs 16.1% ($p = 0.35$)

Cumulative live birth rate 47 vs 48% ($p = 0.99$)

SECONDARY OUTCOMES-

Mean dose of gonadotropins- 3282+/- 913 iu vs 3119 vs 926iu ($p = 0.07$)

Mean duration of stimulation- 11.67 +/- 1.87days vs 11.09 +/- 2days ($p = 0.01$)

Mean number of oocytes retrieved-14.98+/- 7.14 vs 13.9+/-7.9 ($p = 0.15$)

Mean number of mature oocytes-11.19+/- 5.64 vs 10.43+/-6.44 ($p + 0.21$)

Mean fertilisation rate-10.7+/- 5.47 vs 9.81+/-6.08 ($p = 0.12$)

Limitations, reasons for caution: Retrospective design.

Outcomes are assessed in frozen embryo transfer cycles, bur are not compared with fresh embryo transfer cycles.

Wider implications of the findings: The study provides reassuring data that low serum LH on the day of trigger does not affect the blastulation rates, quality of blastocysts and cumulative live birth rates in frozen embryo transfer cycles.

Whether frozen embryo transfer can negate the effect of low pretrigger LH, further studies are needed.

Trial registration number: Not applicable

Abstract citation ID: dead093.975

P-647 Underlying mechanisms in clinical manifestations of polycystic ovarian morphology (PCOM) and PCOS women

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Study question: Does the D19S884 allele 8 (A8) equally affect the pathogenesis and ovarian gene expression of PCOS and PCOM patients?

Summary answer: A8-allele produces metabolic, endocrine, and ovarian alterations regardless diagnose. The mechanisms involved in PCOM alterations are different from those of the ovarian phenotype of PCOS.

What is known already: The specific D19S884 allele 8 of the FBN3 gene may be related to polycystic ovarian syndrome (PCOS) clinical manifestations. The A8 allele participates in alternative splicing of FBN3 and produces Asprosin-3, related to glucose modulation, and Fibrillin-3. Fibrilin-3 is an extracellular matrix protein, and together with a dysregulation of the Hippo pathway, could be responsible for constraining follicular growth in the PCOS ovaries. However, it is still unknown how these pathways act in PCOS, and whether these mechanisms are also involved in pathophysiology of polycystic ovarian morphology (PCOM) in apparently normal women with regular menses.

Study design, size, duration: Cross-sectional and descriptive study with 139 women (24-39 years-old) undergoing an IVF cycle between 2019-2022 at Hospital La Fe (Valencia, Spain). Thirty-one patients were considered PCOS, twenty-eight were classified as PCOM while the remaining eighty were controls. After recruitment women were screened for A8 allele, metabolic status, hormone profile, follicular fluid protein determination and expression of Hippo pathway and extracellular matrix genes. The IVF cycle parameters were recorded to determine their relationship with study variables.

Participants/materials, setting, methods: Women with two or more Rotterdam-criteria were considered as PCOS. PCOM patients were defined by polycystic ovarian morphology on ultrasound with regular ovulatory cycles. Genomic DNA was isolated from blood samples to assess the presence of A8 allele by capillary electrophoresis and FBN3 concentration was measured on follicular fluid by ELISA. TaqMan qPCR assay was used to analyze the expression of Hippo pathway (BIRC1 and CCN2) and extracellular matrix (ECM) genes in cumulus cells.

Main results and the role of chance: PCOS patients showed statistically significant metabolic and endocrine alterations with higher BMI, free androgen index (FAI), and glucose levels compared to controls. Despite having increased AMH levels (PCOS:45.0 ± 21.7; PCOM:33.8 ± 21.5; Control:17.6 ± 7.19pmol/L, $p < 0.05$), and AMH/AFC ratio (PCOS:1.9 ± 0.9; Control:1.2 ± 0.5, $p = 0.008$), PCOS showed lower fertilization rates (PCOS:64 ± 26; Control:80 ± 18%, $p = 0.008$), reduced good quality (PCOS:0.6 ± 1.0; Control:1.3 ± 1, $p = 0.003$) and transferred embryos (PCOS:1.3 ± 0.9; Control:1.9 ± 1.0, $p = 0.018$).

In PCOM, metabolic and androgen profiles were not different from controls. Although AMH, AFC (PCOM:24.4 ± 13.7; Control:15.5 ± 7.1, $p = 0.007$) and aspirated follicles (PCOM:17.3 ± 5.7; Control:14.3 ± 7.0, $p = 0.026$) were increased, fewer embryos were transferred (PCOM:1.2 ± 1.1; Control:1.9 ± 1.0, $p = 0.027$). PCOM women also showed ovarian downregulation of the Hippo-pathway (CCN2-PCOM:-4.8 ± 10.8; Control:0.1 ± 3.0, $p = 0.010$) and increased FBN3 concentration (PCOM:19.6 ± 8.8; Control:14.7 ± 5.6ng/ml, p=N.S).

The A8 allele was detected in 17% of our patients: 4% PCOS, 50% PCOM and 46% controls. Overall, A8 presence associated a Hippo-pathway downregulation (BIRC1-A8+:-2.7 ± 4.5; A8-:0.3 ± 2.2, $p = 0.034$) and increased ECM expression (EMILIN1-A8+:2.1 ± 1.0; A8-:1.2 ± 1.5, $p = 0.04$). Interestingly, in controls, higher glucose (A8+:95.1 ± 6.7; A8-:83.5 ± 10.1mg/dl, $p = 0.002$), cholesterol (A8+:182.5 ± 11.1; A8-:165.4 ± 27.3mg/dl, $p = 0.029$), LDL (A8+:117.8 ± 10.4; A8-:83.9 ± 29.4mg/dl, $p = 0.002$), and DHEAS levels (A8+:2633.0 ± 670.7; A8-:1585.8 ± 670.0ng/ml, $p = 0.026$) were found, consistent with a reduction in HDL (A8+:51.8 ± 8.8; A8-:67.4 ± 15.43mg/dl, $p = 0.026$) and a downregulation of the Hippo-pathway (BIRC1-A8+:-4.8 ± 4.6; A8-:0.2 ± 4.6, $p = 0.013$). In PCOM, A8+ promoted higher FAI (A8+:1.6 ± 1.5; A8-:0.8 ± 0.7, p=N.S), and less embryos obtained (A8+:5.9 ± 3.8; A8-:10.7 ± 3.8, $p = 0.04$).

Limitations, reasons for caution: Due to the low prevalence of A8 in our PCOS population, its effects cannot be evaluated in this group. Further validation of gene expression results by RNA-seq will be required to assess the broad spectrum of ovarian transcriptomic changes induced by both, the PCOM phenotype and the A8 allele.

Wider implications of the findings: Polycystic ovarian morphology is not unique to clinical disorder of PCOS and the triggering mechanisms appear to be distinct from those observed in women with regular cycles. Our findings suggest that the presence of A8 allele impacts on metabolic profile, androgen levels, and ovarian pathway dysregulations, despite the clinical diagnose.

Trial registration number: Not applicable

Abstract citation ID: dead093.976

P-648 Association of severity of menstrual dysfunction with cardiometabolic risk markers in polycystic ovary syndrome

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Study question: Is the severity of menstrual dysfunction related to cardiometabolic risk markers in women with polycystic ovary syndrome (PCOS)?

Summary answer: PCOS women with amenorrhea, compared to those with oligomenorrhea or eumenorrhea, show a higher prevalence of insulin resistance (IR), prediabetes and hypertriglyceridemia.

What is known already: PCOS is associated robustly with a wide range of unfavorable cardiometabolic risk markers, including obesity, glucolipid metabolism disorders and metabolic syndrome. Many epidemiological studies have found compared to the women with regular menstrual cycles, women with a history of irregular menstruation increased the risks of metabolic syndrome and coronary heart disease. Recently, the association between the severity of menstrual cyclicity and hyperinsulinemia and IR has been demonstrated, suggesting menstrual history could be a simple but clinically important marker of metabolic dysfunction risk. But evidence linking the severity of menstrual cyclicity with cardiometabolic risk in PCOS women is very limited.

Study design, size, duration: In this prospective cross-sectional study, one hundred and fifty-four women diagnosed with PCOS by the Rotterdam criteria were recruited from July 2021 to September 2022. The association of menstrual cycle regularity with cardiometabolic risk markers including obesity, hypertension, glycemic abnormalities, dyslipidemia and metabolic syndrome was systematically analyzed.

Participants/materials, setting, methods: In a university-affiliated hospital, PCOS participants with eumenorrheic (Eumeno, n = 24), oligomenorrhea (Oligo, n = 73) and amenorrhea (Ameno, n = 57) underwent history and physical examination, gonadal steroid hormone measurement, lipid profile, oral glucose tolerance test (OGTT) and homeostasis model assessment of IR.

Main results and the role of chance: Adjusting for age, BMI and waist circumference, a trend toward an increase of unfavorable cardiometabolic risk markers including obesity, hypertension, prevalence of IR, prediabetes, hypertriglyceridemia and prevalence of metabolic syndrome was observed in Ameno group than in other menstrual categories. A significantly higher prevalence of IR, prediabetes and hypertriglyceridemia was demonstrated in Ameno group compared to the Eumeno or Oligo group. In logistic regression, adjusting for age, BMI, and waist circumference, Ameno women were more likely to have: IR than Eumeno women (odds ratio [OR]: 2.90; 95% CI: 1.01 to 8.31); prediabetes than Eumeno women (OR: 4.06; 95% CI: 1.05 to 15.77) and hypertriglyceridemia than Oligo women (OR: 4.43; 95% CI: 1.74 to 11.26). Moreover, the prevalence of IR, prediabetes and hypertriglyceridemia was still significantly higher in Ameno group than that in the Oligo or Eumeno group when further adjusted for free androgen index in the logistic

regression. The proportion of Type 2 Diabetes, high total cholesterol, high low-density lipoprotein (LDL) cholesterol and low high-density lipoprotein (HDL) cholesterol was similar across the three groups.

Limitations, reasons for caution: Our study was limited by its cross-sectional nature, and another limitation was the cycle regularity question relied on the participant's interpretation of irregular, although we have acquired the detailed menstrual cycle history and further reviewed at consultation, yet some exposure misclassification might also be present.

Wider implications of the findings: Our study used menstrual cycle as a readily obtainable marker for identification of PCOS women at greatest risk of IR, prediabetes and hypertriglyceridemia, which would emphasize the importance of menstrual cycle regularity and help refine the current recommendations concerning the screening of PCOS subjects for cardiometabolic diesases.

Trial registration number: 20210728

Abstract citation ID: dead093.977

P-649 Circadian serum progesterone variations on the day of frozen embryo transfer in artificially prepared cycles

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Study question: What is the intra-day variation of serum progesterone related to vaginal progesterone administration on the day of frozen embryo transfer (FET) in an artificial cycle?

Summary answer: We observed a statistically significant intra-day variation of serum progesterone (P) levels on the day of FET in artificially prepared cycles (AC).

What is known already: The use of FET cycles has increased enormously. In an attempt to further optimize pregnancy outcomes after FET, recent studies have focused on the importance of correct serum luteal P levels in both natural and AC-FET cycles. Despite the different cut-off values proposed to define low serum P, it is generally accepted that lower serum P values(<8.8 ng/ml) around the day of FET are associated with lower live birth rates and higher miscarriage rates. However, a single luteal serum P measurement can be misleading given the diurnal variation and the impact of patient characteristics such as age, BMI, parity, ethnicity.

Study design, size, duration: A prospective cohort study was conducted at a tertiary university-based hospital encompassing twenty-two patients undergoing a single blastocyst transfer in an AC from August to December 2022. Sample size calculation was performed using a paired t-test resulting in twenty-two patients required to detect a difference of 15% between the first and the last daily progesterone value with a false-positive rate of 5%(two-sided),a power of 80%,assuming a standard deviation of change in the outcome of 0.250.

Participants/materials, setting, methods: Patients with a normal BMI,aged between 18 and 40 years old, were included. Endometrial preparation was achieved by administering estradiol valerate(6 mg/day) until an adequate endometrial thickness was reached. Consecutively, micronized vaginal progesterone(MVP, 800 mg/die) was started five days prior to blastocyst transfer. P levels were evaluated on the day of FET at 08:00h, 12:00h, 16:00h, and 20:00h. The first and last blood samples were collected just before the intake of MVP,at 8:00h and 20:00h.

Main results and the role of chance: Mean age of the study population was 33.95 ± 3.98 years and BMI 23.10 ± 1.95 kg/m^2. Basal FSH was 7.84 ± 2.31 IU/L and estradiol concentration at 8:00h on the day of FET was 206.04 ± 93.32 ng/L. Mean P values at 08:00h, 12:00h, 16:00h and 20:00h were 11.72 ± 4.99 µg/L, 13.59 ± 6.33 µg/L, 10.23 ± 3.81 µg/L and 9.28 ± 3.09 µg/L,respectively. Statistically significant differences in P values were observed between measurements performed at 08:00h and 20:00h ($p = 0{,}007$), 8:00h and 12:00h($p < 0{,}001$), 12:00h and 16:00h ($p < 0{,}001$), and 16:00h and 20:00h ($p = 0{,}004$). The proportion of patients encountering low P values, defined as P < 8.8 ng/ml, was 27,3% at 8:00h, 13,6% at 12:00h, 40,9% at 16:00h, and 36,4% at 20:00h. Moreover, the difference in patients with normal P values (> 8.8 ng/ml) was statistically significant between samples performed at 8:00h and 12:00h and 12:00h and 16:00h ($p = 0.009$ and $p = 0.01$ respectively). No difference was observed in normal P values between 16:00h and 20:00h($p = 0.3$) and 8:00h and 20:00h($p = 0{,}07$). Additionally, no association was found either between age and BMI and the first progesterone evaluation of the day, neither between these parameters and the difference in P levels between 8:00h and 20:00h.

Limitations, reasons for caution: The strict inclusion criteria applied in this study could potentially imply a bias when extrapolating the results to a wider infertile population undergoing AC-FET cycles. A confirmation of the findings in larger prospective studies including a more heterogeneous patient population is recommended.

Wider implications of the findings: The results of this study highlight the importance of a standardized procedure for the timing of progesterone measurements, partly taken into account the last MVP intake. This is especially important when clinical actions, such as additional P supplementation, are considered when low or high P values are encountered.

Trial registration number: not applicable

Abstract citation ID: dead093.978

P-650 Follicular extracellular vesicles of older women inhibit oocyte maturation

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Study question: Whether follicular extracellular vesicles of older women interfere with the quality of oocytes.

Summary answer: OLD-EVs induced the oxidative stress in the oocytes and inhibited oocyte maturation by increasing the abnormal mitochondria distribution and abnormal spindle rates.

What is known already: Ovary is a critical regulator of female fertility and the source of oocytes. With the modern tendency of delaying childbearing, ovarian aging has become an age-related disease. Ovarian aging is generally characterized by a gradual decrease in both the quantity and quality of oocytes. The pregnancy outcomes of older women were far from satisfying with higher abortion rates, birth defects rates, and lower live birth rates even after the assistance of IVF. However, the detailed mechanisms of decreased oocyte quality of aging women have not been clarified.

Study design, size, duration: Extracellular vesicles (EVs) were isolated from follicular fluid of older women (aged 40-50, n = 7, OLD-EVs) and younger women (aged 25-30, n = 6, YOUNG-EVs) via ultracentrifugation. Germinal vesicle (GV) oocytes from female ICR mice were co-cultured with OLD-EVs, YOUNG-EVs, or PBS (blank control), respectively. GV breakdown (GVBD) rate and maturation rate were calculated at two-hour and fourteen-hour of co-culture, respectively. Besides, oocyte mitochondria distribution, meiosis spindle morphology, and oxidative status were assessed in different groups.

Participants/materials, setting, methods: EVs were determined by western blotting, nanoparticle tracking analysis, and transmission electron microscopy. Fluorescence labeled EVs were used to visualize internalization by oocytes. Oocytes mitochondria and mitosis spindles were stained with fluorescence, and abnormal mitochondria rate or abnormal spindle rate was calculated. Reactive oxygen species (ROS) level was detected in the differently treated oocytes. Moreover, the expression of CAT, GSS, and SOD was determined in the oocytes using quantitative reverse transcription polymerase chain reaction.

Main results and the role of chance: Both OLD-EVs and YOUNG-EVs are bilayered vesicles, ranging from 100 to 150 nm and enriched in Alix, TSG101, and CD9. EVs could be internalized by oocytes within one hour. After coculture, GVBD rate was similar among the three groups; whereas

maturation rate was significantly decreased in the OLD-EV group compared with YOUNG-EV group or PBS group. In addition, the abnormal mitochondria distribution rate or abnormal spindle rate were significantly increased in the OLD-EV group compared with PBS or YOUNG-EV group. The ROS level was increased in the PCOS-EV group compared with YOUNG-EV group, and the expression of CAT, GSS, and SOD was increased in the OLD-EV-treated oocytes.

Limitations, reasons for caution: Our study did not identify the contents of OLD-EVs and YOUNG-EVs, and the molecular mechanisms of dysregulations induced by OLD-EVs need further researches to investigate.

Wider implications of the findings: This work confirmed that EV-conducted cellular communication played an important role in oocyte maturation. The dysregulation of oocytes induced by OLD-EVs might be related to the poor oocyte quality of aging women, which provide a novel target to improve pregnancy outcomes of these patients.

Trial registration number: not applicable

Abstract citation ID: dead093.979
P-651 The role of FMR1-associated miR-323a and its impact on SMAD-signaling pathway in relation to the regulation of ovarian function

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Study question: Is *FMR1* (Fragile-X-Mental Retardation 1 Gene)-targeting miR-323a related to poor ovarian response and what impact does it have on the folliculogenesis-regulating *SMAD*-signaling pathway?

Summary answer: In poor ovarian responders, miR-323a is significantly upregulated, FMRP – downregulated. Human granulosa cells, transfected with a miR-323a mimic, show an upregulation of *SMAD* proteins.

What is known already: Folliculogenesis is a complex process, regulated, among others, by *FMR1* and its protein FMRP. MicroRNAs are involved in translational repression, controlling ovarian function and follicular development. miR-323a suppresses *FMR1* in tumor cells, but their relationship has not been evaluated in the human ovary. miR-323a also interacts with components of *SMAD* pathways, which regulate cell proliferation, differentiation and apoptosis. *SMADs* belong to the downstream signaling of bone morphogenetic protein-receptor-2, and our previous data shows that its expression is affected by FMRP. Thus, researching miR-323a together with its target molecules may provide more insight into the pathogenesis of poor ovarian response.

Study design, size, duration: For *ex vivo* experiments, we investigated human granulosa cell (HGC) samples of poor (POR, N = 22) and normal (NOR, N = 32) ovarian responders, with an ongoing sample collection which began in 2013. Our *in vitro* experiments were performed on COV434 cell line, transfected with a microRNA-323a-3p mimic. RNA extraction and RT-qPCR were performed to determine the relative expression levels of the targets, with NOR serving as the control group *ex vivo* and non-transfected COV434 cells *in vitro*.

Participants/materials, setting, methods: 54 patients who underwent IVF/ICSI treatments were recruited and divided into two groups based on their ovarian response according to the Bologna criteria. HGCs were collected during oocyte retrieval, and COV434 cell line was used for miRNA mimic transfection experiments. Briefly, we performed RNA extraction, cDNA synthesis and TaqMan RT-PCR, as well as protein extraction and ELISA. The collected data was analysed using the $2^{-\Delta\Delta Ct}$method and Student's t-test.

Main results and the role of chance: We found that miR-323a was significantly upregulated in HGCs of POR ($p = 0.0324$). *FMR1* expression in this patient group showed a slight downregulation that did not reach statistical significance ($p = 0.1054$). Protein expression level analysis of 22 patients from this collective (N_{NOR}=11, N_{POR}=10) demonstrated a significant downregulation of FMRP in POR ($p = 0.0373$), suggesting its participation in the pathogenesis of poor ovarian response. Additionally, *in vitro* upregulation of miR-323a resulted in altered expressions of *SMAD*-signaling pathway components. Namely, *SMAD3* expression increased by 5.7 fold, *SMAD4* – 1.6 fold, *SMAD5* – 2.5 fold, and *SMAD8* – 4.1 fold, compared to non-transfected COV434 cells, with *SMAD3* results requiring further investigation due to an unexpected 2 fold elevation of its expression in the negative control (scramble) sample. *FMR1* and *SMAD1* expression levels remained stable between transfected cells and control samples. These findings show that *FMR1*-targeting miR-323a may play a critical role in regulating the *SMAD* pathways, thus affecting folliculogenesis, HGC proliferation and, ultimately, ovarian reserve, which is reduced in case of poor ovarian response.

Limitations, reasons for caution: Larger patient sample size is needed to confirm the current results. An age-related influence cannot be ruled out because of the existing difference between the groups. More repeats of miR-323a-3p mimic transfection and miR-323a-3p inhibitor transfection should be carried out to support the *in vitro* findings.

Wider implications of the findings: For the first time, we researched the expression levels of miR-323a in HGCs. Our results suggest that miR-323a, which, like FMRP, is downregulated in POR, is involved in the *FMR1*/FMRP regulatory loop, and its upregulation affects the *SMAD*-signaling pathway, deepening our understanding of the pathogenesis of poor ovarian response.

Trial registration number: not applicable

Abstract citation ID: dead093.980
P-652 RCT to evaluate the necessity of routine luteal phase support after ovarian stimulation by oral ovulogens in intrauterine insemination (IUI) cycles

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Study question: 1. Is it beneficial to provide luteal phase support in each IUI cycle stimulated by oral ovulogens ?

Summary answer: 1. Luteal phase support with progesterone makes no significant difference in clinical pregnancy rate in oral ovulogen stimulated IUI cycles.

What is known already: ART (assisted reproductive techniques) involves superovulation to achieve the development of multiple follicles. This produces supraphysiological E2 levels that causes feedback inhibition of FSH and LH in luteal phase leading to luteal phase defect. In IUI,only few follicles develop ,so the above phenomenon is rarely seen. Today widespread use of progesterone in clinical practice has become a habit, instead of the need, adding to burden of medication and cost to the patient without much evidence to recommend it.

Study design, size, duration: Prospective Randomized control over 1 year

200 IUI cycles randomized to either supplementing luteal phase with tab dydrogesterone (Group A ,no.100) or not supplementing (Group B, No. 100) as per inclusion(Unexplained infertility,Mild male factor,Donor sperm IUI, PCOD,Coital factors,Mild endometriosis) and exclusion criteria(IUI with gonatotropin, age more than 38 years, thin endometrium ,previous two or more IUI failures, history suggestive of luteal phase defect, recurrent pregnancy loss, structural uterine anomaly, history of endocrine or autoimmune diseases)

Participants/materials, setting, methods: Transvaginal scan was conducted on day 2 , followed by ovarian stimulation with letrozole 2.5 mg for 5 days. HCG trigger (10000IU) was given When follicle reached 18- 24mm with endometrium >7mm and IUI timed 36 hours after. Computer generated random allocation was done with one group receiving luteal phase support with tab dydrogesterone 10mg twice a day(Group A)and other not receiving LPS(Group B).Outcome of study was studied in terms of clinical pregnancy on USG.

Main results and the role of chance: Among the patients recruited, infertility was observed to be primary infertility in 74% cases in Group B and 72% in Group A , whereas secondary infertility was observed in 26% cases in group B and 28% in group A ($P = 0.75$). Group B included mild male factor in 29%, 24% of polycystic ovarian syndrome, 17 % tubal, 15% poor ovarian reserve, 9% of unexplained and 6% had endometriosis. Whereas in group A,

PCOS (25%),male factor (21%),poor ovarian reserve and unexplained in 20%, endometriosis in 8% and 6% had tubal infertility. Among patients, mean endometrium thickness on trigger day of group Band group A were 8.46 ± 1.36mm and 8.44 ± 1.17mm respectively. Conception was reported by a positive urine pregnancy test . Among Group B , 23% had a positive urine pregnancy test. Whereas, in controls Group A 21% had a positive test, the difference being statistically non-significant (p value = 0.733) Outcomes were expressed terms of clinical pregnancy described as the presence of intrauterine sac with foetal heart rate. Clinical pregnancy rate was 22% of group B and 21% in group A , difference was statistically nonsignificant (p = 0.755).

Limitations, reasons for caution: Due to limited period of study and hence restricted sample size, the trial needs to be extended over a larger sample size for further interpretation. Further subgroup analysis needs to be conducted when a larger study group is recruited.

Wider implications of the findings: This study suggested that luteal support does not significantly affect the clinical pregnancy rate in IUI cycles with oral ovulogens .Our study could be a basis for further analysis to determine whether treatment burden in the form of luteal phase support can be reduced in such patients.

Trial registration number: not applicable

Abstract citation ID: dead093.981
P-654 The effect of low birth weight on the level of Anti Mullerian hormone in umbilical cord blood of female newborns

Abstract withdrawn by the authors

Abstract citation ID: dead093.982
P-655 A prospective, observational, multivariate study to evaluate the best predictor of ovarian response, between AMH measured with fully automated assay and AFC.

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Study question: Which of AMH (measured with fully-automated assay) or AFC is the best predictive biomarker of ovarian response to exogenous gonadotropins in IVF/ICSI cycles?

Summary answer: In patients >35 years, AMH predicts the number of oocytes retrieved better than AFC and it has greater discriminating ability to detect suboptimal response.

What is known already: Individual variability in ovarian response to a starting dose of gonadotropins is a well-known aspect during controlled ovarian stimulation and many efforts have been made for obtaining the personalization of the treatment, identifying different biomarkers (age, basal FSH, BMI, AMH, AFC) that may predict the ovarian response. Among these biomarkers, AMH and AFC demonstrated the best performance in predicting ovarian response. Nevertheless, approximately one in five patients in clinical practice has a discordance between AMH and AFC; in these cases, the clinicians do not know which indicator should be chosen to individualize the protocol and the starting dose of gonadotropins.

Study design, size, duration: This prospective, observational, multivariate study compared fully-automated AMH with AFC in the prediction of ovarian response, defined as the number of oocytes retrieved and it involved 161 couples attending their first IVF/ICSI cycle between 2019 and 2022 (*ClinicalTrials.gov NCT04168892*). The patients underwent a GnRH antagonist protocol and received a HMG starting dose exclusively based on their age (150 IU if ≤ 35 years, 225 IU if > 35 years). Two physicians performed all the AFC determinations.

Participants/materials, setting, methods: The study was conducted at ANDROS Day Surgery Clinic, Palermo, Italy.

Inclusion criteria were: female age 18-40 years, BMI 18-30 kg/m^2, FSH ≤15 IU/l, normal menstrual cycles, normal uterine cavity, presence of both ovaries. Exclusion criteria were: PCOS, severe endometriosis, previous ovarian surgery, ovarian cysts, endocrinological diseases. Considering a power (1-β) of 90% and an alpha of 0.01, a total sample size of 160 couples was considered sufficient to verify the study hypothesis.

Main results and the role of chance: Three out of 161 patients were excluded after randomization. Eighty-eight out of 158 patients (55.7%) received 150 IU as starting dose (age=31.03 ± 3.33; range 21-35 years); 70 patients (44.3%) received 225 IU (age=37.68 ± 1.31, range 36-40). 9/158 cycles (5.6%) were suspended for hypo-response.

The two groups were different in number of oocytes retrieved ($p < 0.05$), with higher number in ≤ 35 years group ($M_{\leq 35}$=11.30 ± 7.14, $M_{>35}$=8.77 ± 5.30).

Univariate analyses revealed that the number of oocytes retrieved was correlated, in both groups, with AMH (ps < 0.01) and AFC (ps < 0.01), and in ≤ 35 years group with basal FSH (p = 0.03). No relationships were found with cause of infertility, BMI, smoking. Correlation coefficient (ρ) between AMH and AFC was 0.74 (p < 0.01) in ≤ 35 years group and 0.57 (p < 0.01) in > 35 years group.

Multivariate Poisson regressions confirmed that AMH and AFC significantly predicted the number of oocytes retrieved in ≤ 35 years group (p < 0.01, Exp(B)=1.08; and p < 0.01, Exp(B)=1.03, respectively), while only AMH was a significant predictor in > 35 years group (p < 0.01, Exp(B)=1.18) (AFC: p = 0.34, Exp(B)=1.01).

For prediction of suboptimal ovarian response (<8 oocytes retrieved), ROC analysis showed that AMH was not different from AFC in ≤ 35 years group (AUC_{AMH}=0.811, AUC_{AFC}=0.767; p = 0.331) but significantly better in > 35 years group (AUC_{AMH}=0.858, AUC_{AFC}=0.675; p < 0.001).

Limitations, reasons for caution: This is a single-center study; a multicenter trial could be useful for a further validation of the results. Moreover, many laboratories do not use automated assays for measuring AMH and this could make the results of this study hardly applicable in all contexts.

Wider implications of the findings: The results of this prospective trial highlight that, in patients >35 years, automatized AMH predicts the ovarian response better than AFC and it shows greater discriminating ability to detect suboptimal response. This could open new possibilities of individualizing treatment with AMH-based algorithms, especially in patients with the worst reproductive prognosis.

Trial registration number: NCT04168892

Abstract citation ID: dead093.983
P-656 Machine learning algorithm automatically manages and accurately predicts ovulation in natural frozen-thawed embryo transfer cycles.

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Study question: Can an Artificial Intelligence (AI) algorithm automatically manage frozen-thawed embryo transfer (NC-FET) treatment cycles and give an accurate prediction of ovulation day.

Summary answer: An AI algorithm automatically managed and predicted the ovulation of NC-FET treatment cycles with 94.8% accuracy using an average of 3.01 test days.

What is known already: Today the preferred method for frozen embryo transfer is natural cycle based on ovulation detection. Currently, there is no software capable of managing the treatment cycle automatically and identifying the time of ovulation to support doctor decisions. The aim of this study is to develop a physician support AI software for determining ovulation time reliably with high accuracy.

Study design, size, duration: 2083 NC-FET cycles from September 2018 to June 2021 were used to develop the ovulation detection and treatment management algorithms.

Each cycle had data from at least 2 visits including: hormonal levels (Estrogen/Progesterone/LH) and follicle sizes.

The dataset was divided into a train set and two test sets. In the 1st test set ovulation was determined by experts' opinions and the 2nd test set included cycles in which follicle rupture was documented in consecutive ultrasounds.

Participants/materials, setting, methods: Two algorithms were developed, an ovulation prediction algorithm based on an NGBoost model and a treatment management algorithm that used the model to determine if and when to call for a new test or declare the ovulation day.

Both algorithms were jointly tuned to reach the highest success rate, defined as providing the correct day of ovulation using the available cycle data, with as few test days as possible.

Main results and the role of chance: On the first test set, which consisted of 176 cycles in which ovulation was determined through the majority decision of 2 independent experts and the attending physician, the treatment management algorithm required on average 3.01 tests to reach a prediction and successfully predicted the ovulation day in 94.8% of cycles.

In the second test set, which consisted of 29 cycles in which ovulation was determined through the follicular rupture in two consecutive ultrasounds, only the ovulation prediction model was tested. To ensure that the model provides a reliable answer and does not rely solely on the follicular disappearances, examined cycles were tested twice: Once using the ovulation day without the day prior to it, and again using only the day prior to ovulation without the ovulation day itself. The algorithm accurately predicted ovulation in 28 out of 29 instances (96.6%) using the day of ovulation and in 28 out of 29 instances (96.6%) using the day before ovulation.

Limitations, reasons for caution: The main drawback is this being a retrospective study: while the algorithm was trained to maximize accuracy when it selects the test days, the dataset test days were selected by the attending physicians. Statistical methods were used to overcome this, however further prospective trials are needed to validate the results.

Wider implications of the findings: This is the first AI algorithm designed to automatically manage NC-FET IVF treatment cycles and predict ovulation. The high accuracy and low average tests count might improve treatment outcomes, reduce the patients' life disruption, and allow physicians to spend less time monitoring their patients' treatments.

Trial registration number: not applicable

Abstract citation ID: dead093.984

P-657 Elecys anti-Müllerian hormone thresholds for defining ovarian response categories; comparison of published and novel values in 7,988 treatment naïve women

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Study question: Test the accuracy of published and novel AMH thresholds in predicting (low, sub-optimal, optimal and high) ovarian response categories in first treatment cycles.

Summary answer: Published AMH thresholds for poor and excessive ovarian response perform adequately, however, prediction of suboptimal or optimal oocyte yields is difficult reflecting their limited range.

What is known already: AMH is an established predictor of ovarian response, and can identify women at risk of poor (<4 oocytes) or excessive (>15 oocytes) response. With the development of suboptimal (4 to 9 oocytes) and optimal (10 to 14 oocytes) response categories, a series of AMH ranges for the Roche Elcesys AMH assay has been proposed to predict response for all four categories (AMH <6.4pmol/l, 4.9–11.3 pmol/l, 11.3–20.9 pmol/l, and >14.2 pmol/l). To date there has been no internal or external validation of these single centre derived thresholds, and it is unknown whether they are generalisable to other clinic populations.

Study design, size, duration: Observational cohort study from 8 UK clinics, incorporating 7,998 women undertaking their first ovarian stimulation cycle for assisted conception or fertility preservation between 2016 and 2022, with a pre-treatment AMH measured using the Roche Elecsys AMH assay. Both GnRH agonist and antagonist cycles were included, with triggering by either recombinant hCG or GnRH agonist once 3 follicles were ³18mm in size with oocyte retrieval performed 36h later.

Participants/materials, setting, methods: To give equal opportunity to the published and novel thresholds, patients were divided into training (5,330) and testing (2,668) sets. Novel cut-off points for a low, sub-optimal, optimal and high response were derived in the training set. The performance of these novel thresholds and those of published values were then assessed and compared in the testing set by their respective AUC, and precision as defined as the fraction of correct predictions for a certain class.

Main results and the role of chance: Baseline patient characteristics (mean, SD) were 35.1 ± 4.7 years; BMI 25.5 ± 4.4 kg/m^2, with a median AMH 15.2 pmol/l (IQR 8, 26) and a median of 11 (IQR 6,16) oocytes retrieved. These baseline characteristics and outcomes were similar after splitting in the training and testing datasets. Baseline AMH and oocytes collected were moderately correlated ($r = 0.54$, $p < 0.001$). 465 (5.8%) cycles were cancelled due to not meeting trigger criteria and classed as poor/low responders. The novel thresholds for low oocyte yield (≤10.05 pmol/l) provided similar performance (AUC 0.70 95%CI 0.66-0.74, sensitivity 0.69, specificity 0.74) to the published thresholds (AUC 0.67, 95%CI 0.62-0.72) in the testing population. Similarly, the novel high (15.9pmol/l) threshold (AUC 0.78, 95%CI 0.75-0.81, sensitivity 0.78, specificity 0.64) exhibited similar performance to the published threshold AUC 0.77, 95%CI 0.74,0.81) in the testing population. Deriving novel thresholds for prediction of 4 categories of response (6.7, 12.9, 18.9pmol) was associated with slightly better precision for low, suboptimal and high categories ($p < 0.001$), with the fraction of correct predictions for a certain category of response for the novel cut-points; 69.0 % low, 11.7% suboptimal, 14.1% optimal and 78.3% high, as compared to 52.0%, 27.8%, 12.9% and 83.8% respectively for the published values.

Limitations, reasons for caution: While designing the ovarian stimulation strategies clinicians were aware of the baseline AMH and targeted a perceived optimal response for cumulative live birth (~15 oocytes), which may have contributed to the performance of both the established and novel AMH thresholds.

Wider implications of the findings: In a large heterogenous population of women undergoing their first treatment cycle, we confirm the appropriateness of the previously published Elecsys AMH values for prediction of low (<6.4pmol/l) and high (>14.2pmol/l) oocyte yields.

Trial registration number: N/A

Abstract citation ID: dead093.985

P-658 Clinical outcomes from ART in predicted hyperresponders: in-vitro maturation of oocytes versus conventional ovarian stimulation

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Study question: Do ongoing pregnancy rates (OPR) differ in predicted hyperresponders undergoing ART after in-vitro maturation of oocytes (IVM) compared with conventional ovarian stimulation (COS)?

Summary answer: One cycle of IVM is non-inferior to one cycle of COS in women with serum AMH levels >10ng/mL.

What is known already: Women with high antral follicle count (AFC) and elevated serum AMH levels, indicating an increased functional ovarian reserve,

are prone to hyperresponse during ART treatment. To avoid iatrogenic complications of COS, IVM has been proposed as a mild-approach alternative treatment in predicted hyperresponders, including women with polycystic ovary syndrome (PCOS) who are eligible for ART. To date, inferior pregnancy rates from IVM compared to COS have hampered the uptake of IVM by ART clinics. However, it is unclear whether the efficiency gap between IVM and COS may differ depending on the extent of AMH elevation.

Study design, size, duration: This study is a retrospective cohort analysis of clinical and laboratory data from the first completed highly purified human menopausal gonadotropin (HP-hMG) primed, non-hCG triggered IVM or COS (FSH or HP-hMG stimulation in a GnRH antagonist protocol) cycle with ICSI in predicted hyperresponders ≤36 years of age at a tertiary referral university hospital. A total of 1707 cycles were included between January 2016 and June 2022.

Participants/materials, setting, methods: Predicted hyperresponse was defined as a serum AMH level >3.25ng/mL (Elecsys® AMH, Roche Diagnostics). The primary outcome was cumulative ongoing pregnancy rate (OPR) assessed 10-11 weeks after embryo transfer. The predefined non-inferiority limit was -10.0%. The analysis was adjusted for AMH strata. Time-to-pregnancy (TTP), defined by the time interval between egg retrieval and date of successful embryo transfer, was a secondary outcome. Statistical analysis was performed using a multivariable regression model controlling for potential confounders.

Main results and the role of chance: Data from 463 IVM cycles were compared with those from 1244 COS cycles. Women in the IVM group more often had a diagnosis of Rotterdam PCOS (434/463, 93.7%) compared to those undergoing COS (522/1193, 43.8%), were significantly younger (29.5 years vs 30.5 years, $p \leq 0.001$), had a higher BMI (25.7kg/m^2 vs 25.1kg/m^2, $p \leq 0.01$) and higher AMH (11.6ng/mL vs 5.3ng/mL, $p \leq 0.001$). Although IVM cycles yielded more cumulus oocyte complexes (COCs) (25.8 COC vs 15.0 COC, $p \leq 0.001$), both groups had similar numbers of mature oocytes (MII) (11.9 MII vs 10.6 MII, $p = 0.9$).

In the entire cohort, non-adjusted cumulative OPR from IVM was significantly lower (198/463, 42.8%) compared to COS (794/1244, 63.8%), $p \leq 0.001$. When analysing OPR across different serum AMH strata, cumulative OPR in both groups converged with increasing serum AMH, and OPR from IVM was non-inferior compared to COS from serum AMH levels >10ng/mL onwards (113/221, 51.1% [IVM]; 29/48, 60.4% [COS]). Time-To-Pregnancy was shorter in the IVM group compared to COS (11.3 weeks vs 21.0 weeks, $p \leq 0.001$). Multivariable regression analysis adjusting for ART type, age, BMI, oocyte number and PCOS phenotype showed that the number of COCs was the only parameter associated with OPR in predicted hyperresponders with a serum AMH >10ng/mL.

Limitations, reasons for caution: These data should be interpreted with caution as the retrospective nature of the study holds the possibility of unmeasured confounding factors. Patients enrolled in a clinical IVM program received one-to-one care, in contrast with COS patients who received standard care, which may have influenced TTP.

Wider implications of the findings: Among subfertile women who are eligible for ART, IVM and COS resulted in comparable reproductive outcomes in a subset of women with a serum AMH >10ng/mL. These findings should be corroborated by an RCT comparing both treatments in selected patients with elevated AMH.

Trial registration number: Not applicable

Abstract citation ID: dead093.986

P-659 Natural cycle versus medically programmed cycle in frozen embryo transfer: a retrospective analysis

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Study question: Does frozen embryo transfer (FET) in natural cycle (NC) and programmed cycle (PC) differ in achieving live birth, and what are the confounding factors?

Summary answer: The probability of live birth was significantly higher in natural cycle FET compared to programmed cycle.

What is known already: FET can be performed in natural or programmed (hormonally modified) cycle but the data regarding the most optimal preparation of the endometrium remains conflicting and inconclusive. The latest Cochrane review (Glujovsky, 2020) including very low- to moderate-quality evidence could not demonstrate superiority of either protocol on life birth rate or other pregnancy outcomes.

Study design, size, duration: This is a retrospective single center registry study including all frozen embryo transfers performed at Helsinki University Hospital Reproductive Medicine Unit during 2016-2020. Main outcome measure was live birth and secondary outcomes were biochemical pregnancy, ectopic pregnancy and miscarriage. The study cohort included 5435 attempted frozen embryo transfers to 1913 women, of which 1735 cycles were cancelled, mostly due a poor cycle or a weekend in a 5-day clinic, and 3700 were performed.

Participants/materials, setting, methods: Study included 2581 FETs in NC and 1119 in PC. The collected data included patient and male partner age, BMI, smoking, underlying health conditions, causes of infertility, endometrial thickness, embryo quality classification and the pregnancy outcome. Embryos from donor oocytes were excluded. To assess the effect of different confounding factors, we performed a regression analysis with a generalized linear mixed model.

Main results and the role of chance: Mean age of the women in the PC group was slightly younger (33.5, 33.0-33.9 years) than in the NC group (34.5, 34.1-34.5 years; $P < 0.001$) years. Mean BMI was not statistically different (NC 24.2 vs PC 25.1 kg/m^2), but significantly more women with BMI above 30 (PC 19.5% vs NC 10.2%; $P < 0.001$), as well as women with ovulation disorder (PC 67.2% vs NC 5.2%) were observed in the PC group. Smoking was more common in the NC group compared to the PC group (5.9 vs 4.0%, $P < 0.001$). Mean endometrial thickness at check-up visit prior to FET was 8.3 (8.1-8.5) mm in the PC group compared to 7.7 (7.6-7.8; $P < 0.001$) mm in the NC group. We observed a lower live birth rate in the PC group (23.3%) compared to the NC group (29.1%, OR 0.74, 95% CI 0.63-0.87). However, when adjusted with most significant confounding factors (female age, embryo quality score and tubal factor) the effect of the FET protocol on LBR was diminished. Incidence of miscarriage was higher in the PC group compared to the NC group, but no statistically significant difference was observed in the other secondary outcomes of biochemical pregnancy or ectopic pregnancy.

Limitations, reasons for caution: Retrospective design may include a selection bias between the study groups, although the demographic characteristics of the groups were quite comparable.

Wider implications of the findings: Confounding factors seem to explain much of the inferiority of programmed cycle compared to the natural cycle in terms of achieving live birth. However, prospective, randomized controlled trials including women with normal, ovulatory cycle would be needed to conclusively assess the optimal FET protocol.

Trial registration number: N/A

Abstract citation ID: dead093.987

P-661 Therapeutic management in women with a diminished ovarian reserve (DOR): a systematic review and meta-analysis of randomized control trials

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Study question: Unlike previous reviews, our meta-analysis investigated protocols involving only women with diminished ovarian reserve (DOR) identified by antral follicle count and/or AMH (POSEIDON criteria).

Summary answer: Testosterone, GH and delayed antagonist starting protocol are associated with better IVF outcomes comparing with respective control groups.

What is known already: The clinical management of women with diminished ovarian reserve (DOR) is a challenge in the field of medically assisted reproduction. Several therapeutic strategies have been proposed, however, with mixed results. This issue is probably linked to the fact that the definition of diminished ovarian reserve used was inconsistent among trials. The POSEIDON criteria define women with DOR, based on antral follicle count and levels of antimullerian hormone (AFC <5 or AMH <1.2 ng/ml).

Study design, size, duration: We conducted a systematic search using the MEDLINE, EMBASE, and ISI WEB OF KNOWLEDGE, to identify relevant studies published up to July 2022. The reference lists were also handsearched to implement the systematic search. We selected only RCTs in which the study population was affected by DOR with basal levels of AMH and AFC consistent with Poseidon criteria. The primary outcome was live birth rate or ongoing pregnancy when data on live birth was unavailable.

Participants/materials, setting, methods: A total of 14,386 articles were identified through the search, and duplications were removed using the EndNote library and manually (n = 6,945). The titles and abstracts of 7,441 articles were scrutinized, and 117 full-text articles were assessed for eligibility. In total, forty-seven RCTs were included. The following interventions were evaluated: DHEA, testosterone; GH high versus low gonadotropin dose regimen, delayed started protocol, letrozole, clomiphene citrate, luteal phase stimulation, dual triggering, LH supplementation, corifollitropin alfa.

Main results and the role of chance: Testosterone, high-dose gonadotropin regimen, and delayed antagonist starting protocol significantly improved the total number of eggs collected. In addition, GH and delayed starting protocol significantly increased the number of metaphase II oocytes. Testosterone, GH, and delayed antagonist starting protocol were associated with improved clinical pregnancy rates among all interventions evaluated. Testosterone and GH were also associated with higher live birth rates comparing with no supplemented women.

Limitations, reasons for caution: The main limitation of the present review relates to the minimal number of studies reporting live birth rates. Another relevant issue is the heterogeneity in women involved and the IVF protocols' diversity. More trials are demanding to corroborate our findings.

Wider implications of the findings: Our study's results might help clinicians to identify the most appropriate approach to managing women with DOR. Furthermore, our findings may stimulate researchers to develop more specific trials centred on specific categories of low-prognosis women, for example, those with a standardized diagnosis of DOR.

Trial registration number: The review protocol was registered at http://www.crd.york.ac.uk/PROSPERO/ (registration number CRD42022346117O).

Abstract citation ID: dead093.988

P-662 Embryo ploidy rates following PPOS or GnRH antagonist protocol. A prospective study with repeated ovarian stimulation

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Study question: Is there any difference in embryo ploidy rates following progesterone primed ovarian stimulation (PPOS) using micronized progesterone or GnRH antagonist protocol?

Summary answer: Pituitary downregulation with a PPOS protocol with micronized progesterone results in a comparable number of euploid blastocysts with a GnRH antagonist protocol.

What is known already: Although the GnRH antagonist is consider by most the gold standard protocol for controlling the LH surge during ovarian stimulation for IVF/ICSI, progesterone-primed ovarian stimulation protocols (PPOS) are being increasing used in freeze-all protocols. Still, despite the promising results of PPOS protocols, an early randomized trial reported potentially lower live births in recipients of oocytes resulting following downregulation with medroxyprogesterone acetate as compared with a GnRH antagonist protocol. The scope of the current prospective study was to investigate whether PPOS with micronized progesterone results in equivalent blastocyst euploidy rates as compared with a GnRH antagonist protocol.

Study design, size, duration: In this prospective study, performed between September 2019 to January 2022, 44 women underwent two consecutive ovarian stimulation (OS) protocols within a period of 6 months in a GnRH antagonist protocol or in a PPOS protocol with oral micronized progesterone.

Participants/materials, setting, methods: Overall, 44 women underwent two OS cycles with identical fixed dose of rFSH (225 or 300 IU) in both cycles. Downregulation in the 1st cycles was performed with the use of a flexible GnRH antagonist protocol and consecutively, after a washout period of one month, control of LH surge was performed with oral micronized progesterone from stimulation day 1. After the completion of both cycles, all generated blastocysts underwent genetic analysis for aneuploidy screening (PGT-A).

Main results and the role of chance: Comparisons between cycles did not reveal differences between the duration of OS neither in the gonadotrophins dose.

Hormonal profile on the day of trigger revealed statistically significant differences between cycles in all the tested hormones except for FSH: with significantly higher serum E2 levels, more elevated LH levels and higher Progesterone levels in PPOS cycles as compared with antagonist cycles, respectively.

PPOS protocol resulted in a significantly higher number of oocytes (10.27 ± 5.84 versus 12.68 ± 8.09), (DBM -2.4 [95% CI -4.1; -0.73]), MIIs (7.34 ± 4.15 versus 9.09 ± 6.12), (DBM -1.8 [95% CI -3.1; -0.43]), and 2PNs (5.66 ± 3.35 versus 7.14 ± 4.99), (DBM -1.5 [95% CI -2.6.1; -0.32]) as compared with the GnRH antagonist protocol.

Nevertheless, no differences were observed regarding the mean number of blastocysts (2.84 ± 2.12 versus 2.91 ± 2.11), (DBM -0.07 [95% CI -0.67;

0.53]) and the mean number of biopsied blastocysts (2.86 ± 2.15 versus 2.93 ± 2.16), (DBM -0.07 [95% CI -0.70; 0.56]). Finally, no difference was observed for the primary outcome, with a mean number of euploid embryos of 0.86 ± 0.90 vs. 1.00 ± 1.12 for the comparison of PPOS vs. GnRh antagonist.

Limitations, reasons for caution: The study was powered to detect differences in the mean number of euploid embryos and not in terms of pregnancy outcomes. Additionally, per protocol there was no randomisation, the first cycle was always a GnRH antagonist cycle and the 2nd a PPOS with one month of washout period in between.

Wider implications of the findings: The current study provides evidence that the PPOS may result in equivalent blastocyst euploidy rates compared with antagonist protocol. This may imply that in case of a freeze-all protocol, clinicians may safely consider PPOS to control the LH surge and patients could benefit from the advantages (cost and oral administration).

Trial registration number: NCT04108039

Abstract citation ID: dead093.989

P-663 Serum anti-Müllerian hormone level to predict the chance of live birth after spontaneous or assisted conception: a systematic review and meta-analysis

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Study question: Is Serum anti-Müllerian hormone (AMH) level predictive of cumulative live birth (CLB) rate after assisted reproductive technologies (ART) or in women trying to conceive naturally?

Summary answer: Serum AMH level is linked to CLB after IVF/ICSI but data are lacking after intrauterine insemination (IUI) or in women trying to conceive without ART.

What is known already: Serum AMH level is a marker of ovarian reserve and a good predictor of ovarian response after controlled ovarian hyperstimulation. Literature is unclear whether its measurement can predict CLB in spontaneous or assisted conception.

Study design, size, duration: A systematic review and meta-analysis, following PRISMA guidelines, was undertaken to assess whether serum AMH level may predict chances of CLB in infertile women undergoing IVF/ICSI or IUI and/or chances of live birth in women having conceived naturally.

Participants/materials, setting, methods: A systematic review and meta-analysis was performed using the following keywords: "AMH", "Anti-Müllerian Hormone", "Live-Birth", "Cumulative live birth". Searches were conducted from January 2004 to May 2021 on PubMed and Embase. Two independent reviewers carried out study selection, quality assessment and bias assessment with QUIPS tool scale and data extraction. Odds ratio were estimated using random-effect model. Pre-specified sensitivity analyses and subgroup analyses were performed. The primary outcome was CLB.

Main results and the role of chance: A total of 32 studies were included in the meta-analysis. After IVF-ICSI, only 4 studies reported CLB rate according to AMH level. No statistically significant differences in mean serum AMH levels was shown between patients with and without CLB, but with a high heterogeneity [Difference in Means (95% CI) = 0.97 (-0.25;2.19) ng/mL; I^2 = 99%; p = 0.12; n = 4]. After exclusion of 2 studies with high risks of bias, there were no more heterogeneity [I^2=0%] and the mean AMH level was statistically significantly higher in women with CLB [Difference in Means (95% CI)=0.86 (0.53;1.19) ng/mL; p < 0.00001; n = 2]. Overall, 27 articles were included in the meta-analysis of the relationship between AMH and CLB or AMH and LB after IVF/ICSI. A non-linear positive relationship was found in both cases. A polynomial fraction was the best model to describe it but no discriminant AMH threshold was shown. There were not enough articles/data to assess the ability of AMH to predict CLB rate or find an AMH threshold after IUI or in women without history of infertility trying to conceive without ART.

Limitations, reasons for caution: Systematic review and meta-analysis have some limitations due to the limits and bias of the studies included. In the present meta-analysis, heterogeneity may have been caused by different baseline characteristics in study participants, different stimulating protocols for ART, different serum AMH level threshold used and various assays of serum AMH.

Wider implications of the findings: Serum AMH level is linked to CLB rate after IVF/ICSI but no discriminating threshold can be established. Data are lacking concerning its predictive value after IUI or in women trying to conceive without ART. Our findings may be helpful to counsel candidate couples to IVF-ICSI.

Trial registration number: not applicable

Abstract citation ID: dead093.990

P-664 Optimizing IVF unit workload by balancing retrievals using artificial intelligence algorithm for trigger day decision

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Study question: Can an algorithm effectively reduce fluctuations in the number of retrievals per day by distributing them more evenly throughout the month without compromising clinical outcome?

Summary answer: A trigger day decision algorithm can evenly distribute retrievals throughout the month, significantly reducing the variations of retrievals per day, without compromising clinical outcome.

What is known already: Whether clinic workload can have an impact on IVF outcomes is controversial with some studies indicating that high procedure volume may lead to lower pregnancy rates. Furthermore, efficient workload management is crucial for fertility clinics to achieve optimal outcomes for both patients and staff. Overwhelming workloads can lead to staff burnout and decreased productivity. Clinics need to be cautious with the number of patients they treat due to the fluctuations in the number of retrievals. Reducing these fluctuations can help the clinic maintain stability in patient flow, reduce waiting lists, and enhance overall efficiency and productivity.

Study design, size, duration: A retrospective cohort study including data of 6,562 retrieval protocol cycles performed in a large center serving over 50 physicians, between November 2021 and October 2022.

The data includes 5,377 (81.94%) antagonist protocol cycles and 1,185 (18.06%) cycles of different protocols. The antagonist protocol cycles also include information on suggested trigger options: a specific single day, two options or three optional days, which were determined by a trigger management algorithm.

Participants/materials, setting, methods: An algorithm was developed to evenly distribute retrievals throughout the month. It divides the cycles into two groups: those with one suggested trigger day and fixed retrieval day (4,730 cycles 72.08%), and those with two/three suggested trigger day options and flexibility in choosing retrieval day (1,832 cycles 27.92%). The algorithm first allocates cycles with two options and then cycles with three options, aiming to minimize the variation in the number of retrievals between days.

Main results and the role of chance: The study showed that the implementation of a balancing algorithm on a clinic with an average of 18 retrievals per day resulted in a decrease of the standard deviation of the number of retrievals per day, from an average of 7.66 to 3.16, narrowing the range (average ± 2 standard deviation) of daily activity from 3-33 to 12-24 daily retrievals.

Furthermore, the study showed that the number of days with more than 150% or less than 50% retrievals than the average of the month, decreased significantly from an average of 8.4 days per month to 0.5 day per month when the balancing algorithm was applied.

Assuming the clinic has the capacity to handle the previous upper range of daily retrievals, the implementation of the balancing algorithm reduced the standard deviation and allowed for an increase in the average number of daily retrievals by approximately 31%, to an average of 23.6 cycles per day, with the clinic's capacity remaining unchanged.

Limitations, reasons for caution: This algorithm was developed specifically for antagonist cycles, which comprise approximately 80% of retrievals. Therefore, it cannot be applied to about 20% of cycles that are limited to a fixed trigger day but may have similar flexibility when an algorithm is available.

Wider implications of the findings: The use of a balancing algorithm has the potential to reduce workload and improve efficiency for medical and laboratory staff, thereby reducing errors and costs. Furthermore, it may enable clinics to treat more patients using the same facilities and resources, thus decreasing waitlists for treatments.

Trial registration number: HMC-0011-22

Abstract citation ID: dead093.991

P-665 Timing of metformin treatment in women with polycystic ovarian syndrome and associated pregnancy outcomes: a systematic review and meta-analysis

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Study question: Does giving women with polycystic ovarian syndrome (PCOS) preconception metformin and continuing it through at least the first trimester improve pregnancy outcomes?

Summary answer: Giving women with PCOS preconception metformin and continuing throughout the first trimester significantly reduces the risk of early miscarriage, and can improve pregnancy outcomes.

What is known already: Metformin, either alone or in combination with other ovulation induction agents, can improve ovulation and clinical pregnancy rates in women with PCOS. Its impact on miscarriage and live birth rate are less well understood. Furthermore the impact of both the timing and duration of metformin treatment on subsequent pregnancy outcomes, in women with PCOS, is unknown. We sought to evaluate the role that preconception metformin, continued until at least the point of positive pregnancy test, may have on pregnancy outcomes in women with PCOS and the effect that stopping metformin once pregnant might have.

Study design, size, duration: We conducted a systematic review and meta-analysis. A search within MEDLINE, Embase and the Cochrane Central Register of Controlled Trials for studies listed from database inception to 13th October 2022 was conducted. Potentially eligible studies identified through other sources (such as the reference lists of the selected primary studies and review articles) were also screened for inclusion. We adopted Grading of Recommendations, Assessment, Development and Evaluation (GRADE) for determining the quality of the evidence.

Participants/materials, setting, methods: The full-text of studies identified as being potentially suitable for inclusion were obtained. Those meeting the study selection criteria, outlined, were included in the systematic review and meta-analysis. Studies were included if they were prospective randomised controlled trials, comparing the use of metformin in women with PCOS, which had been started pre-conceptually and continued at least until pregnant (confirmed urinary pregnancy test), to either placebo or no treatment.

Main results and the role of chance: A total of 12 studies (1517 women) were included in the systematic review and meta-analysis. Women who were given preconception metformin which was continued until at least the end of the first trimester showed a significantly reduced risk of early miscarriage [OR 0.51 95% CI (0.27-0.96), 6 studies, n = 294, I^2=0%], compared to either placebo or no treatment. Continuing preconception metformin until at least the end of first trimester also significantly increased clinical pregnancy rates [OR 1.92 95% CI (1.43-2.59), 5 studies, n = 793, I^2=67%]. Only 3 studies, looked at live birth outcome which showed an increased odds of live birth in the metformin group, but this was not statistically significant [OR 1.38 95% CI (0.65-2.92), 3 studies, n = 212, I^2=0%]. In women who stopped taking metformin following a positive pregnancy test, there was a suggestion of improvement in clinical pregnancy [OR 1.36 95% CI (0.99-1.87), 6 studies, n = 663, I^2=45%] and live birth rates [OR 1.15 95% CI (0.57-2.35), 5 studies, n = 244, I^2=0%]; but these were not statistically significant. Conversely, there was an increased odds of early miscarriage [OR 1.22 95% CI (0.59-2.52), 6 studies, n = 244, I^2=0%], but this was also not statistically significant.

Limitations, reasons for caution: Whilst a role for commencing metformin pre-conceptually and continuing until at least the end of the first trimester has been demonstrated to be effective in improving clinical pregnancy rates and reducing miscarriage risk, the effectiveness and safety of continuing it throughout the remainder of the pregnancy remains unclear.

Wider implications of the findings: Continuing metformin treatment until at least the end of first trimester can increase the clinical pregnancy rate and reduce the risk of miscarriage. Stopping metformin once pregnant could be harmful and increase the risk of miscarriage. These findings should be considered when managing women with PCOS wishing to conceive.

Trial registration number: CRD42017073976

Abstract citation ID: dead093.992

P-666 High embryo yield (and not oocyte yield) despite low ovarian stimulation dose correlates with cumulative livebirth rate in normal-high responders undergoing IVF/ICSI

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Study question: Does a good response i.e., high oocyte or embryo yield with low ovarian stimulation correlate with cumulative live birth outcome in IVF/ ICSI treatment?

Summary answer: High ovarian sensitivity to stimulation (embryos to gonadotropin dose ratio) positively predicts cumulative live birth in IVF, while oocytes to stimulation dose ratio does not.

What is known already: The conventional belief that the higher the number of eggs retrieved, the better for IVF outcome has been challenged. Oocyte yield fails to predict fresh-cycle live birth in presence of high ovarian response. Ovarian sensitivity index (OSI) which is an expression of inherent ovarian responsiveness to stimulation remained overlooked as a predictor of success in IVF. We explore whether attention should be on the oocyte or embryo yield as a function of ovarian stimulation (OSI) can predictor of positive outcomes in IVF.

Study design, size, duration: Retrospective analysis of 750 patients who underwent IVF/ ICSI treatment with ≤150 IU of gonadotropin in an antagonist protocol due to predicted normal or high response. This is a further analysis of a previously published study covering a period between 1 January 2016 to 31 Dec 2018.

Participants/materials, setting, methods: Multi-centre study within a group company using the same treatment protocols. Participants were normal or high responders of IVF/ ICSI, predicted poor responders (according to Bologna criteria) were excluded. All patients received ≤150 IU daily dose of gonadotropin (FSH) in an antagonist cycle. Cumulative LBR calculated. Univariate and multivariate (regression) analysis, adjusting for all significant variables were done to find the predictors of cumulative livebirth.

Main results and the role of chance: When the women who achieved a livebirth were compared with those who did not, there was no difference in the mean BMI (23.7 vs 23.6, $p = 0.81$), antral follicle counts ($p = 0.33$), Anti-mullerian hormone levels ($p = 0,89$), mean starting (149.5 vs 140.5 IU/ day, $p = 0.16$), and total gonadotropin doses (1360.7 vs 1342.8 IU, $p = 0.62$). Women's age (33.5 vs 35.3 years, $p < 0.0001$) and the number of embryos transferred ($p < 0.0001$) had significantly correlation with LBRs. There was no difference in the mean number of oocyte (9.1 vs 8,6, $p = 0.21$) or oocyte yield per unit of gonadotropin (7.4 vs 6.7, $p = 0.32$)..

The mean number of embryos (5.4 vs 4.1, $p < 0.0001$), transferable embryos (2.5 vs 1.9, $p = 0.0006$) and embryo yield per unit of gonadotropin (4.1 vs 3.2, $p < 0.0001$) were significantly higher in the livebirth group.

After adjusting for woman's age and the number of embryos transferred, the embryo yield (both total ($p < 0.0001$) and transferable ($p = 0.01$) embryos and embryo yield per gonadotropin unit ($p = 0.0007$) were predictors of cumulative livebirth whereas oocyte yield and oocyte number per unit of gonadotropin were not.

The above findings remained the same when only IVF cycles excluding male infertility or only the cycles with a fixed 150 IU/d gonadotropin dose were analysed.

Limitations, reasons for caution: A moderate-sized study population. The poor responders have been excluded to minimise variations in the protocol and stimulation dose.

Wider implications of the findings: Our study is a proof of concept of 'ovarian sensitivity index' indicating the inherent ovarian responsiveness rather than the strength of stimulation determines the IVF/ICSI outcome. Embryo yield appeared to be a predictor of cumulative livebirth while oocyte yield is not, in normal and high responders of IVF.

Trial registration number: Not applicable

Abstract citation ID: dead093.993

P-667 The attenuated response to elevated serum progesterone levels on pregnancy outcomes of fresh IVF/ICSI cycles in older patients

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Study question: Whether there are different impacts of serum progesterone (P) levels on the day of trigger on pregnancy outcomes in patients of different age groups.

Summary answer: Our results suggest that the pregnancy outcome was less sensitive to progesterone elevation in older patients.

What is known already: It is now generally believed that the P elevation at the late follicular phase reduces the pregnancy rate by opening the implantation window prematurely and disrupt the embryo-endometrial synchrony. Previous studies further investigated the effect of developmental stages of embryo, number of oocytes retrieved, cause of infertility and different stimulation regimens on the association of P level and the likelihood of pregnancy. However, the study of maternal age on such association is scarce. A previous study found that LH receptor (LHCGR) and progesterone receptor (PGR) were up regulated in advanced age group.

Study design, size, duration: This was a non-interventional, retrospective cohort study of patients undergoing routine practice in a university-based fertility center between 2015 and 2021. One thousand five hundred and seventeen IVF/ICSI cycles among 1305 patients were included in the analysis.

Participants/materials, setting, methods: Fresh ET using autologous oocytes with serum P level measured on the day of hCG administration were included. Exclusion criteria include cycles without P measurement, using frozen oocytes, PGT cycles, freeze-all cycles, oocyte donation and no embryo transfer cycles. The patient age was divided into 3 groups: group 1 included patients at age 35 or below, group 2 included patients at age 36 to 39, group 3 included those at 40 years old or above.

Main results and the role of chance: We performed logistic regression analysis for each group to determine the contributing factors to the live birth outcome. In group 1, age, serum P level, number of usable embryos, embryo grading and endometrial thickness were found to be significantly associated with live birth. In group 2, age and number of usable embryos were significantly associated with live birth. However, total dose of gonadotropin was found to be the sole contributing factor to live birth in group 3. We also divided the P levels into low (≤0.50 ng/mL), median (0.51-1.00 ng/mL) and high (>1.00 ng/mL) and compared the live birth rate in each age groups. The live birth rate was found to be significantly reduced with increasing P levels (40.3% vs. 35.5% vs. 23.6%, $p = 0.005$). No significant difference in live birth rate between different P levels was found in age group 2 and 3.

Limitations, reasons for caution: The limitation of the study is its retrospective nature which may have led to bias in the interpretation of the data. There were also significantly higher proportion of cleavage-stage embryo to blastocyst transfer in the older group.

Wider implications of the findings: The negative association of premature progesterone rise at pre-ovulatory phase to the IVF fresh ET outcome was more prominent for younger patients. This may be one of the contributing factors that accounts for the great heterogeneity in the cut-off of P and its predicting power in different studies.

Trial registration number: Not applicable

Abstract citation ID: dead093.994

P-668 Effects of the active site domain of visfatin on the improvement of ovarian function in 4-vinylcyclohexene diepoxide-induced ovarian failure mice

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Study question: Can only the active site of domain of visfatin improve ovarian function in mice with 4-vinylcyclohexene diepoxide (VCD)-induced ovarian failure?

Summary answer: The active site domain of visfatin improves ovarian function and fertility potential in VCD-induced mice by stimulating the ovarian angiogenesis and early follicular development.

What is known already: Ovarian aging is a representative unmet need in infertility treatment and many studies have attempted to improve oocytes quality by activating ovarian angiogenesis. Visfatin has been reported to have angiogenic activity. Our previous study reported that visfatin improved ovarian function and fertility potential in aged female mice. However, visfatin has many limitations in developing as a drug due to large molecular weight. Visfatin is composed of two structural domains, and the active site is located in the second structural domain (amino acid residues 181 to 390), which were defined as the active site domain (hereafter A-domain peptide) in this study.

Study design, size, duration: This is a controlled experimental study using a total of mice with 60 VCD-induced ovarian failure. The duration of this study was from January to November 2022.

Participants/materials, setting, methods: C57BL/6 female mice were randomly divided into six groups: Five groups received 160mg/kg VCD and one group received an equal volume of vehicle. Four VCD groups were intraperitoneally injected three times at intervals of 2 days with 500 and 1000 ng/ml of full-size visfatin and A-domain peptide, respectively. The remaining one group served as a negative control. After final treatment, follicular development, gene expression, AMH level, and fertility potential were examined.

Main results and the role of chance: In hematoxylin and eosin (H&E) staining, numbers of functional follicles including primordial, primary, secondary, and antral follicles were significantly decreased in the VCD-vehicle group compared to the control. However, treatment of full-size visfatin and A-domain peptide increased the number of primordial and primary follicle. On immnohistochemistry (IHC), expression of BMP15, C-KIT, VEGF, and visfatin in ovarian tissues were significantly reduced in the VCD-vehicle group, but immunoreactivity of these factors strongly stained in the treatment of full-size visfatin and A-domain peptide. This effect was more remarkable in 500 ng/ml of A-domain peptide compared to other treatments. The serum AMH level was significantly lower in the VCD-vehicle group compared to the control group, but it was increased after the treatment with A-domain peptide and full-size visfatin.

Limitations, reasons for caution: This study does not elucidate a clear mechanism how A-domain peptide increases the ovarian function and fertility of VCD-induced ovarian failure mice although it stimulated the expression of genes associated with early follicular development and angiogenesis. Also, generalizability to humans may be limited because the study was conducted in mice.

Wider implications of the findings: This study suggests that administration of A-domain peptide could be applied as a new treatment strategy for ovarian aging, presumably by activating ovarian angiogenesis and dormant primordial follicles.

Trial registration number: Not applicable

Abstract citation ID: dead093.995

P-669 The effect of ovarian follicle size on oocyte fate and embryology outcome

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Study question: Does ovarian follicle size influence ultimate oocyte fate and embryology outcome?

Summary answer: Per punctured follicle, higher oocyte yield, MII oocytes, 2PN fertilisation, and utilisable blastocyst is observed as follicle increases in size up to threshold of 15.5mm

What is known already: It is well known that oocyte maturity and probability of successful fertilisation is correlated to the size of follicles where oocyte originated from. However the true cutoff and ideal size at oocyte retrieval remains elusive. Furthermore, there is no conclusive evidence that embryological outcome in terms of blastocyst morphology and utilisation differs in the mid to large ovarian follicular sizes.

Study design, size, duration: Prospective observational study undertaken in a tertiary fertility referral centre over 10 months. 50 participants recruited and underwent conventional controlled ovarian stimulation as per unit protocol. A total of 288 follicular punctures performed in the study period.

Participants/materials, setting, methods: 50 oocyte retrieval (OR) procedures were performed. The size of ovarian follicles was individually measured using transvaginal ultrasound. 288 follicular punctures were performed and up to 3 oocytes per patient were collected for the study to be individually cultured to allow for tracking of oocyte and embryo outcomes. Follicular fluid and spent culture media were collected separately for subsequent metabolomics analysis. Follicular sizes were divided into 6 groups (<=9.5, 10-12.5, 13-15.5, 16-19.5, 20-23.5, >=24).

Main results and the role of chance: Oocyte fate included 113 mature oocytes, 83 bipronuclear (2PN) oocytes, 7 cleavage stage embryos, and 39 good quality blastocysts suitable for utilisation in fresh or frozen transfer cycles.

When compared to overall average per punctured follicle, there is an association of higher oocyte yield, proportion of MII oocytes, 2PN fertilisation, and utilisable blastocyst as follicle size increases. The threshold for a change in fate appears to be when follicle size increases beyond 15.5mm where optimal follicle size may reach plateau.

Proportion of good-quality blastocysts appears to be associated with size of follicles at rates as follow- 0% (less than 10mm), 11.1% (10-12.5mm), 13.2% (13-15.5mm), 15.9% (16-19.5mm), 14.5% (20-23.5mm), and 20% (above 24mm). 23 embryos have been utilised to date, yielding a 50% positive pregnancy rate. Of these utilised embryos resulting in a positive pregnancy test, 81.8% of embryos originated from follicles above 15.5mm.

Metabolomics analysis for follicular fluid and spent culture media is ongoing.

Limitations, reasons for caution: The observational nature of the study along with a small sample size are limitations that did not allow clinical significance to be observed.

Wider implications of the findings: Oocyte retrieved from follicles less than 10mm is futile. There appears to be a plateau in optimal follicle size where no real detriment is demonstrated. This is an important findings to guide stimulation, especially in cases of asynchronous growth where sacrifice in larger follicles is commonly assumed.

Trial registration number: Not applicable

Abstract citation ID: dead093.996

P-670 Altered immunological conditions in women with PCOS and its correlation with the development and progression in infertile women with severe polycystic ovaries

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Study question: What is the impact of the dysfunctional lymphoid cells on the development and progression in infertile women with severe polycystic ovaries syndrome (SPCOS).

Summary answer: The dysfunctional lymphoid cells in immune system was deeply correlated with follicular remodeling, which prompting the progression of SPCOS.

What is known already: PCOS has been confirmed as a low-level chronic inflammation impacting on ovulation and luteinization regulated by a cytokine-mediated inflammatory response orchestrated by lymphocytes, granulocytes, and macrophages. However, the exact relationship between immunologic dysfunction and the pathogenesis of PCOS is still not clarified.

Study design, size, duration: A prospective cohort study including a total of 129 women aged 18 to 35 years were enrolled. PCOS patients with polycystic ovaries (ultrasound measurement of the number of $\geq$ 80 follicles $\geq$3 mm in diameter) were referred to SPCOS in this study. Peripheral Blood (PB) were collected to detect the immunological changes by using flow cytometry: CD8+ T cell (CD3+,CD8+); CD4+ T cell (CD3+,CD4+); NK cell (CD45+,CD3-,CD56+); NKT cell (CD45+,CD3+,CD56+); Treg cell (CD4+CD25hi); B cell (CD3-CD19+).

Participants/materials, setting, methods: PB from SPCOS cohort (n = 64) and healthy controls (n = 115) were collected to detect the immunological conditions. Furthermore, the discarded tissues from SPCOS patients (n = 5) who needed to avoid OHSS by surgical ovarian wedge resection and women undergoing fertility preservation procedures (n = 3) were digested to single cell suspension, which were used to outline the ovarian homeostasis changes by using scRNA-seq.

Main results and the role of chance: Peripheral blood NK cells from SPCOS patients were significantly decreased than those from healthy group (10.7% vs. 14.9 ± 6.2%, p = 0.04). Conversely, NKT cells in the PB of the SPCOS women were 11.2% vs. 6.9 ± 3.5% (P = 0.02), while B lymphocytes were 15.6% vs. 12.6 ± 4.2% (P = 0.04), compared to healthy controls. Furthermore, the immune cells and distinct cellular clusters of SPCO were disturbed in severe polycystic ovaries. The CD8+ T cell populations in ovarian of SPCOS group were strongly decreased (32.49% vs.22.35%, $p < 0.001$) and T cell activation signaling was downregulated by lowly expressed PTPRC, APBB1IP, LCP11 and MX12. Besides, the cytotoxicity-associated genes IFNG and TNF were highly expressed, especially in CD4+ T cells. The chemokines released by activated CD4+ T cells may increase the production and recruitment of CD8+ T cell and induce the compositional and functional alteration of ovarian, thereby prompting the progression of PCOS.

Limitations, reasons for caution: The study group is limited and transcriptome analysis covers much, but not enough. Although suggestive associations between immunological conditions and the severity of PCOS have concluded, additional animal models studies should be conducted to confirm our findings further.

Wider implications of the findings: The expression profiles of lymphoid cells provide deep insight into the molecular mechanisms of PCOS. Signaling receptors and gene data set will aid in future studies on the interorgan communication. It also facilitated discovery of novel biomarker for PCOS diagnosis and new immunological treatment.

Trial registration number: Not applicable

Abstract citation ID: dead093.997

P-671 Comparing clomiphene citrate/ flexible GnRH antagonist vs ultrashort flare GnRH agonist protocols for controlled ovarian stimulation in poor ovarian reserve patients

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Study question: How do Clomiphene citrate (CC)/ flexible GnRH antagonist and ultrashort flare GnRH agonist protocols compare in poor ovarian reserve patients (POSEIDON groups 3 & 4)?

Summary answer: In poor ovarian reserve patients, irrespective of age, use of CC / flexible antagonist vs ultrashort flare protocol significantly reduces gonadotrophin consumption and stimulation duration.

What is known already: Poor responders constitute 47% of ART patients and up to 65% of these have diminished ovarian reserve. Despite the various treatment options available, lack of good quality evidence to enable recommendation of a particular protocol or adjuvant continues to pose a challenge to their effective management. The need for multiple stimulations for pooling up embryos also makes effective cost of treatment an added concern in them.

Study design, size, duration: An open label, multi centric, randomized controlled study was conducted over a period of 1 year recruiting 116 women having AMH <1.2ng/dl and antral follicle count (AFC) <5 who were further analysed in POSEIDON 3 and 4 subgroups. Patients were randomized to 1 of 2 groups for controlled ovarian stimulation. Those with severe endometriosis, adenomyosis, autoimmune or metabolic disorders, sustained hyperprolactinaemia, congenital uterine malformations and with male partners having severe oligoasthenoteratozoospermia were excluded.

Participants/materials, setting, methods: Group A (55 women) received CC 100mg from D1-D5 with 300 IU human menopausal gonadotrophin (hMG) in a flexible GnRH antagonist protocol. Group B (61 women) underwent ultrashort flare protocol. 1mg leuprolide was administered from D1-D4 and 300 IU hMG given D2 onwards followed by flexible GnRH antagonist administration. Dual trigger with triptorelin and recombinant hCG was administered 35-36 hours before oocyte retrieval. Embryos cultured till D5 were vitrified for transfer at a later stage.

Main results and the role of chance: The two groups were well matched for baseline characteristics like age, BMI, AMH, duration of infertility, D2 AFC, estradiol and FSH levels. The mean total gonadotrophin consumption (3387.29 + 1082.5 vs 4407 + 1224.2, *p value <0.01)* and the duration of stimulation (10.71 + 2.52 vs 11.95 + 2.43, *p value <0.01*) were significantly lower in group A vs group B. The primary outcomes of median number of oocytes retrieved (4 vs 4, *p value- 0.71)* , M2 rate (3 vs 3, *p value-0.91*), fertilisation rate were similar in the two groups. The secondary outcomes of oocyte retrieval rate (103.05 + 42.97 vs 97.14 + 42.96, *p value- 0.63*), FORT (0.81 +0.68 vs 0.96 + 0.48, *p value- 0.37*) FOI (0.91 + 0.55 vs 0.92+ 0.54, *p value 0.92*), top quality embryo rate (0.53 + 1.1 vs 0.41 + 0.84, *p value 0.61*), cycle cancellation rate (26.22% vs 29.09%), implantation rate (23.63% vs 24.59%) and clinical pregnancy rate (21.31% vs 20%) were also similar in group A vs B respectively. A subgroup analysis of POSEIDON 3 versus POSEIDON 4 patients revealed similar outcomes with both the protocols in the two groups.

Limitations, reasons for caution: Small sample size of the cases limits the current statistical power of the study.

Wider implications of the findings: CC /flexible GnRH antagonist protocol is an effective alternative to ultrashort flare protocol in patients with poor ovarian reserve irrespective of their age. The lower gonadotrophin dosage required and shorter duration of stimulation observed make it a viable option in patients who might need multiple stimulations for pooling up embryos.

Trial registration number: Not applicable

Abstract citation ID: dead093.998

P-672 Vaginal microbiome changes in women with polycystic ovary syndrome: a large cross-sectional study

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Study question: How the vaginal microbiome changes in women with polycystic ovary syndrome?

Summary answer: The PCOS women had a higher diversity in the vaginal microbiome and showed an enhanced level of heterogeneity.

What is known already: PCOS is a common reproductive endocrine disorder with a highly heterogeneous clinical presentation. Recently, few studies with limited sample size have reported that the inconsistent vaginal microbiota composition changes in PCOS.

Study design, size, duration: The cross-sectional study conducted at a single academic university-affiliated center. A total of 1,446 subjects, including 713 PCOS cases and 733 controls, were recruited.

Participants/materials, setting, methods: Vaginal swabs were collected for subsequent 16S rRNA gene sequencing. The microbiome diversities, community distances, vaginal bacterial relative abundances, and microbial interactions network were compared between the PCOS group and control group.

Main results and the role of chance: The PCOS group had a higher alpha diversity in the vaginal microbiome than the control ($P < 0.05$), while higher intra-group variability was observed in PCOS ($P < 0.05$). At the genus level, the abundance of *Lactobacillus* in the PCOS group decreased, while the abundance of *Gardnerella* and *Ureaplasma* increased (FDR<0.2). *Gardnerella vaginalis*, *Prevotella buccalis*, and *Prevotella timonensis* were identified as differential species and were significantly associated with clinical parameters of PCOS, like AMH. The microbial interaction network analysis revealed that *Prevotella* and *Lactobacillus* could be key drivers for network rewiring in PCOS. Notable alterations of predicted pathways, which significantly differed between PCOS and control women, mainly enriched in amino acid metabolism.

Limitations, reasons for caution: The study only included Chinese Han population data. Due to the structure of the vaginal flora may vary between ethnic groups, generalizing the results of this study to other populations should be taken caution.

Wider implications of the findings: This study with large samples could enhance our understanding of the PCOS vaginal microbiome, and provide a basis for future research on the potential mechanism by which pathogenic bacteria are involved in PCOS vaginal microbial imbalances.

Trial registration number: Not applicable

Abstract citation ID: dead093.999

P-674 Automatizing ovarian follicle counting and measurement with artificial intelligence

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Study question: Can ovarian follicle counting and volumetric measurement in ultrasound cine-loops be automated using deep learning methods?

Summary answer: A deep-learning model achieved an Area Under Precision-Recall Curve (AUC-PR) of 77.8% for identifying follicles of diverse sizes, in cine-loops from everyday clinical practice.

What is known already: The measurement of ovarian follicles via transvaginal ultrasound is an established procedure in infertility treatment. The high predictive value of antral follicle count (AFC) is utilized in hormonal dosing algorithms, scheduling of triggering and oocyte retrieval, as a criterion to assess hyperstimulation risk, etc. As it is repeatable and time-consuming, it is a good candidate for automatization. However, traditional methods fail to distinguish follicles from acoustically shadowed areas, ovarian cysts, or anechoic extraovarian structures. Existing approaches assume manual ovary outlining or 3D volume acquisition, which requires additional operator training. Moreover, existing evaluations rarely define criteria for correct follicle identification.

Study design, size, duration: A retrospective study was conducted on 331 ultrasound cine-loop videos from 100 patients (mean age 35 ± 5.6, AMH level 3.3 ± 2.9 ng/ml) undergoing an IVF or donor cycle between February and December 2021 at six IVF centers in Poland. For training the model, 1903 more cine-loops from 350 other patients were used. To reflect everyday clinical practice, there was no selection based on patient condition or video quality.

Participants/materials, setting, methods: Folliscan (MIM Solutions) is a model developed based on 3D neural networks architectures. It analyzes a cine-loop without any manual preprocessing and presents a list of follicles together with exact 3D outlines and confidence scores. A total of 24711 follicles were manually annotated and reviewed by sonography experts to ensure exhaustive enumeration. A detected follicle is considered correct if it sufficiently overlaps (Intersection over Union above 35%) with a single expert annotation.

Main results and the role of chance: The precision was 85.8% (95% Confidence Interval: 83.7–87.7) and recall was 74.2% (CI 71.7–76.6). The area under the precision-recall curve (AUC-PR) was 77.8%. We note that 19.7% of annotations were only added during review, indicating that even expert sonographers fail to annotate a certain number of follicles.

For studies performed on days 7-12 of stimulation (N = 163 cine-loops), when only follicles ≥10mm were taken into account, accuracy was significantly higher: precision 95.4% (CI 93.0–96.8), recall 87.7% (CI 73.1–94.2).

We observe that the smallest anechoic areas, about 1mm in diameter, often are not imaged in enough resolution to be unanimously classified by experts. Common reasons for errors in medium-sized follicles are poor image quality (acoustic shadowing, unclear boundaries between nearby follicles), ambiguous volumes (where two outlines can seem equally reasonable, but do not overlap sufficiently), non-convex follicle shapes (due to adjacent follicles).

When measuring follicle diameters, the Mean Average Error (MAE) was 0.76mm, only slightly larger than the inter-observer MAE of 0.62mm (calculated on 32 cine-loops for which two experts independently measured all follicles). Moreover, 3D outlines enable a more physiological measurement than 2D diameters.

Limitations, reasons for caution: Follicle recognition is highly dependent on image quality and patient group. To contrast our model with manual methods, more cine-loops would need to be independently annotated by multiple experts. A future study on 3D acquisitions would be useful to compare with software that requires it, such as SonoAVC (GE Healthcare).

Wider implications of the findings: Automatizing follicle measurement can speed-up the process and diminish acquisition requirements (examination time, operator experience). Deep learning methods consider each follicle's context, enabling more reliable recognition of ovarian structures. They have potential to increase the predictive value of follicle counting beyond what is already possible with human observers.

Trial registration number: N/A

Abstract citation ID: dead093.1000

P-675 Analysis of a digitally integrated ovulation date kit to determine ovulation precisely

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Study question: Do digitally integrated ovulation prediction kits (OPK) predict the fertile window and the ovulation date accurately? And can this prediction be proven by sonography?

Summary answer: Particularly for subfertile women with irregular cycles, OPKs can help predict their fertile window and ovulation day, in accordance with vaginal sonography.

What is known already: The fertile window is defined as the period of five days before ovulation including the day of ovulation itself. Prediction based only on cohort observations or historical datasets may be inaccurate for women with irregular cycles, particularly when unforeseen changes of ovarian function occur during reproductive life. Urinary luteinizing hormone (LH) assays and smartphone applications are already widely used to detect the optimal time for conception, but can only predict a likelihood of ovulation without proof.

Study design, size, duration: 100 healthy women between the ages of 21 and 45, cycle length up to 42 days, trying to conceive and without hormone intake were to be recruited in the gynecological outpatient clinics of the Technical University of Munich (TUM) and of the University of Ulm. Each participant entered the study for up to three consecutive cycles. Two vaginal ultrasound scans were performed per cycle – before and after the ovulation date prognosis by the app.

Participants/materials, setting, methods: After informed consent, participants downloaded the smartphone application "Pearl Fertility" and received OPKs consisting of four different sets of lateral flow immunoassay test strips (LFIA): for luteinizing hormone, follicle-stimulating hormone, progesterone and human chorionic gonadotropin. Home measurements with LFIAs were performed with morning urine. The phone camera feed analyzed the color information of the LFIAs. Three differently prioritized algorithms calculated the ovulation date. Two questionnaires evaluated patient baseline characteristics and their perception of the app.

Main results and the role of chance: 89 women were recruited. Mean age was 34.6 yrs. (SD ± 4.2). On average, the included women had tried to conceive for 7.5 (± 5) months before joining the study. 8% (n = 7) became pregnant before the first cycle started.140 completed cycles in 71 remaining women were analyzed so far. 46% of the recruited women completed the study after 3 cycles, the drop-out-rate was 32.4%.

The app predicted ovulation for cycle day 14.3 (mean, SD ± 3.5). In 25 cycles the date of ovulation was before day 12 and in 33 cycles on day 16 or later.

The first ultrasound was done on cycle day 13.5 (mean, SD ± 3), the second ultrasound on cycle day 17.6 (mean, SD ± 3.6). In several cycles at least one (up to three) corrections of the prognosis occurred by the app, resulting in the day of predicted ovulation being moved either backward (retrospectively) or forward by 2.5 (mean, SD ± 1.9) days. Urinary progesterone measured with the OPK and post-ovulatory ultrasound were in accordance in the majority of cycles.

17% (n = 12) of the women became pregnant in the current analysis (mean duration to pregnancy: 1.3 cycles). In the ITT population (after informed consent) the rate was 23%.

Limitations, reasons for caution: This study was performed without a control group. Therefore, as an intraindividual control, a follow-up is planned to calculate the pregnancy rates and outcomes with and after the use of the app.

Wider implications of the findings: Our study shows that digitally integrated OPK are able to predict the fertile window and the ovulation date in a slightly subfertile cohort unselected for mild cycle irregularities. An early and accurate prediction may help couples to conceive, particularly if the date of ovulation is divergent from standard information.

Trial registration number: Not applicable

Abstract citation ID: dead093.1001

P-677 Letrozole inhibits ovulation via reducing responsiveness to luteinizing hormone in in vitro-grown mouse follicles

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Study question: Does letrozole reduce responsiveness to luteinizing hormone (LH) in *in vitro*-grown mouse follicles?

Summary answer: We found that letrozole reduced responsiveness to LH via reducing of luteinizing hormone/chorionic gonadotropin receptor (*Lhcgr*) transcription in *in vitro*.

What is known already: The continuous dosing of letrozole, the third-generation aromatase inhibitor, is used for the controlled ovarian stimulation (COS) of fertility preservation cycles in women with hormone-sensitive breast cancer to prevent the transient rise of serum estrogen levels. Recently, lower oocyte maturation rates and higher abnormal fertilization rates in COS with letrozole compared to standard COS have been reported. The studies using aromatase knockout mice and estrogen receptor beta knockout mice showed that intra-follicular estrogen is essential for responsiveness to LH of granulosa cells. However, whether letrozole reduces responsiveness to LH of follicles remains unknown.

Study design, size, duration: We evaluated the effect of letrozole on responsiveness to LH using *in vitro* mouse follicle culture system. Preantral follicles (150-200 μm) with a small number of stromal cells were isolated from 3 weeks old C57BL/6jjcl mouse ovaries and cultured in the presence of letrozole. We analyzed *in vitro* ovulation ability, and expression levels of ovulation-related genes to clarify the effect of letrozole on responsiveness to LH of follicles.

Participants/materials, setting, methods: The follicles were cultured with letrozole (0.01μM, 0.1μM, or 1μM) or vehicle (0.1% DMSO) for 5 days followed by 5 IU/ml hCG and 5 ng/ml hEGF stimulation as ovulation induction. The diameter and survival rate of follicles were assessed. After16 hours after ovulation induction, we assessed the ovulation and measured the transcription of *Lhcgr, Ptgs2, Runx1* and *Actb* (internal control) in the follicles by RT-qPCR. We conducted a collateral experiment in the presence of estradiol.

Main results and the role of chance: The follicle growth and survival rate did not change by letrozole *in vitro*. However, *in vitro* ovulation was reduced by the addition of letrozole in a dose-dependent manner (Cochran-Armitage trend test, $P < 0.0001$): 41.2% in the 0.01μM letrozole treated group (n = 23), 10.5% in the 0.1μM letrozole treated-group (n = 17), and no ovulation in the 1μM letrozole treated-group (n = 19) compared with 69.6% in the vehicle-group (n = 18). Exogenous estrogen addition restored the ovulation ability of 1μM letrozole-treated follicle to the same levels as the vehicle group. RT-qPCR revealed that 0.1 μM letrozole significantly reduce the transcription levels of *Lhcgr* ($P = 0.0027$), *Runx1* ($P = 0.0124$), and *Ptgs2* ($P = 0.0067$) compared with vehicle-treated follicles. Exogenous estrogen addition restored the transcription of these genes to levels observed in vehicle-treated follicles. We also observed a positive correlation between the transcription of *Lhcgr* and *Ptgs2* ($R = 0.55$, $P = 0.0003$), *Lhcgr* and *Runx1* ($R = 0.71$, $P < 0.0001$), and *Ptgs2* and *Runx1* ($R = 0.77$, $P < 0.0001$). Our results suggested that letrozole-treated follicles impaired ovulation due to dysregulation of *Lhcgr* transcription and its downstream cascade caused by estrogen deficiency.

Limitations, reasons for caution: This is an in vitro experiment using a mouse model. Further research is needed to determine whether our findings are applicable to humans.

Wider implications of the findings: Our findings imply that letrozole may have disadvantages in not only ovulation, but also subsequent reproductive processes affected by the effectiveness of the LH surge, such as oocyte maturation, fertilization, and preimplantation embryo development. When evaluating oocyte cryopreservation for fertility preservation, it may be necessary to consider the COS protocol.

Trial registration number: Not applicable

Abstract citation ID: dead093.1002

P-678 Predictive value of intra-cycle FSH measurements for blastocyst euploid rate: a longitudinal cohort study

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Study question: Can intra-cycle FSH levels measured at baseline, mid-cycle, and on trigger day predict blastocyst euploid rate?

Summary answer: Repeat measurements of FSH were not useful for predicting the euploid rate of blastocysts after accounting for the effects of female age.

What is known already: During the follicular phase of ovarian stimulation cycles, blood FSH levels are kept at supraphysiologic levels for a longer period compared to naturally occurring ovulation cycles during which FSH levels recede in response to rising estradiol. Some studies suggested maintaining higher than necessary blood FSH concentrations may have a detrimental effect on embryo quality and ploidy and recommended intra-cycle FSH monitoring. Whether intra-cycle measurements of FSH can be used to predict euploid rate is yet to be determined.

Study design, size, duration: Retrospective study was performed at tertiary IVF refereral center, including 373 cycles between April 2017 and February 2022. All GnRH (Gonadotropin-Releasing-Hormone) antagonist stimulation cycles using only recombinant FSH were included. Included patients were between 19 and 44 years old and all embryos underwent aneuploidy testing with Next Generation Sequencing using trophectoderm biopsy. Patients with PCOS, surgical sperm extraction, or warmed oocytes were excluded.

Participants/materials, setting, methods: Patients with primary or secondary infertility and an indication for ovarian stimulation for IVF/ICSI with PGT-A were included. Ovarian stimulation cycles were monitored according to the clinical routine by ultrasound and repeated measurement of FSH, E2, and progesterone at baseline, mid-cycle, and on trigger day. FSH changes were evaluated as delta differences between basal to mid-cycle levels and from mid-cycle to trigger levels.

Main results and the role of chance: 1,119 intra-cycle measurements of FSH were obtained from 373 cycles of 344 women. The median age of included women was 31 years (IQR:27-34), with AMH levels of 3.28ng/ml (IQR: 2.13-4.24). The euploid rate per fertilized oocyte was analyzed in categories of low (≤33.3%), normal (33.3 to 66.6%), and high (>66.6%). Delta change in serum FSH values from baseline to mid-cycle was not significantly different between groups (median change: 7.6 IU, 7.4 IU, and 6.2 IU; low, normal, and high euploid rate groups, respectively, P = 0.481). Again, delta change in mid-cycle to trigger day FSH was not significantly different between groups either (median change: -0.2 IU, 0.0 IU, 0.6 IU; low, normal, and high euploid rate groups, respectively, P = 0.151). After adjusting for the effect of age, basal FSH values (OR: 1.11, 95% CI:0.91-1.34, P = 0.248), delta FSH change to mid-cycle (OR: 0.99, 95% CI:0.91-1.07, P = 0.951) or delta change to trigger day (OR: 1.14, 95% CI:0.99-1.31, P = 0.067) was not significantly associated with high euploid rates. Performance of age alone compared to age and delta of FSH measurements combined for predicting high euploid rates, as measured with area under the curve (AUC) values, were AUC: 0.63 (95% CI: 0.54-0.71) and AUC: 0.66 (0.58-0.73), respectively (P = 0.369).

Limitations, reasons for caution: This was a retrospective study including only recombinant FSH cycles and findings represented here may not be generalizable to larger populations. Sample power may not be adequate to detect some effect sizes.

Wider implications of the findings: We could not demonstrate a direct relationship between euploid rate and delta change in intra-cycle FSH measurements. More studies using more diverse populations and larger sample sizes may be needed to further investigate this association.

Trial registration number: Not applicable

Abstract citation ID: dead093.1003

P-679 Controlled ovarian stimulation using follitropin delta results in higher cumulative live birth rate compared to follitropin alfa/beta in a large prospective real world dataset

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Study question: Does IVF/ICSI-treatment using follitropin delta (hrFSH) result in higher pregnancy rates (PR) or live birth rates (LBR) compared to follitropin alfa/beta (recFSH)?

Summary answer: Real world data (RWD) from a large prospective German registry study shows that using hrFSH results in higher pregnancy rates compared to recFSH.

What is known already: Follitropin delta represents a new recombinant FSH derived from a human cell line. Contrary to follitropin alfa or beta, follitropin delta has a glycolysation pattern consisting of α2,3- and α2,6-linked sialic acids which is more similar to native human FSH. Follitropin delta is approved for use with an individualized dosing algorithm based on serum AMH and body weight, targeting an optimal ovarian response (8-14 oocytes). Efficacy and safety have been demonstrated in numerous clinical trials. Moreover, Follitropin delta seems to reduce the risk of OHSS although its safety with respect to ovarian hyperstimulation using RWD remains to be investigated.

Study design, size, duration: The German IVF Registry collects prospectively data about IVF/ICSI-treatments from 140 German IVF centers. The underlying analysis of RWD includes controlled ovarian hyperstimulation cycles that have been performed in Germany between 2017-2021. Study groups were built subject to the gonadotropin used for stimulation, irrespective of previous treatments: (1) hrFSH, n = 3002, (2) recFSH, n = 135293. "Pregnancy" was defined as clinically assessed, intrauterine pregnancy, including miscarriages. Biochemical pregnancies were not defined as "pregnant". Ectopic pregnancies (n = 558) were excluded.

Participants/materials, setting, methods: PR and LBR were calculated subject to the number of embryo transfers (ET) (n = 2062 (rFSH) vs. n = 103357 (recFSH)) after excluding freeze-all cycles, (n = 529 (hrFSH) vs. n = 13682 (recFSH)) and cycles that ended without ET (n = 411 (hrFSH) vs. n = 18254 (recFSH)). The collected data were saved in compliance with the applicable data processing regulations. Statistical analysis was performed using Fishers-exact- and students-t-test and Microsoft Excel, whereas $p < 0.05$ was defined as significant.

Main results and the role of chance: There was no difference between the study groups regarding age (33.9 ± 4.00y (hrFSH) vs. 33.8y ± 4.34y (recFSH), p = 0.12) or infertility diagnosis. Stimulation with hrFSH resulted in a higher number of oocytes (11.15 ± 7.2 vs. 10.39 ± 7.01, $p < 0.01$). Antagonist protocol was used less often in the hrFSH-group (78% (2344/3002 vs. 80% (109217/135293), $p < 0.01$). PR in 2021 were higher (39.2% (222 pregnancies / 567 embryo transfers) vs. 35.2% (6837/19420), OR = 1.18 [0.99-1.41] p = 0.05) while using hrFSH compared to recFSH. The effect was even stronger when comparing patients aged 30-34y (46.2% (98/212) vs. 38.6% (2967/7865), OR = 1.41 [1.07-1.88], p = 0.01). Another quantitative difference could be observed for patients aged 30-34y in their first IVF/ICSI-cycle (PR 48.5% (63/130) vs. 39.5% (1926/4873), OR = 1.44 [1.00-2.07], p = 0.05). Including all cycles between 2017-2021 resulted in higher PR for couples treated with hrFSH (38.3% (790/2062) vs. 36.2% (37439/103357), OR = 1.09 [1.00-1.20], p = 0.049). Cumulative PR including consecutive frozen embryo transfers after the first stimulation showed significantly higher PR while using hrFSH (81.6% (422/517) vs. 71.3% (17373/24367), OR = 1.79 [1.42-2.23], $p < 0.01$). Finally, cumulative LBR per ET was significantly increased if hrFSH was used for ovarian stimulation (60.0% (310/517) vs. 51.9% (12648/24367), OR = 1.39 [1.16-1.66], $p < 0.01$) compared to recFSH.

Limitations, reasons for caution: Since we analyzed RWD, comparison with large clinical trials should be considered carefully. PR and LBR was calculated only using stimulations which successfully generated ET. Moreover, individual AMH values are not transmitted to the German IVF Registry. Therefore, difference in ovarian reserve between study groups can't be excluded.

Wider implications of the findings: In this large, prospective RWD, higher cumulative LBR and PR using hrFSH compared to recFSH, irrespective of age or infertility diagnosis, supports the use of individualized fertility treatment approach based on hrFSH. These results are consistent with previous retrospective findings.

Trial registration number: Not applicable

Abstract citation ID: dead093.1004

P-680 Oocyte maturation triggering protocols in in vitro fertilisation treatment: a systematic review and network meta-analysis

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Study question: What is the relative effectiveness and safety of final oocyte maturation trigger protocols in women undergoing in vitro fertilisation (IVF) treatment?

Summary answer: OHSS rate was lowest with GnRH agonist trigger and no difference in LBR was observed comparing various trigger protocols in normal, high and poor responders.

What is known already: Oocyte maturation trigger prior to oocyte retrieval is a crucial component of ovarian stimulation(OS). Pituitary suppression is an important component of OS in IVF, involving the use of GnRH analogues. Currently, the GnRH antagonist regimen is predominantly used given its comparable efficacy and lower risk of OHSS compared with GnRH agonist (GnRHa) regimens. Whilst only hCG trigger can be used with GnRH agonist regimens, GnRH antagonist regimen enables use of hCG trigger, GnRH agonist trigger or combinations of both as dual or double trigger. However, there is no consensus on how these trigger protocols compare in their effectiveness and safety.

Study design, size, duration: The following databases were searched until August 2022: MEDLINE, EMBASE, CINAHL, CENTRAL and ClinicalTrials.gov. Randomised controlled trials (RCTs) comparing at least two trigger protocols: hCG trigger, GnRH agonist trigger, dual trigger (hCG and GnRH agonist administered at the same time) and double trigger (GnRH agonist followed by hCG after a time interval) with the antagonist regimen were included. Primary outcome was live birth (LBR) per participant. Secondary outcomes included number of oocytes and OHSS rates.

Participants/materials, setting, methods: Two reviewers independently screened, selected studies and extracted data. Pairwise and network meta-analyses (NMA) were conducted according to ovarian response groups (normal, high and poor response). Effect estimates were presented as weighted means difference (WMD) and risk ratio (RR) with 95% confidence interval (CI) for continuous and dichotomous outcomes respectively. Quality assessment was performed using GRADE

Main results and the role of chance: Initial searches identified 4225 studies, of which 54 RCTs involving 5838 women met the inclusion criteria to be included in the analysis.

In normal responders, there is no difference in LBR with the GnRHa vs hCG (RR:1.11,95%CI:0.83-1.49;3 studies,430 women,I^2=33%,low-certainty evidence, direct comparison(DC)),dual trigger vs hCG(RR:1.14,95%CI:0.99-1.31;4 studies,1007 women,I^2=33%,low-certainty evidence, DC),double trigger vs hCG(RR:0.53, 95%CI:0.27-1.06;indirect comparison(IC)),dual vs GnRHa (RR:0.97, 95%CI:0.70-1.34, IC),double vs GnRHa (RR:2.07, 95%CI:0.98-4.38, IC),dual vs double (RR:2.14, 95%CI:1.06-4.32;IC).

In high responders, there is no difference in LBR with the GnRHa vs hCG (RR:1.04, 95%CI:0.84-1.29; 3 studies,178 women, I^2 = 44%, low-certainty evidence, DC, dual trigger vs hCG (RR:1.82, 95%CI:1.25-2.67; 4 studies,117 women, I^2 = 37%, low-certainty evidence, DC), double trigger vs hCG (RR:2.10, 95%CI:1.29-3.43, IC), dual vs GnRHa (RR:0.57, 95%CI:0.37-0.88, IC),double vs GnRHa (RR:0.49,95%CI:0.29-0.88, IC),dual vs double (RR:0.87, 95%CI:0.64-1.18; 1 study,57 women, low-certainty evidence,DC).

In poor responders, there may be a difference in LBR when comparing dual trigger to hCG (RR:1.12,95%CI:1.12-2.89 1 study,112 women,low-certainty evidence,DC).

OHSS rates were lowest with the use of GnRHa in high responders (RR:0.39,95%CI:0.04-3.78;9 studies;960 women;I^2 =43%, low-certainty evidence,DC) and normal responders (RR:0.88, 95%CI:0.78 to 0.99;7 studies; 2246 women; I^2=0%, low-certainty evidence,DC).

There was no significant difference in number of oocytes or miscarriage risks with the use of any triggers.

Limitations, reasons for caution: Stratifying results by predicted ovarian response resulted in disconnected networks, limiting our ability to perform NMA for certain groups and outcomes. The certainty of the evidence was limited by high risk of bias.

Wider implications of the findings: Our results suggest that the use of short GnRH agonist trigger results in reduced OHSS rates in women with predicted normal or high ovarian response. There is no difference in LBR and number of oocytes comparing the different trigger protocols in all response groups (normal, high, poor responders)

Trial registration number: Not applicable

Abstract citation ID: dead093.1005

P-681 Embryo utilisation rate and blastocyst formation rate correlate negatively with the oocyte yield and positively with live birth rate: an analysis from HFEA's national database

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Study question: Do embryo utilisation rate (EUR) and blastocyst formation rate (BFR) correlate with oocyte yield and livebirth rate (LBR) in fresh IVF/ICSI cycles?

Summary answer: EUR and BFR correlate positively with livebirth rate (LBR) but decline with increasing number of oocytes to a nadir when the fresh cycle LBRs plateaus.

What is known already: LBR in fresh IVF cycles plateaus once a certain number of oocytes are retrieved. While impaired endometrial receptivity with high ovarian response appears to be an underlying cause, other factors, such as decreasing competency of oocytes or embryos cannot be ruled out. Cytoplasmic dysmorphism of oocytes and chromosomal disorders have been shown to rise with increasing number of oocytes. Consequently, cumulative livebirth rate per oocytes has been reported to decline with increasing oocyte yield. When obvious male factor is excluded, the competency of oocytes can be reflected in the embryo grading.

Study design, size, duration: Retrospective analysis of 8015 fresh IVF/ICSI cycles that met the inclusion criteria from the national database published by Human Fertilisation and Embryology Authority (HFEA) in the United Kingdom. We analysed 2 years of published data between January 2015 and December 2016.

Participants/materials, setting, methods: Population included couples at their first IVF treatment with single fresh blastocyst transfer, due to tubal or unexplained infertility among women aged <40 years. Only cycles that produced at least 1 transferable blastocyst were included to determine the blastocyst formation rate. Inclusion criteria were designed to minimise other co-factors that may influence embryo quality. Linear regression analysis was done to find the correlation between EUR/ BFR and oocyte yield; multiple regression to predict livebirth

Main results and the role of chance: EUR (correlation coefficient = -0.156605, $p < 0.0001$) and transferable BFR correlation coefficient = -0.156605, $p < 0.0001$) declined with increasing number of oocytes. The findings were no different whether IVF or ICSI was performed.

Both EUR (OR 1.002, CI 1.001- 1,004) and (transferable) BFR (OR 1.005, CI 1.003-1.006) correlated positively with LBR ($p < 0.0001$, for both), after adjusting for other confounders including age and method of insemination.

Both (transferable) BFR and EUR declined with the increasing number of oocytes falling to a nadir when around 8 oocytes were retrieved then remained static with higher oocyte yield. LBR in fresh cycles also reaches its peak at around 8 oocytes then plateaus.

Limitations, reasons for caution: Information on patient's BMI or stimulation dose is not available in the HFEA database. However, it might have little impact in our findings given large sample size. Cycles with no blastocyst formation through extended culture were not included, as this intention to treat information was not available

Wider implications of the findings: Declining proportion of competent embryos may result in flattening of LBR graph in fresh cycles after certain number of oocytes retrieved. Increasing absolute number of competent embryos fails to boost fresh cycle LBR after a saturation point, We have to improve embryo selection than increasing ovarian stimulationto improve fresh LBR

Trial registration number: NA

Abstract citation ID: dead093.1006
P-682 Androgen and Inhibin B levels during ovarian stimulation before and after eight weeks of low dose hCG priming in women with low ovarian reserve

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Study question: Does eight weeks of low dose human chorionic gonadotropin (hCG)-priming affect androgen or Inhibin B levels in serum and follicular fluid during IVF/ICSI treatment?

Summary answer: Inhibin B was reduced on stimulation day 1 in the IVF/ICSI cycle following hCG-priming. Androgen levels in serum and follicular fluid was not increased.

What is known already: In our recently published pilot study, eight weeks of low-dose hCG-priming was used to stimulate the intraovarian androgen synthesis in women with AMH <6.29 pmol/L. Priming resulted in more retrieved oocytes and more follicles sized 2-5 mm and less sized 6-10 mm at the start of stimulation. Stimulation time and FSH consumption was increased after priming. HCG priming potentially stimulate the intraovarian androgen synthesis causing upregulation of FSH receptors on granulosa cells, and therefore it was unexpected that the antral follicles were smaller and stimulation time longer following priming. This might indicate a different mechanism of action than previously thought.

Study design, size, duration: A prospective, paired, non-blinded, single-center study including 20 women conducted between January 2021 and July 2021 at a tertiary referral hospital. Participants underwent two identical IVF treatments: a Control cycle including elective freezing of all blastocysts and a Study cycle with fresh blastocyst transfer. The Control and Study cycles were separated by eight weeks (two menstrual cycles) of hCG-priming by daily injections of 260 IE hCG (details in our previously published paper).

Participants/materials, setting, methods: Women aged 18-40 years with cycle lengths of 23-35 days and AMH <6.29 pmol/L. Control and Study IVF cycles were performed in a fixed GnRH-antagonist protocol. Blood samples were taken on stimulation day 1, stimulation day 5-6, trigger day, day of oocyte pick up (OPU), and trigger day + 7 days in the Control and Study cycles. Follicular fluid was collected from the first aspirated follicle on both sides during OPU in both cycles.

Main results and the role of chance: Inhibin B serum levels were significantly lower on stimulation day 1 after hCG-priming, but no other significant differences in serum Inhibin B or androgen levels were seen. The concentrations of Inhibin B and androstenedione in the follicular fluid from the Study and Control cycle did not vary but testosterone was significantly lower in the Study cycle. A lower Inhibin B in the Study cycle corresponds with the antral follicles being significantly smaller after priming, and this probably led to a longer stimulation time in the Study cycle. This contradicts the theory that androgen priming causes more FSH receptors on developing (antral up to preovulatory) follicles. Instead, we hypothesize that androgen priming rescued some small antral follicles that would have otherwise undergone atresia by the end of the previous menstrual cycle. The mechanism of action was likely an increase in androgen levels and FSH receptor expression within these small follicles, however, not enough to elevate androgen levels in serum. We retrieved significantly more oocytes in the Study cycle and the production of estradiol per follicle ≥10 mm on trigger day was comparable in the Study and Control cycle, suggesting that the rescued follicles were competent in producing oocytes and steroid hormones.

Limitations, reasons for caution: The sample size was small, and androgen and Inhibin B levels were not the primary outcome. We have not demonstrated a higher androgen level or FSH receptor expression in small antral follicles following hCG-priming.

Wider implications of the findings: The results make us question the mechanism of action behind hCG-priming prior to IVF. It is important to design a study with retrieval of small antral follicles immediately after priming to investigate the proposed hypothesis and to confirm improved cycle outcomes i.e., more retrieved oocytes in a larger study population.

Trial registration number: NCT04643925

Abstract citation ID: dead093.1007
P-683 Live birth rate following medicated frozen-thawed embryo transfer with or without GnRH-antagonist (Cetrotide) pituitary suppression. A randomised, controlled pilot trial

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Study question: Does the administration of GnRH-antagonist (Cetrotide) in medicated frozen-thawed embryo transfer (FET) cycles improve live birth (LBR) or cycle cancellation rate?

Summary answer: Live birth and cycle cancellation were similar with or without Cetrotide. However, cancellation for threatened ovulation was statistically significantly lower when Cetrotide was administered.

What is known already: During medicated FET, embryo and endometrial development must be synchronized, with endometrial window of implantation dependent on duration of progesterone. Suppression of spontaneous ovulation is key to maintaining synchrony. There are insufficient data regarding the use of supplementary pituitary suppression in medicated FET.

A recent Cochrane review identified one small RCT comparing live birth rate between medicated FET with or without GnRH-agonist pretreatment. The study suggested benefit to GnRH-agonist, but cycles were not monitored for ovulation. One RCT compared GnRH-antagonist with agonist showing better clinical pregnancy rate with GnRH-antagonist. No published RCT has compared medicated FET with versus without GnRH-antagonist.

Study design, size, duration: We conducted an open, two-arm, single-centre, randomised, controlled trial comparing medicated FET with or without Cetrotide. Target recruitment was 300. However, the study was stopped due to the Covid-19 pandemic. Recruitment occurred between 23rd January 2019 and 11th March 2020 and ended at 161 participants. Analysis was undertaken on an intention-to-treat basis, with live birth rate (LBR) as the primary outcome.

Participants/materials, setting, methods: Patients were given oestradiol 2mg orally TDS. Once the endometrium was ≥7mm, vaginal progesterone was commenced, with FET on the sixth day of progesterone. The Cetrotide group were given Cetrotide 0.25mg SC OD from days 1-7. Urine pregnancy test was done 11 days following FET, with oestradiol and progestogen

continued to 10 weeks gestation. Cycles were cancelled for "threatened ovulation" if daily urinary LH-surge testing was positive or an ovarian follicle (≥12mm) was growing.

Main results and the role of chance: 161 patients were randomised (Cetrotide: 76, no Cetrotide: 85). Six were withdrawn after randomisation (three withdrew consent, two did not start, one natural pregnancy). 155 participants started treatment (Cetrotide: 73, no Cetrotide: 82) and 140 had embryo transfer (Cetrotide: 68, no Cetrotide: 72).

The LBR was similar between groups (Cetrotide: 39% (30/76), no Cetrotide (29/85): 34%; OR 0.79, 95% CI 0.42 - 1.51), as were pregnancy rate (42% versus 38%; OR 0.87; 95% CI 0.47-1.63), clinical pregnancy rate (52% versus 49%; OR 0.83; 95% CI 0.44 – 1.56), biochemical pregnancy rate (20% versus 24%; OR 1.25; 95% CI 0.44 - 3.58) and miscarriage rate (7% versus 10%; OR 1.55; 95% CI 0.24 – 9.97).

In the Cetrotide group, 7% of cycles were cancelled, compared to 12% in the no Cetrotide group (NS). However, cancellation due to threatened ovulation was statistically significantly lower in the Cetrotide group, with 0% of cycles cancelled compared to 5% in the no Cetrotide group (OR 1.05; 95% CI 1.001 – 1.105).

Baseline characteristics (age at oocyte retrieval, age at FET, parity, subfertility diagnosis, number of oocytes retrieved, number of viable blastocysts, proportion of IVF/ICSI, endometrial thickness, number of embryos transferred, embryo quality) were similar between groups.

Limitations, reasons for caution: The study is limited by the fact it was unblinded, undertaken at a single site and was underpowered for live birth due to early trial cessation.

Wider implications of the findings: Results suggest no effect of Cetrotide on LBR in medicated FET. There was a significantly higher risk of cycle cancellation due to risk of ovulation in patients not taking Cetrotide. This is clinically significant and requires further study, as cancelled cycles increase costs and duration to birth.

Trial registration number: EudraCT Number: 2018-001915-63

Abstract citation ID: dead093.1008
P-684 Subclinical hypothyroidism and ovarian reserve indices in women with normogonadotropic anovulation

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Study question: Does subclinical hypothyroidism (SCH) affect ovarian reserve indices in women with Polycystic Ovary syndrome (PCOS) or other forms of Hypothalamic-Pituitary-Ovarian Axis Dysfunction (HPOD)?

Summary answer: SCH increases Anti-Müllerian hormone (AMH) concentration, but has no influence on Follicle-stimulating hormone (FSH).

What is known already: In PCOS, anovulation is caused by the arrest of growing follicles due to increased frequency and amplitude of luteinizing hormone (LH) pulses, resulting in increased number of pre-antral and antral follicles, and concentration of AMH. In other cases of HPOD, the causes of anovulation are more diverse and have not yet been clearly identified. Thyroid hormones can modify the function of the HPO axis, affecting the maturation of follicles. While overt hypothyroidism is treated to improve ovulation and fertility, the effect of subclinical hypothyroidism (SCH) and the presence of circulating antithyroid antibodies (ATA) on ovarian function is uncertain.

Study design, size, duration: A prospective cohort tertiary single-center study (consent no. 1072.6120.172.2022) included women aged 18-45, examined due to menstrual disorders and/or infertility, from July to August 2022. Only women without previously diagnosed thyroid dysfunction were included. All enrolled women gave informed written consent to participate in the study.

Participants/materials, setting, methods: Women underwent routine gynecological examination and pelvic ultrasound. Blood sample was tested for AMH, FSH, Luteinizing hormone (LH), Thyroid-stimulating hormone (TSH), anti-thyroid peroxidase (anti-TPO) and anti-thyroglobulin (anti-TG) antibodies concentrations. The Rotterdam-ESHRE-ASRM criteria were used to diagnose PCOS and its phenotypes. HPOD was diagnosed according to WHO classification. SCH was defined as TSH > 2.5 uIU/ml with normal thyroid function. The above parameters within subpopulations were calculated using regression analysis, Kruskal-Wallis and post-hoc tests.

Main results and the role of chance: The study included 51 euthyroid women aged 18-40. PCOS was diagnosed in 42/51 (82.35%) women, including PCOS-A phenotype in 31/51 (60.78%), PCOS-B in 5/51 (9.8%), PCOS-D in 6/51 (11.76%) women, and HPOD in 9/51 (17.65%) women. There was a significant positive correlation between the concentration of TSH and AMH in the studied population - with the increase of TSH value, the concentration of AMH increased ($r = 0.4$, $p = 0.0035$). The mean AMH concentration significantly differed between the groups and equaled to 55.4, 39.4, 31.1, 24.0 pmol/l in PCOS-A, PCOS-B, PCOS-D and HPOD ($p = 0.05$), respectively. The mean anti-TPO concentration was significantly different between the groups and was equal to 18.2, 19.0, 14.6, 10.9 U/ml in PCOS-A, PCOS-B, PCOS-D and HPOD ($p = 0.05$), respectively. There was no significant difference in FSH, LH, TSH, anti-TG concentrations between the groups.

Limitations, reasons for caution: The limitations of the study are small study group and single-center nature.

Wider implications of the findings: SCH increases the concentration of AMH, which in PCOS may exacerbate the symptoms of anovulation. Whether treatment of subclinical hypothyroidism affects ovarian reserve indices and improves ovarian function remain a subject of further research.

Trial registration number: 1072.6120.172.2022

Abstract citation ID: dead093.1009
P-685 Extracellular vesicles of human follicular fluid as biomarkers of ovarian disease

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Study question: Our aim is to analyze the morphology of follicular fluid extracellular vesicles (EVs) according to ovarian pathology by labeling the vesicles with luminescent gold nanoclusters.

Summary answer: Cryo-TEM analysis reveals that EVs from the follicular fluid of patients presenting with polycystic ovary syndrome have a particularly complex morphology compared to normo-ovulatory women.

What is known already: EVs are particles enclosed by a lipid membrane and secreted by cells. Previously considered as cellular debris, they are in fact involved in a multitude of biological functions and are potential biomarkers. Development of an optical biosensor based on luminescent nanoparticles would make it possible to analyze them in biological fluids.

Study design, size, duration: Samples were provided by GERMETHEQUE, Biobank dedicated to human fertility. Samples from 32 patients presenting with ovarian pathology (ovarian failure, polycystic ovary syndrome) and 32 normo-ovulatory patients were analyzed.

Participants/materials, setting, methods: Luminescent gold nanoclusters (1 to 2 nm in diameter) have been synthesized and functionalized in order to interact with extracellular vesicles. Analysis of the morphology of EVs was carried out by cryoTEM. Qualitative results were analyzed by Chi2 or Fisher tests. The quantitative results were analyzed by the Kruskal Wallis test.

Main results and the role of chance: The interactions between luminescent gold nanoclusters and lipid membranes show that it is possible to induce electrostatically and in a controlled manner either significant structural changes in the membranes or membrane labeling. Patients presenting with ovarian

pathology have more oval vesicles ($p = 0.0014$). Differences are especially significant in polycystic ovary syndrome showing that simple vesicles are less abundant than controls ($p < 0.0001$) and large tubules more frequent ($p = 0.0023$).

Limitations, reasons for caution: The first results concerning the interactions between luminescent nanoclusters and vesicles demonstrate their potential for labeling these membrane structures

Wider implications of the findings: The characterization of these anomalies could allow a better understanding of ovarian pathology and open up new therapeutic fields. An efficient method of encapsulation in liposomes of gold nanoclusters has been developed.

Trial registration number: GERM 20220705

Abstract citation ID: dead093.1010

P-686 Long photoperiod exposure results in glycometabolism disorder in the cumulus-oocyte complex in SD rats

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Study question: Does long photoperiod exposure cause glycometabolism changes in the cumulus-oocyte complex?

Summary answer: The levels of key enzymes and substrates involved in glycometabolism have changed in the cumulus-oocyte complex in SD rats due to circadian rhythm changes.

What is known already: Circadian rhythm is essential for maintaining the required reproductive activities in mammalians. In our previous research, long photoperiod exposure results in the reduction of ovulation number and the increase of ROS level in oocytes. In the process of oocyte maturation, the tricarboxylic acid cycle and energy metabolism gradually increase, while the ability of the oocyte to absorb glucose is low. It is mainly metabolized by cumulus granulosa cells into pyruvate and then transported to oocytes for subsequent metabolism. Metabolomics is a comprehensive analysis of small molecules, which provides key information about cellular metabolic states.

Study design, size, duration: Female SD rats, 6-8 weeks old, were randomized into two groups. The rats ($n = 20$) were exposed to the control photoperiod (12 hours light/12 hours dark) or long photoperiod (16 hours light/8 hours dark) for 12 weeks.

Participants/materials, setting, methods: Cumulus-oocyte complexes were collected after ovarian stimulation and numbers were counted. The parameters compared include MII rate, fertilization rate, and ovarian follicle number. We used mass spectrometry-based metabolomics to directly measure metabolite abundance. The glycometabolism pathway and its regulatory signaling molecules were analyzed by qPCR and/or Western blots.

Main results and the role of chance: Long photoperiod exposure significantly reduced the ovulation rate, MII rate and fertilization rate. Interestingly, we find secondary follicle and antral follicle were decreased ($p < 0.01$), while primordial follicle and primary follicle were increased ($p < 0.01$). We identified a total of 318 metabolites in COC. Among them, 59 metabolite levels have significant differences between the two groups (FDR < 0.05). The metabolical function analysis suggested that the phosphatidylinositol phosphate metabolism, pyruvate metabolism, phosphatidylcholine biosynthesis, and glycolysis pathways were influenced by circadian interference. In addition, hexokinase, glucose-6-phosphate isomerase, phosphofructokinase glyceraldehyde 3-phosphate expression level were down regulated.

Limitations, reasons for caution: More studies are required to address how clock gene regulating glycometabolism process. And Translating results in rat model to the human setting is a limitation of this study.

Wider implications of the findings: Our study findings are first to demonstrate that glycolmetabolism is regulated by long photoperiod exposure in follicle development. This knowledge could help to develop strategies to modulate specific recovery strategies based on metabolic regulator to improve human IVF.

Trial registration number: Not applicable

Abstract citation ID: dead093.1011

P-687 Serum Anti-Müllerian hormone to antral follicle count ratio does not predict euploidy rate in young patients who had at least 1 zygote after fertilization

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Study question: Does serum Anti-Müllerian hormone to antral follicle count ratio predict euploidy rate in young patients who had at least 1 zygote after fertilization?

Summary answer: Serum Anti-Müllerian hormone to antral follicle count ratio (AMH/AFC) does not predict euploidy rate in young patients who had at least 1 zygote after fertilization.

What is known already: AMH is produced by the granulosa cells of the growing follicles. It has been demonstrated that the antral follicles are the main contributors to its serum values.

AMH's role as a quantitative predictor of the ovarian response to stimulation is undeniable. However, studies looking at its correlation with oocyte quality, assessed as embryo ploidy, have yielded conflicting results. All of them include only patients who had at least one blastocyst for biopsy obviating those who had all embryos arrested, which might be genetically abnormal. Besides, no study before has considered the per-follicle production of AMH when evaluating serum AMH levels.

Study design, size, duration: Retrospective analysis of women undergoing Preimplantation Genetic Testing for Aneuploidy at the blastocyst stage according to standard clinical procedures in a tertiary referral IVF center from April 2017 to August 2022.

As linear regression excluded an effect of age on euploidy rate until 35 years, 570 patients below this age were included.

AMH/AFC ratio was calculated as a marker of per-follicle AMH production. Patients were classified into quartiles. Euploidy rates/zygote were compared among them.

Participants/materials, setting, methods: Patients who had at least 1 zygote were included. AMH was measured by Elecsys within 6 months before start of stimulation. Scans were performed by 3 experienced sonographers.

Women with an AMH > 5,98 ng/ml, endometriosis, autoimmune disease, non-functional ovarian cyst, hormonal treatment prior to AMH measurement or history of ovarian surgery, men with less than 1 million of sperm/milliliter of ejaculate, and couples with an abnormal karyotype or history or gonadotoxic treatment were excluded.

Main results and the role of chance: Included patients had a median (+ interquartile range) AMH of 2.73 ng/ml (1.68-3.77), median BMI of 25.7 kg/m2 (22.8-28.9) and median antral follicle count of 14 (11-19). Median number of oocytes collected was 14 (10-20) and median number of metaphase II oocytes was 11 (7-15).

Thresholds for p25th and p75th AMH/AFC ratios were 0.13 and 0.24 respectively. The number of patients included in the <p25th, p25th-p75th and >p75th groups were 134, 297 and 139, respectively.

Whereas median age and BMI weren't statistically different among groups, significant differences were found in median AMH (<p25th: 1.38 ng/ml (0.8-2.1); p25th-p75th: 2.75 ng/ml (2-3.7); >p75th: 3.9 ng/ml (3.1-4.8), $p < 0.001$) and AFC (<p25th: 14 (9.2-20) p25th-p75th: 16 (12-20); >p75th: 13 (10-16), $p < 0.001$).

No significant differences were found in the fertilization rate (<p25th: 0.75 (0.6-0.8); p25th-p75th: 0.75 (0.6-0.9); >p75th: 0.78 (0.7-0.9)) nor in the biopsy rate/zygote (<p25th: 0.64 (0.5-0.8), p25th-p75th: 0.6 (0.4-0.8), >p75th: 0.6 (0.5-0.7)).

Euploidy rate/zygote wasn't statistically different among groups: <p25th: 0.36 (0.2-0.5); p25th-p75th: 0.33 (0.2-0.5); >p75th: 0.33 (0.2-0.5). When compared with p25th-p75th, patients with an AMH/AFC <p25th showed a tendency towards a higher risk of not having an euploid embryo, due to culture arrest or aneuploidy (19.4% vs 10.8%, p 0.065).

Limitations, reasons for caution: The retrospective design of the study might restrict an adequate control of confounding factors. The small size of the lower quartile group might compromise the accuracy of the findings.

Wider implications of the findings: The association between AMH levels and live birth rate after In Vitro Fertilization remains debated. Whereas this study didn't find a correlation between AMH/AFC ratio and euploidy rate/zygote, further research will have to evaluate other markers of oocyte quality that might by affected by a low per-follicle production of AMH.

Trial registration number: Not applicable

Abstract citation ID: dead093.1012

P-688 The relationship between higher levels of serum AMH and in vitro fertilization outcomes

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Study question: What is the relationship between increased serum antimullerian hormone (AMH) levels and in vitro fertilization (IVF) outcomes in women with infertility?

Summary answer: High levels of AMH are associated with higher oocytes and embryo number, but a lower proportion of GQE from the transferred and the total number.

What is known already: Although serum AMH level is considered a predictor of ovarian response to controlled ovarian stimulation, the predictive value of higher AMH levels is not clearly established due to contradictory reports, with both positive and negative associations with IVF outcomes.

Study design, size, duration: We performed a retrospective study in the Department of Reproductive Medicine of a private hospital. The medical records of all consecutive patients who underwent IVF between January 2014 and December 2021 with all causes of infertility were reviewed. The study group included 1401 patients with a median age [interquartile range] of 35 [6] years and median AMH of 1.99 [2.79] ng/mL.

Participants/materials, setting, methods: Patients with various causes of infertility undergoing IVF-ICSI and normal ovarian reserve (defined as a serum level of AMH above 1.1 ng/mL) were included in the study. Only patients with serum levels of AMH and age available for analysis were included in the study. The outcomes included were oocytes and obtained embryos number, the proportion of good-quality embryos from the total number of embryos obtained (GQE/T) and from the transferred number of embryos (GQE/Tr).

Main results and the role of chance: Patients were divided according to their serum AMH level into 3 groups: group 1 with AMH between 1.1 ng/ml and 5 ng/mL, group 2 with AMH between 5 and 8 ng/mL and group 3 with AMH more than 8 ng/mL. After adjustment for age, patients in groups 2 and 3 had a higher number of oocytes (beta 0.231, $p < 0.0001$ and beta 0.206, $p < 0.0001$) and embryos (beta 0.143, $p < 0.0001$ and beta 0.158, $p < 0.0001$). After adjustment for age, patients in group 2 had a lower proportion of GQE/T in comparison with patients in group 1 (beta -0.077, p 0.037) and patients in both groups 2 (beta -0.119, p 0.001) and 3 (beta -0.103, p 0.004) had a lower proportion of GQE/Tr in comparison with group 1. Being in groups with higher AMH (2 and 3) was not associated with the number of GQE.

Limitations, reasons for caution: Patients included in this study are infertile patients with an indication for IVF treatment. Therefore, the results of this study should be used with caution in other populations

Wider implications of the findings: Our study suggests that having higher levels of serum AMH (above 5 ng/mL) is not associated with better IVF outcomes in terms of the number of good-quality embryos. Moreover, obtaining a higher number of embryos is not associated with a proportional increase in good-quality embryo availability.

Trial registration number: NA

Abstract citation ID: dead093.1013

P-689 Reproductive outcomes in letrozole-stimulated vs artificial frozen-thawed embryo transfer cycles in women with PCOS and/or oligo-anovulation: a systematic review and meta-analysis.

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Study question: Does stimulation with letrozole improve reproductive outcomes compared to artificial endometrial preparation before frozen-thawed embryo transfer (FET) in women with PCOS and/or oligo-anovulation?

Summary answer: Live birth rate (LBR) was significantly improved with letrozole stimulation compared to artificial endometrial preparation before FET in PCOS and oligo-anovulatory women.

What is known already: Letrozole, an aromatase inhibitor reducing oestradiol levels, has been shown to increase live LBR compared to clomiphene citrate in PCOS/anovulatory women before intrauterine insemination. There is now growing interest in its potential value in improving LBR in FET cycles as well. Furthermore, recent studies indicate an increased risk of hypertensive disorders in pregnancies that have arisen following FET cycles in the absence of a corpus luteum. Letrozole stimulation offers an alternative approach compared to artificial cycle FET with oestradiol and progesterone in women with oligo-anovulation and preserved ovarian reserve, ensuring a corpus luteum, which may improve reproductive outcomes.

Study design, size, duration: A systematic review of studies published in PubMed or the Cochrane library up until the 14th of October 2022. Randomised controlled trials and cohort studies comparing letrozole-stimulated FET and artificial cycle FET were included. Screening, data extraction, and quality assessment was done independently by two reviewers using the Robins-I tool and Cochrane Handbook. The study is registered in PROSPERO, and data collection and reporting followed the PRISMA guidelines.

Participants/materials, setting, methods: Women with PCOS according to the Rotterdam Criteria and/or oligo-anovulation disorders and FET cycles were included. Letrozole-stimulated FET cycles with or without adjuvant gonadotrophins and/or luteal phase support were included in comparison to artificial endometrial preparation for FET. Random-effect meta-analyses were conducted with Review Manager. Dichotomous variables were summarised using the Mantel-Haenszel risk ratio, and heterogeneity was evaluated with Forest plots and I^2-statistic. Funnel plots were used to assess publication bias for all outcomes.

Main results and the role of chance: The systematic search identified 62 studies, 23 relevant full-text studies were scrutinized, and a total of 10 studies fulfilled inclusion criteria with an appropriate control group. Five observational studies of women with oligo/anovulation, and one randomised controlled trial and four observational studies of women with PCOS according to the Rotterdam criteria, but no further information on ovulation pattern. Except three studies, adjuvant gonadotrophins were used as a standard or if the follicular response to letrozole was poor. In all the studies letrozole-stimulated women received luteal phase support. The live birth rate (LBR) was 7% higher in the letrozole vs artificial FET cycle groups (95% CI: (3%;10%), $I^2 = 56\%$, $P = 0.0001$) with similar effects in the subgroup analysis (7 studies, $n = 3931$ letrozole-treated women). Furthermore, the ongoing pregnancy rate increased by 10% (95% CI: (4%;16%), $I^2 = 1\%$, $P = 0.001$) in women with PCOS and/or oligo-anovulation (4 studies, $n = 297$ letrozole-treated women). Definition criteria of miscarriage and biochemical pregnancy loss varied to an extent where a meta-analysis could not be performed. Nevertheless, seven studies reported on miscarriage rate, six showed similar rates and one significantly lower miscarriage rate with letrozole. No outcomes raised concerns of publication bias in the funnel plots.

Limitations, reasons for caution: Most included studies were observational studies with a high risk of bias, and substantial heterogeneity was observed amongst the studies, which could dilute the evidence. Some studies

included women with PCOS without clarifying if ovulatory or anovulatory. The funnel plot of each outcome raised no suspicion of publication bias.

Wider implications of the findings: Letrozole stimulation FET in PCOS and/or oligo-anovulatory women significantly improves LBR and ongoing-pregnancy-rate compared to artificial endometrial preparation. However, robust evidence is lacking and RCTs restricted to women with oligo-anovulatory women are urgently needed. The evidence is insufficient to conclude whether luteal phase support is required after letrozole stimulation

Trial registration number: Not applicable

Abstract citation ID: dead093.1014
P-690 RESEarch on ovarian reseRVE among Neurologic patients: the RESERVEN study

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Study question: Could Multiple Sclerosis (MS) reduce the levels of main ovarian reserve markers, measured through automated systems?

Summary answer: There is no statistically significant difference in ovarian reserve markers between women affected by Multiple Sclerosis and healthy controls.

What is known already: Few studies evaluated the issue of ovarian reserve in women affected by MS, so far. The majority of them observed no differences in ovarian reserve markers between women affected by MS and healthy controls. However, interestingly, women with MS in case of a more active disease were observed to have lower levels of ovarian reserve markers. In fact, evidence suggests that an inflammatory status may reduce the ovarian reserve, where autoimmune-related diseases are considered a possible cause of Premature Ovarian Insufficiency (POI).

Study design, size, duration: This is a single-center multidisciplinary prospective case-control (44 cases and 72 controls) study performed from September 2019 to September 2022.

Participants/materials, setting, methods: Cases were reproductive-age women affected by MS recruited at our MS unit, controls were age- and body mass index (BMI) - matched healthy women recruited at our assisted reproductive technology (ART) unit. During the early follicular phase, a transvaginal ultrasound was performed with sonoAVC software (GE medical systems) for 3D antral follicle count and a blood sample taken for hormonal assays (Anti-Müllerian hormone levels was measured using the Roche Elecsys AMH Plus).

Main results and the role of chance: There are no differences in age ($p = 0.11$), BMI ($p = 0.96$) as per protocol. Moreover, differences in FSH ($p = 0.14$), LH ($p = 0.13$), estradiol ($p = 0.92$), and AMH ($p = 0.99$) levels were not statistically significant. The 3D antral follicle count showed a statistically significant difference ($p = 0.03$), but following a re-evaluation of pictures in post-processing, removing the small antral follicles (2-4 mm) the difference was not statistically significant ($p = 0.14$).

Limitations, reasons for caution: We could not assess women before they started on medication for MS. Therefore, we could not exclude that medication have an impact in these results, also because the sample is small and patients were not treated with the same drug. Moreover, all patients were in disease remission at enrollment.

Wider implications of the findings: This is the first study to evaluate ovarian reserve markers with automated systems. Our results confirmed the ones previously published. However, the interesting data on 3D antral follicle count increased in MS women should be confirmed and studied in order to evaluate if follicular dynamics are altered in Multiple Sclerosis.

Trial registration number: Not applicable

Abstract citation ID: dead093.1015
P-691 Does serum Kisspeptin levels discriminate between fertile and infertile women with polycystic ovary syndrome?

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Study question: Does serum Kisspeptin (KP) levels discriminate between fertile and infertile women with polycystic ovary syndrome (PCOS)?

Summary answer: Serum KP levels can be used as a potential discriminatory biomarker for infertility in women with PCOS.

What is known already: PCOS is the most common cause of anovulatory infertility and not fully elucidated pathology. A persistent rapid gonadotropin-releasing hormone (GnRH) pulse frequency in patients with PCOS exist throughout ovulatory cycle. KP is a neuropeptide that increases GnRH pulsatile release during ovulation. Therefore, KP may have a key role as a central regulator of fertility in PCOS patients. Although, recent studies showed that the KP concentration is higher in PCOS patients, the evidence is limited as to whether serum KP levels determine infertility in PCOS.

Study design, size, duration: This was a single center prospective cohort study conducted at Goztepe Prof. Dr. Suleyman Yalcin City Hospital Affiliated to Istanbul Medeniyet University, Istanbul, Turkey between January 2023 to February 2023. Our study enrolled 30 fertile and 30 infertile women with PCOS, who were aged between 18 and 45 years. PCOS was defined according to the criteria of the Rotterdam ESHRE- ASRM sponsored consensus group (2004).

Participants/materials, setting, methods: Venous blood samples from the participants were obtained after fasting and between 8 a.m. and 10 a.m. on days 2-5 of the menstrual cycle. Samples were thawed, and a human KISS1 (Kisspeptin 1) ELISA kit (Elabscience, USA,lot no:E-EL-H5618) was applied to measure KP serum concentrations. Patient characteristics and KP concentrations were compared among the two groups. Statistical analysis was performed by Mann-Whitney, Spearman correlations, and linear regression analysis. A p-value <0.05 was considered significant.

Main results and the role of chance: The mean age of the patients was 27.57 ± 5.39 years in the fertile PCOS group and 26.80 ± 4.85 years in the infertile PCOS group ($p = 0.63$). There was no significant difference among the groups in terms of body mass index (BMI), duration of infertility, serum FSH, LH, LH-to-FSH ratio, E2, antimullerian hormone (AMH), TSH, prolactin, 17OHP, total testosterone, SHBG, HbA1c, HOMA-IR, neutrophil–lymphocyte ratio levels and antral follicle count (AFC). DHEA-S was significantly higher in the infertile PCOS group (306.73 ± 94.869 mcg/ dL), compared to the fertile PCOS group (258.08 ± 84.29 mcg/ dL, $p = 0.037$). The mean KP level was significantly higher in the infertile PCOS group (444.80 ± 136.61 ng/ mL), compared to the fertile PCOS group (333.02 ± 131.10 ng/ mL, $p = 0.001$). The KP level was positively correlated with AFC, AMH, and total testosterone levels ($R = 0.495$, $R = 0.548$, $R = 0.362$, $p < 0.05$, respectively) in the infertile PCOS group. Furthermore, ROC analysis showed that the optimal cut-point value was found to be 285.59. When this cut-off value of serum KP levels was taken, the sensitivity was 0.96 and the specificity was 0.50. Linear regression analysis revealed that AMH was positively associated with KP levels ($p = 0.022$).

Limitations, reasons for caution: Due to the limited number of women with available samples, we were not able to analyze KP serum levels according to specific PCOS phenotypes

Wider implications of the findings: Serum KP levels are higher in infertile women with PCOS compared to fertile women with PCOS. Infertility caused by PCOS can be predicted by serum KP levels. In the future identification, KP in PCOS may be applied to develop potential therapeutic agents.

Trial registration number: Not applicable

Abstract citation ID: dead093.1016

P-692 Effect of intra-ovarian instillation of autologous platelet rich plasma on live birth rate in POSEIDON 3 versus POSEIDON 4 group: A prospective follow-up study

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Study question: What is the effect of intra-ovarian platelet rich plasma(PRP) on live birth rate(LBR) in POSEIDON-3 versus POSEIDON-4 group?

Summary answer: Intra-ovarian PRP improves the live birth rate in low prognosis patients irrespective of age, this giving them hope of their own biological child.

What is known already: With the emerging regenerative medicine promising various treatment modalities, PRP is considered a novel therapeutic option. Various anecdotal cases have been reported, demonstrating successful pregnancy outcomes after the PRP treatment. But the data still remains elusive, especially for women of Asian ethnicity, who have a rapid decline in ovarian reserve when compared to the Caucasian population. Consequently, we attempt to compare the effects of autologous PRP on LBR in POSEIDON-3 versus POSEIDON-4 groups.

Study design, size, duration: After ethical approval, a prospective interventional study was conducted at ART Centre, Department of Obstetrics and Gynaecology of a tertiary care institute during a period from July 2020 to April 2022. 72 women between the ages of 20 and 40 years with idiopathic poor ovarian reserve (AMH 1.2<ng/ml; AFC<5) and a normal adequate uterine cavity were enrolled, and those with a history of radiotherapy, chemotherapy, endometriosis, or ovarian/tubal surgery were excluded.

Participants/materials, setting, methods: Women between 20-40 years of age with low ovarian reserve received 1.5 ml-PRP prepared from 30 ml venous-blood, was instilled in each ovarian stroma between day 6-10 of menses. Patients were followed up for 3-consecutive months to assess ovarian-reserve parameters (FSH, AMH, AFC). Patients showing significant improvement (AFC>5) were enrolled in antagonist-IVF cycles and categorised into two groups: POSEIDON-3 vs 4 groups. Paired/signrank test and Independent/ranksum test compared clinical parameters between the two groups.

Main results and the role of chance: The mean age and BMI of the recruited women were 31.7 + 4.2 years and 24.89 + 3.73 kg/m^2. Out of 72 participants, 16.67% responded in 1st month of follow-up(AFC: 5 vs 3,p<0.001), 43% in 2nd month(AFC: 6 vs 3,p<0.001)(AMH: 1.02 vs 0.99,p=0.02) and 12.5% in 3rd month(AFC:4 vs 3,p<0.001)(AMH:0.89 vs 0.85,p=0.01). 20 participants showed no improvement in AFC, AMH, or FSH. Excluding one, 43 underwent antagonist cycle. Despite the evident age-difference between the POSEIDON-3 and 4 groups(29.6 vs 36.6 years), the POSEIDON-4 group women were more obese (27.4 + 1.30 vs 23.3 + 0.49,p=0.0007). Although the number of oocytes retrieved was similar (6.35 + 1.83 vs 5.75 + 1.35,p=0.21), the E2 levels on the day of trigger were significantly low in POSEIDON-4 patients (1082.33 + 273.03 vs 1727.16 + 867.79,p=0.01). Also, the dose of HMG requirement was higher in POSEIDON-4 (1450 + 96.53 vs 1190.3 + 66.49,p=0.04) thus depicting the effect of advanced age. Otherwise, there was no significant difference in dose of rFSH requirement (2705.6 + 48.93 vs 2737 + 54.74,p=0.71), fertilisation rate (60.86% vs 57.86%,p=0.66), biochemical pregnancy rate (30.7% vs 29.03%,p=0.78), CPR (25% vs 25.8%,p=0.95) and LBR (22.6% vs 16.66%,p=0.66) between the two-groups. No adverse events reported.

Limitations, reasons for caution: Our study was a non-randomized study with no control/sham, arm as doing so would be unethical and impose a financial burden on the patients with no guaranteed clinical success.

Wider implications of the findings: With the impetus to provide a biological child to the women with poor ovarian reserve, ovarian-rejuvenation with PRP seems to be a promising treatment. It can be concluded that PRP improves the ovarian reserve which translates into better reproductive outcomes in women who were planned and counselled for donor-oocyte IVF.

Trial registration number: Not applicable

Abstract citation ID: dead093.1017

P-693 Retrospective evaluation of the effect of the implementation of the DIVINE dose-calculator in daily practice on ovarian hyperstimulation treatment risk and live birth rate

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Study question: Does the implementation of the DIVINE dose-calculator in routine care decrease treatment risk due to ovarian hyperstimulation whilst maintaining treatment efficacy?

Summary answer: The implementation of the DIVINE dose-calculator significantly decreases treatment risks caused by ovarian hyperstimulation, without affecting treatment efficacy in terms of live birth.

What is known already: Meta-analyses show that tailoring the gonadotropin starting dose did not affect live birth rates, but reduced treatment risks. The ORT iOS IPD-MA study group used individual participant data to develop and validate a model for ovarian stimulation in IVF/ICSI: the DIVINE dose-calculator. This model scored adequate for assessing hyperstimulation treatment risk and poor for predicting live birth. It provides clinicians an overview of risk percentages per gonadotropin starting dose, which aids in selecting an appropriate, personalized gonadotropin starting dose. This model should be implemented in routine care to confirm the true clinical effect on treatment risk and live birth results.

Study design, size, duration: Retrospective cohort study using pseudonymized data in two fertility centers in The Netherlands. The dose-calculator was implemented on 15th February 2021. Data from 36 months before and 12 months after implementation were collected. The dose-calculator included female age, AMH and GnRH-type in order to select a starting dose between 100-225 IU. Baseline characteristics and fresh cycle treatment outcomes were extracted from the patient file, checked and cleaned. Missing data were imputed 50 times.

Participants/materials, setting, methods: Subfertile women younger than 38 years, starting their first IVF/ICSI treatment cycle with follitropin alfa or beta were included. Couples who did not plan to receive a fresh embryo transfer, women diagnosed with PCOS and couples undergoing limited insemination or PGT were excluded. Intention-to-treat and per protocol analyses were completed. Inverse propensity weighting was applied to balance confounders. Weighted regression was then performed using general linear models or ordered logistic regression models.

Main results and the role of chance: 493 women were included, 401 in the standard group and 92 in the dose-calculator group (per protocol analysis). There was no difference in live birth rate before and after implementation: 25.4% vs 25.0% respectively (OR: 1.01, 95% CI: 0.6 – 1.71). Ovarian hyperstimulation treatment risk clearly decreased after the implementation of the dose-calculator from 11.3% in the pre-implementation period to 4.3% in the post-implementation period (OR: 0.27, 95% CI: 0.08 – 0,91), of which OHSS incidence declined from 6.8% to 0% after implementation. Pre-implementation, women stimulated 1.0 day shorter as compared to the post-implementation period (MD, CI: 0.25 – 1.75), and used 200 IU gonadotropins less per cycle (MD, 95% CI: 48.68 – 352.11). The cancellation rate due to low ovarian response were equal pre- versus post-implementation (OR: 0.94, 95% CI: 0.45 – 1.98) as well as the cancellation rate due to high ovarian response (OR: 0.56, 95% CI: 0.11 – 2.84). Post-implementation 1.05 less oocytes were collected during follicle aspiration (MD, 95% CI: -2.51 – 0.41), which resulted in 0.41 less usable embryos (MD, 95% CI: -1.04 – 0.17). The usable embryo per oocyte was comparable (MD: 0.003, 95% CI: -0.061 – 0.067). The intention-to-treat analysis confirmed these results.

Limitations, reasons for caution: Results are preliminary and based on single center data. We expect to finalize the analyses including approximately 100 patients from the second center and a cost-effectiveness analysis before ESHRE 2023. Unfortunately, cumulative cycle results including cryopreserved embryo transfers and subsequent treatment cycles are not available.

Wider implications of the findings: The dose-calculator aids in optimizing ovarian stimulation, without compromising live birth prospects. It is an easy

to use model, which substantially reduces risks caused by ovarian hyperstimulation. A free of charge mobile application will be developed to stimulate implementation. Future research should address cumulative cycle results and temporal/geographical validation.

Trial registration number: Not applicable

Abstract citation ID: dead093.1018

P-694 Desogestrel did not affect Follicular Output Rate (FORT) during controlled ovarian stimulation for oocyte vitrification: prospective cohort study

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Study question: Does desogestrel an option for LH suppression during controlled ovarian stimulation for oocyte vitrification?

Summary answer: This new option for LH suppression did not affect FORT and, consequently, the number of collected oocytes for vitrification.

What is known already: Traditionally, LH suppression is achieved by using a gonadotropin-releasing hormone (GnRH) analogue. However, improved cryopreservation techniques and freeze-all strategies are introducing new concepts, including the possibility of using progesterone instead of GnRH analogues. Progesterone compounds are currently wide-used during ovarian stimulation for oocyte freezing for LH suppression. The use of oral medroxyprogesterone was previously shown to effectively prevent the LH surge, without impacting embryo development or pregnancy rates after frozen embryo transfer. The use of dydrogesterone with the same purpose was also effective. Desogestrel is a progesterone broadly known and accepted, and has good tolerability and low cost.

Study design, size, duration: A prospective cohort study was performed during the period of 2019-2021 including 75 patients submitted to oocyte freezing and 173 women matched by age as the controls (patients submitted to IVF for the first time).

Participants/materials, setting, methods: Patients from the control group used a flexible GnRH antagonist for LH suppression, and patients in the desogestrel group utilized desogestrel 75 mcg oral twice a day (starting from the beginning of gonadotropin to hCG/agonist day). All patients received the same gonadotropin regimen (recombinant FSH). The final oocyte maturation was equal for both groups (recombinant hCG and GnRH analogue agonist). The primary endpoint was the antral follicle responsiveness to follicle-stimulating hormone, measured by FORT.

Main results and the role of chance: Age [control group= 36 years (median 28-45); desogestrel group= 36 years (median 23-43); $p = 0.793$; Mann Whitney U test], baseline antral follicle count [control group= 8 (2-23); desogestrel group= 9 (2-21); $p = 0.741$; Mann Whitney U test] and anti-Mullerian hormone [control group= 1.5 (0.05-7.95); desogestrel group= 1.00 (0.01-16.00); $p = 0.540$; Mann Whitney U test] were similar between both groups. However, the controlled ovarian stimulation length was shorter in the control group [10.4 ± 1.6 vs. 11.0 ± 1.6; $p = 0.044$; t-student test,] and those patients utilized less gonadotropin than the desogestrel group [2736 UI ± 745 vs. 2933 UI ± 785; $p = 0.047$; t-student test,]. Finally, Follicular Output Rate (FORT) [control group= 45%; desogestrel group= 52%; $p = 0.217$; t-student test], number of collected oocytes [control group= 6 (1-33); desogestrel group= 8 (1-20); $p = 0.293$; Mann Whitney U test] and MII [control group= 5 (1-27); desogestrel group= 6 (1-19); $p = 0.156$, Mann Whitney U test] were the same between both groups.

Limitations, reasons for caution: The small sample size and the absence of randomization are the main limitations of our study. Since the first results were encouraging, a randomized study should be performed.

Wider implications of the findings: Desogestrel is a cheap and easy option to be used during controlled ovarian stimulation for oocyte vitrification, since the final results are the same. This is the first scientific report showing the use of desogestrel did not affect FORT and, consequently, the number of collected oocytes for vitrification.

Trial registration number: Not applicable

POSTER VIEWING
REPRODUCTIVE (EPI)GENETICS

Abstract citation ID: dead093.1019

P-697 Pre-pregnancy complications - associated factors and wellbeing in early pregnancy: A Swedish cohort study

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Study question: Which factors are associated with pre-pregnancy complications and poor well-being in early pregnancy?

Summary answer: We identified different risk profiles for different pre-pregnancy complications. Besides age and body mass index, exposure to certain prescribed drugs was a modifiable risk factor.

What is known already: Many couples experience difficulties to become pregnant or carry a pregnancy to term due to unknown causes. Complications including recurrent pregnancy loss (RPL), subfertility (failure to conceive within one year of unprotected intercourse) and the need for artificial reproductive technologies (ART) are increasing worldwide.

Previously identified risk factors for involuntary childlessness include higher age, irregular menstruation, endometriosis and polycystic ovary syndrome – however, none of these are modifiable and there are still many cases unexplained.

Study design, size, duration: Online questionnaires were collected for 5330 unique pregnancies in Sweden from November 2017 – February 2021, collected before 20 weeks of gestation.

Participants/materials, setting, methods: Participants were defined as having pre-pregnancy complication(s) if they had any of the following: prior RPL (> 3) or late miscarriage, subfertility, or the use of ART.

Multivariable logistic regression modelling was used to investigate potential risk factors for pre-pregnancy complications and poor well-being in early pregnancy, reported as odds ratios (OR) with 95% confidence intervals.

Main results and the role of chance: Pre-pregnancy complications were identified in 1142 participants (21%), with the most common one being subfertility (n = 790, 69%).

Risk factors included diagnosed endometriosis (OR 4.43, CI 3.33-5.91), thyroid medication (OR 2.20, CI 1.76-2.74), opioids and other strong pain medication (OR 1.97, CI 1.19-3.19), asthma and allergy medication (OR 1.23, CI 1.03-1.46), body mass index > 25 kg/m^2 (OR 1.35, CI 1.07 for BMI 25-29.9 and OR 1.58, CI 1.19-2.07 for BMI ≥ 30) and age over 35 years (OR 1.35, CI 1.07-1.72). Different subgroups of pre-pregnancy complications had unique risk factors.

The groups also experienced different symptoms in early pregnancy. Women that had experienced recurrent pregnancy loss were at higher risk of depression during their current pregnancy (OR 1.71, CI 1.21-2.38). All groups

were at higher risk for reporting any complications in their current pregnancy (OR 1.33, CI 1.11-1.59), as well as vaginal bleeding (OR 1.72, CI 1.15-2.55).

Limitations, reasons for caution: We have a high prevalence of participants with RPL, and participants that needed in vitro fertilization (IVF), which indicates a selection bias towards participants with pre-pregnancy complications.

Furthermore, we did not collect information on the partner's medical history, which might play a role in the need for ART.

Wider implications of the findings: The identified risk factors could be used to identify women at risk for pre-pregnancy complications and help them earlier in the process of trying to conceive, thus assisting them to reduce stress and depression during the pregnancy.

Trial registration number: Not applicable

Abstract citation ID: dead093.1020
P-698 Genetics and reproductive health outcomes: An Asian perspective

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Study question: Does genetics play a role in the reproductive outcomes in women undergoing assisted reproductive technology (ART)?

Summary answer: We describe a trend towards poorer reproductive outcomes associated with Asian women and ART. Subsequently, we posit a potential biological mechanism.

What is known already: In the last four decades, advances in assisted reproductive technology (ART) have afforded the possibility of conceiving a child to individuals who encounter fertility complications. However, a closer examination of the clinical outcomes of ART shows a stark contrast in Asian women compared to Caucasians, with the majority of studies reporting lower reproductive success among Asian women. In this study, a systematic review was performed to elucidate the genes associated with ART clinical outcomes, with a focus on Asian ethnicities. Concomitantly, we highlight the need for personalized medicine to bridge the gaps in female reproductive health.

Study design, size, duration: We performed a Pubmed and Embase database search, from inception to December 2022, to identify all studies which investigated the differential reproductive outcomes in Asian women compared to their Caucasian counterparts, genetic mutations contributing to fertility in Asian women and personalized medicine. Following the PRISMA workflow, the preliminary search yielded a total of 2,145 papers to be reviewed.

Participants/materials, setting, methods: Following the inclusion/exclusion criteria screening, 128 studies were analyzed (ART outcomes in Asians, n=75; Genetics on women's reproductive health, n=38; Personalized medicine in Asians, n=15). As each research paper identified one or more genes/SNPs involved in female fertility and reproductive health, we took a step further and performed a pathway analysis of gene-sets using STRING in Cytoscape v3.4.0. Network analysis and biological process associations were performed using GSEA and MSigDB. Significant gene-sets were annotated if FDR<0.05.

Main results and the role of chance: We observed that age at menarche(AAM) was found to be correlated with the timing of the first pregnancy, with Hawaiians having the lowest age(22.2yrs) and Japanese women having the highest age(25.0yrs). LIN28 mutations were highly associated with AAM and prevalent in both Chinese and American populations. FMR1 was most associated with ovarian reserve, with BMP15, ESR1, INHA, PRIM, TMEM150B to a lesser extent. Finally, FSHR polymorphisms were found to affect IVF outcomes most significantly. Network analysis highlighted a close association between 5 genes; FMR1, FSHR, ESR1, BMP15 and INHA, through biological functions of ovarian follicle development, menstrual cycle and hyperpituitarism. Network analysis revealed 4 major biological pathways (ovarian follicle development, oocyte maturation, ovulation, steroidogenesis), as well as key proteins which are involved in female fertility and reproductive health outcomes. PI3K/Akt are critical regulators of ovarian function through primordial follicle maturation and granulosa cell proliferation. MAPK signaling is involved with gonadotrophin-releasing hormone which controls FSH and LH secretion while Gq/11 signaling is activated through LH receptor. Therefore, PI3K/Akt, MAPK and Gq/11 protein signaling pathway appear to be key components to a broad range of reproductive outcomes and represent potential pathways to target in future studies investigating ovarian, follicular and steroidogenesis dysfunction.

Limitations, reasons for caution: A large majority of the papers reviewed relied on the self-reporting of ethnicities, which can vary considerably in accordance to how an individual defines his or her own ethnicity. Hence, we could not exclude the possibility of reporting bias in the studies.

Wider implications of the findings: Leveraging these findings,we propose the development of a biomarker panel which would enhance patient stratification,moving towards personalized medicine to address every woman's unique reproductive potential.With several countries reporting low fertility rates due to shifts in socio-economic and life priorities, this has strong implications for those who wish to delay childbearing.

Trial registration number: Not applicable

Abstract citation ID: dead093.1021
P-699 A clinical predictive model for live birth in women of advanced age undergoing PGT cycles

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Study question: In 37–45-year-old women undergoing preimplantation genetic testing, who will benefit from repeated cycles until birth?

Summary answer: The chance of delivering after repeated cycles is higher in those with at least one top-quality unaffected embryo in their first preimplantation genetic testing cycle.

What is known already: PGT for monogenic disease or structural chromosomal rearrangement (PGT-M and PGT-SR) is offered to couples when one or both carry a monogenic mutation or chromosomal rearrangement that put their future offspring at risk of having a genetic disorder so they can select an unaffected embryo for uterine transfer.

The tendency to delay childbirth has led to an increase in advanced-aged women seeking PGT treatments. This poses a major challenge to PGT treatments.

Study design, size, duration: A retrospective cohort study was conducted at a university hospital reproductive center. The computerized database of 158 women aged 37–45 undergoing 753 PGT-M/SR cycles between 2010 and 2021 was analyzed.

Participants/materials, setting, methods: The reproductive outcomes of women who were 37–45 years of age, starting a PGT-M/SR cycle, were analyzed until the first live birth or until the patient reached the age of 45.

Data were analyzed using univariate analysis, multivariable stepwise logistic regression, Kaplan–Meier method, and decision tree analysis. The cumulative live birth rate was calculated in a conservative manner because the assumption that patients who did not return for subsequent cycles had negative results was made.

Main results and the role of chance: The analysis included 158 women undergoing 753 preimplantation genetic testing cycles. The cumulative live birth rate was 37.342% (59/158). Decision tree analysis revealed that women aged $\leq$ 40.1 or women $>$ 40.1 with one or more top-quality embryos in their first cycle had the best chance for a live baby (41% and 56%, respectively). Those older than 40.1 without top-quality embryos and seven or fewer dominant follicles had no live births. A Kaplan–Meier curve showed that for autosomal dominant diseases, there was a negligible increase in live birth rate after three cycles, compared to six cycles in autosomal recessive inheritance.

Limitations, reasons for caution: This study is limited by its retrospective design and the low birth rate in this age group, which reduces its power to detect differences in birth rates between different modes of inheritance.

Wider implications of the findings: The predictive model for live births in women aged 37-45 developed in this study can be used for clinical decision making and patient consultation.

Additional PGT cycles after three in carriers of an autosomal dominant disorder and six in those with an autosomal recessive disorder should be considered prudently.

Trial registration number: Not applicable

Abstract citation ID: dead093.1022

P-700 preimplantation genetic testing for aneuploidy failed to improve cumulative live birth rate in patients with limited good-quality embryos

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Study question: To evaluate whether preimplantation genetic testing for aneuploidy (PGT-A) can improve pregnancy and neonatal outcomes for patients with limited good-quality embryos.

Summary answer: PGT-A failed to improve cumulative live birth rate or shorten time to pregnancy, but optimized pregnancy outcomes per transfer for patients with limited good-quality embryos.

What is known already: PGT-A is currently widely used to screen for aneuploidy with the goal of improving live birth rates. Remarkably, previous studies have focused on the effect of PGT-A on cumulative clinical outcomes in women with good pregnancy prognosis. However, it is still unclear whether PGT-A can improve the cumulative pregnancy outcomes in patients with limited good-quality embryos.

Study design, size, duration: A retrospective cohort study was performed among 1553 women who intended PGT-A for the first time but obtained only two or less good-quality embryos on day 3 after oocyte retrieval from March 2017 to June 2021.

Participants/materials, setting, methods: A total of 1553 patients were divided into two groups: 997 in the PGT-A group and 556 in the drop-out group of withdrawing PGT-A due to poor embryological outcome. Multivariable logistic regression was performed to adjust for potential confounders when comparing the clinical outcomes between two groups.

Main results and the role of chance: After adjusting for potential confounding factors, PGT-A group exhibited significantly lower cumulative rates of biochemical pregnancy (19.96% vs. 30.22%, P-adj < 0.001), clinical pregnancy (17.55% vs. 23.38%, P-adj < 0.001) and live birth (14.14% vs. 16.19%, P-adj $= 0.005$) per oocyte retrieval and longer median time to pregnancy and live birth compared with drop-out group. However, significant increases in rates of biochemical pregnancy (72.16% vs. 35.50%, P-adj < 0.001), clinical pregnancy (61.86% vs. 26.98%, P-adj < 0.001), and live birth (48.45 vs. 18.26%, P-adj < 0.001) per transfer were found in the PGT-A group. No significant differences were observed in cumulative miscarriage and ectopic pregnancy rates, number of ETs needed per live birth and neonatal outcomes.

Limitations, reasons for caution: This study was limited by the inherent potential bias of retrospective studies and small sample size. Thus, additional studies with larger sample sizes are needed to confirm our findings.

Wider implications of the findings: PGT-A failed to improve cumulative live birth rate or shorten time to pregnancy, but optimized pregnancy outcomes per transfer for patients with limited good-quality embryos.

Trial registration number: Not applicable

Abstract citation ID: dead093.1023

P-701 Human embryos diagnosed as mosaic and aneuploid after preimplantation genetic testing for aneuploidy are associated with increased levels of apoptosis in trophectoderm cells

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Study question: Are there cell lineage-related differences in the apoptotic rates of human blastocysts classified as euploid, mosaic and aneuploid after preimplantation genetic testing for aneuploidy (PGT-A)?

Summary answer: In human blastocysts, apoptosis is more frequent in trophectoderm (TE) cells and is associated with the presence of mosaic and uniform chromosomal alterations.

What is known already: Embryos diagnosed as mosaic after PGT-A can develop into healthy infants. However, the reason why these embryos achieve reproductive competence needs further research. One hypothesis is that aneuploid cells are negatively selected during embryo development through cell competition mechanisms, such as apoptosis or differential proliferation. While these mechanisms have been demonstrated in mouse embryos, in which apoptosis occurs more frequently in the inner cell mass (ICM), evidence in human embryos is scarce. In fact, only one previous study has shown an association between mosaicism and aneuploidy after PGT-A and increased levels of apoptosis, which was more frequent in TE cells.

Study design, size, duration: Prospective cohort study performing colocalization of cell-lineage and apoptotic expression markers by immunofluorescence (IF). A total of 53 vitrified human blastocysts with a previous PGT-A diagnosis on day 5 (D5) or day 6 (D6) of development were analysed: n = 13 euploid embryos (n = 11 on D5; n = 2 on D6), n = 22 mosaic embryos (n = 16 on D5; n = 6 on D6), and n = 18 aneuploid embryos (n = 11 on D5; n = 7 on D6). All embryos were donated for research purposes.

Participants/materials, setting, methods: Following warming and re-expansion, blastocysts were fixed in 4% paraformaldehyde, processed for IF and imaged by confocal microscopy. Primary antibodies: 1:80 goat anti-human Nanog (AF1997, R&D-Systems), 1:100 mouse anti-human Gata3 (MAB6330, R&D-Systems) and 1:1000 rabbit anti-human Caspase-3 (NB10056113, Novus Biologicals). 165 μM DAPI was used for nuclei staining. Total cells: DAPI+ cells. TE: Gata3+ cells. ICM: Gata3-/Nanog+ cells. Incidence of apoptosis: % Caspase-3+ cells (per embryo). Data were analysed using Chi-square or ANOVA ($p < 0.05$ significant).

Main results and the role of chance: Total cell number was similar among euploid embryos (59.2 ± 20.6 on D5; 78.5 ± 0.7 on D6), mosaic embryos (49.6 ± 15 on D5; 58.8 ± 16.9 on D6) and aneuploid embryos (48.6 ± 14.2 on D5; 59.6 ± 23.5 on D6) ($P > 0.05$). Nanog and Gata3 expression evidenced the establishment of ICM and TE cell lineages during the blastocyst stage: while the proportion of Nanog+ cells decreased from 38.2% (755/1979) on D5 to 20.3% (188/927) on D6 ($P < 0.0001$), the proportion of Gata3+ cells increased from 77.8% (1540/1979) on D5 to 83.1% (770/927) on D6 ($P < 0.01$). The persistence of Nanog expression in TE cells was associated with the chromosomal status of the embryo, as evidenced by the significantly higher proportion of Gata3+/Nanog+ cells found in aneuploid embryos (27.9%=149/534 on D5; 4.6%=19/417 on D6) compared to mosaic embryos (13.1%=104/794 on D5; 3.4%=12/353 on D6) and euploid embryos (9.7%=63/651 on D5; 0%=0/157 on D6) ($P < 0.05$). The incidence of apoptosis in the TE was significantly higher in aneuploid embryos (45.9% ± 16.1 on D5; 49% ± 15.1 on D6) and mosaic embryos (44.1% ± 19.6 on D5; 43% ± 16.8 on D6), compared to euploid embryos (26.6% ± 16.6 on D5; 17.5% ± 14.8 on D6) ($P < 0.05$). Conversely, apoptosis was much less frequent in the ICM (2.2% ± 7.7), and no differences were found among the study groups ($P > 0.05$).

Limitations, reasons for caution: This is a descriptive, single-centre study with a limited sample size. The karyotype concordance between ICM and TE cells was not tested.

Wider implications of the findings: Our findings demonstrate that apoptosis is common in human blastocysts, particularly in TE cells. The increased

apoptotic rates found in the TE of embryos with chromosomal abnormalities support its role as a mechanism to eliminate aneuploid cells. This mechanism may indeed be related to the reproductive potential of mosaic embryos.

Trial registration number: Not applicable

Abstract citation ID: dead093.1024

P-702 Secretion of beta-human chorionic gonadotropin (b-hCG) by post-implantation embryos differs according to different chromosomal status.

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Study question: To evaluate secretion of b-hCG hormone by human post-implantation embryos cultured in-vitro up to day 10 according to their chromosomal status.

Summary answer: Secreted b-hCG levels differs among embryos with different chromosomal load, being severely diminished in monosomic embryos when compared either to euploid or trisomic embryos.

What is known already: Exploring early post-implantation embryo development is subject of growing interest because errors in correct embryo development at this period might cause early pregnancy losses or development of pathologies affecting postnatal health. Down's Syndrome (DS) is one of the most common examples of chromosomal supernumerary (trisomy 21; T21) aneuploidy that leads to live birth but associated to pathology in postnatal life while monosomy of chromosome 21 (M21) is more likely to cause embryo developmental arrest. Furthermore, conclusions extracted from our previous studies put forward the idea of different gene and metabolite expression according to different chromosomal load (Sanchez-Ribas et al., 2019).

Study design, size, duration: This is an exploratory comparative study which includes a total of 33 supernumerary science-donated human blastocysts on day 6 from in-vitro fertilization cycles followed by preimplantation genetic test for aneuploidies. Euploid (n = 10), pure T21 (n = 13) and pure M21 (n = 10) were included in the study. All day 6 blastocyst included were good-quality embryos and were initiating hatching at the time of vitrification. Embryos degenerated during culture were not used for b-hCG quantifications.

Participants/materials, setting, methods: Euploid, T21 and M21 day blastocysts were thawed and cultured in-vitro until day 10. Embryos were cultured in culture medium specifically designed to support post-implantation embryo development. Each day of culture, half volume of culture media was replaced by fresh media. Levels of b-hCG in conditioned embryo media were quantified by ELISA immunoassay at day 7, 9, and 10. Results are expressed as mean ± standard deviation. Embryo morphology was tracked by bright-field images.

Main results and the role of chance: All euploid and T21 embryos thawed managed to hatch completely and expand the blastocoel cavity, while 40% of M21 embryos failed to hatch being arrested inside the zona pellucida on day 7. On day 7, mean concentration of b-hCG secreted did not differ among euploid, T21 and M21 embryos (37,29 ± 6,27; 36,05 ± 6,61 and 27,16 ± 9,95 ng/mL respectively). Interestingly, euploid and T21 embryos reported a progressive time-dependent increase in b-hCG levels along post-implantation in-vitro culture while secreted levels of b-hCG by M21 embryos showed a decreasing trend, reporting diminished b-hCG secretion at the end of the culture (day 10) compared to day 7 (14,86 ± 2,57 vs 27,16 ± 9,95 ng/mL; $p < 0.05$). No significant differences in b-hCG secretions were observed between euploid and T21 embryos neither on day 9 d.p.f. (61,05 ± 8,47 and 56,08 ± 8,10 ng/mL respectively) nor on day 10 (74,88 ± 6,36 and 71,75 ± 7,01 ng/mL respectively). However, b-hCG secreted by M21 embryos on day 9. and day 10 (27,11 ± 11,45 and 14,86 ± 2,57 ng/mL respectively) were significantly reduced ($p < 0.01$) compared with euploid or T21 embryos. Besides, while all euploid and T21 embryos developed until day 10, 16,67% of expanded M21 embryos degenerated before day 9.

Limitations, reasons for caution: This research focused only on embryo development, no maternal tissues were considered. Further proteins should be investigated to reveal other pathways implicated in healthy embryo development and not only trophoblast proliferation.

Wider implications of the findings: Monosomic embryos, associated to implantation failures, secrete lower b-hCG when compared to either euploid or trisomic embryos, which show similar behavior. Besides, b-hCG in culture media could become a non-invasive marker to track embryo progression along post-implantation stages and detect embryos with increased likelihood of developmental arrest or early miscarriage.

Trial registration number: Not Applicable

Abstract citation ID: dead093.1025

P-703 The role of maternal polymorphic karyotype variants among embryonic ploidy and mosaicism status in patients undergoing IVF treatments

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Study question: Is there a relationship between the chromosomal status of biopsied embryos and the presence of polymorphic variants in the female karyotype in IVF cycles?

Summary answer: The chromosomal status of biopsied embryos is prejudiced because of the presence of specific maternal polymorphic variants (qh-, qh+ and ps+) or combinations of them.

What is known already: Due to the increasing average age of patients who undergo IVF cycles with their own oocytes, more cycles are performed with embryo biopsy for preimplantation genetic test of aneuploidies (PGT-A). Thus, advanced maternal age has been defined as the main factor causing embryonic chromosomal abnormalities.

However, few studies have established direct relationships with other factors. The prevalence of chromosomal polymorphic variants has a higher incidence in the infertile population, which is why there is a need to know the role they play in the genetic inheritance of embryos.

Study design, size, duration: Retrospective evaluation of a cohort of women who underwent autologous IVF cycles and karyotyping. The sample included 162 IVF cycles with embryo biopsy on D5 and/or D6 for PGT-A, performed between July 2017-December 2021: control group (CG) with normal karyotype (86) and study group (SG) with presence of polymorphisms (76).

We studied the connection between maternal karyotype polymorphisms and molecular genetic outcomes in terms of euploid, mosaic, and aneuploid blastocyst rates.

Participants/materials, setting, methods: The analysis of the karyotype of women, prior to the IVF cycle, was performed using the guidelines of the International System for Human Cytogenetic Nomenclature (ISCN).

The trophoectoderm biopsies on D5 and/or D6 blastocysts were analysed by NGS using the Illumina platform (VeriSeq Illumina®, San Diego, CA, USA).

The association between the different polymorphic variants in the female karyotype and chromosomal status of embryos was analysed using R (v. 4.2.0) statistical software.

Main results and the role of chance: We analyzed the results of 483 embryos from 162 IVF-PGT-A cycles. Mean female age was 39.05 ± 2.72 (CG) and 38.97 ± 2.90 (SG). Mean number of mature oocytes (MII) was 7.16 ± 3.94 (CG) and 8.01 ± 5.33 (SG). Mean number of biopsied embryos was 2.97 ± 2.15 (CG) and 2.93 ± 1.88 (SG).

No statistically significant differences were found between the CG and SG groups in terms of the rate of euploid embryos: 26.99% vs 21.50% (p = 0.3), transferable mosaic embryos: 8.90% vs 6.76% (p = 0.5), aneuploid embryos: 61.98% vs 69.96% (p = 0.12) and non-informative embryos: 2.13% vs 1.78% (p = 0.2), respectively.

However, an increase in the rate of chromosomally abnormal embryos was observed when analysing each polymorphism individually. In females carrying the qh+ variant, we found a statistically significant decrease in the euploid embryo rate: 10.97% vs 26.00% found in CG (p = 0.036). Moreover, the variants qh+ and ps+ increased the rate of aneuploid embryos: 84.17% vs 63.48% in CG (p = 0.013) and 73.52% vs 61.30% in CG (p = 0.021), respectively. Furthermore, it is found that the qh- variant increased the rate of transferable mosaic embryos: 20.00% vs 8.90% in CG (p = 0.025).

The results were corrected for confounding variables such as maternal age, oocyte origin and male factor variables, including polymorphic variants in the male karyotype.

Limitations, reasons for caution: Presence of unmeasured variables that may influence the results. Larger prospective studies including homogeneous cohorts are needed in order to corroborate our initial results.

Wider implications of the findings: The results show that polymorphic variants in the female karyotype may have a direct effect on the embryonic ploidy and mosaicism status. Therefore, it would be advisable for patients who undergo IVF-PGT-A cycles to have a karyotype performed and be informed of the implications that it may entail.

Trial registration number: Not applicable

Abstract citation ID: dead093.1026
P-704 Genomic DNA damage in individuals with sex determination defects and germ cell cancer

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Study question: Does genomic instability contribute to common mechanisms underlying variable defects of gonadal differentiation and susceptibility to germ cells cancer?

Summary answer: Genomic DNA damage and associated phenotypes in leukocytes and gonadal tissue indicate systemic dependence on DNA repair mechanisms, connected to germ cell tumorigenesis when defective.

What is known already: Variabilities related to dysfunctional sex chromosomes are associated to Differences of Sex Development (DSD), characterized by dysgenic gonads, deregulated gender specification and failed germ cell maturation. This causes infertility and elevated risk of germ cell tumors (GCT). DSD was described in individuals with Swyer syndrome (46,XY, females), complete or partial androgen insensitivity syndrome (CAIS, PAIS, 46,XY, females), Turner (45,X0, females) and Klinefelter (47,XXY, males) syndromes, characterized by malignant tumor development. Unbalanced karyotype is associated with genome-wide changes, including proliferation delay, defects in proteostasis, DNA damage and activation of innate immune response, that contribute to genome instability phenotypes.

Study design, size, duration: Individuals with DSD were enrolled into study during routine examination in our clinic. Samples from men with testicular germ cell tumors (TGCT) were used as positive controls for malignancy. The samples were collected with written patients' informed consent. For this study we analyzed 40 individuals with DSD and 19 samples with GCT. Control group contained samples from fertile men and women of appropriate age interval.

Participants/materials, setting, methods: Study groups included individuals with Swyer (n = 7), Turner (n = 11), Klinefelter (n = 6), CAIS (n = 9) syndromes and men with TGCT (n = 14). Blood was collected in EDTA and DNA, RNA and protein were isolated from leukocytes. Individuals with Swyer and CAIS, that underwent gonadectomy, formed additional group with histologically proven GCT (DSD-GCT, n = 6). Samples were analyzed with quantitative real-time PCR (qRT-PCR), immunoblotting, mass spectrometry, immunofluorescence.

Main results and the role of chance: We described an increase of DNA damage compared to unaffected controls via γH2AX, labeling double strand brakes in DNA, in leukocytes (immunoblotting: Swyer p = 0,0438; DSD-GCT p = 0,0185; CAIS NS; Turner p = 0,0117; Klinefelter p = 0,0068; TGCT p = 0,0207) and gonads (immunofluorescence, γH2AX+ puncta per cell: Swyer n = 75, p < 0,0001; DSD-GCT n = 46, p < 0,0001; CAIS n = 33, NS; TGCT n = 40, p < 0,0001, n-number of cells analyzed). We associated malignancy in our study groups with DNA damage associated phenotypes by evaluating innate immune response activation via *IFNβ* and *ISG15* RNA levels (DSD-GCT p = 0,0350 and p = 0,0411; TGCT p = 0,0007 and p = 0,0437) and inhibition of autophagy via decrease of LC3 and accumulation of P62 proteins (DSD-GCT p < 0,0001 and p = 0,0453; TGCT p = 0,036 and p = 0,0219). This was supported by whole proteome analysis. Compromised genomic DNA integrity suggested an involvement of DNA repair pathways, illustrated by upregulation of deltaTP53 (Swyer p = 0,0104; DSD-GCT p = 0,0478; CAIS p = 0,0046; Klinefelter p = 0,0426; TGCT p = 0,006). TP53 exhibited significant increase in missense mutations in sequences, encoding transactivation domains, which compromised protein stability, particularly, in samples with GCT. We also illustrated that leukocytes of individuals with DSD-GCT restore DNA damage upon direct DNA repair mechanisms activation by Enoxacin (p = 0,0056) or autophagy inhibition by Bafilomycin A1 (p = 0,0034). T test was used for statistical analysis.

Limitations, reasons for caution: This study is based on a limited number of samples available from the individuals with the rare genetic syndromes, for some observed only in 1 out of 10,000 clinical cases. Therefore, development of *in vitro* study models will promote the research in this area.

Wider implications of the findings: This study elucidated possibilities for prophylactic treatments of DSD-individuals as well as new diagnostic approaches of GCT. It additionally emphasized the importance to address genome integrity in infertility research.

Trial registration number: NA

Abstract citation ID: dead093.1027
P-705 Homozygous missense variant in MEIOSIN causes premature ovarian insufficiency

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Study question: Are the gene variants involved in meiosis initiation responsible for premature ovarian insufficiency (POI)?

Summary answer: *MEIOSIN* variant participates in the pathogenesis of human POI by impairing meiosis due to insufficient transcriptional activation of essential meiotic genes.

What is known already: Meiosis is the key event for the establishment of the ovarian reserve, and several gene defects impairing meiotic homologous recombination have been found to contribute to the pathogenesis of POI. However, the genes involved in meiosis initiation have not been associated with POI.

Study design, size, duration: Retrospective genetic study. An in-house whole exome sequencing (WES) database of 1,030 idiopathic POI patients was screened for variations of meiosis initiation genes.

Participants/materials, setting, methods: Gene variations involved in meiosis initiation were screened in the in-house WES database of 1,030 POI cases. The pathogenicity of the variation was verified by *in vitro* experiments, including protein structure prediction and dual-luciferase reporter assay. The effect of the variant on ovarian function and meiosis was demonstrated through histological analyses in a point mutation mouse model.

Main results and the role of chance: A homozygous variant of *MEIOSIN* that initiates meiosis via the retinoic acid dependent pathway was identified in a patient with idiopathic POI. The dual-luciferase reporter assay revealed that the variant adversely affected the transcriptional function of MEIOSIN in upregulating meiotic genes. Further knock-in mice with the homologous mutation

confirmed that the variation impacted the meiotic prophase I program and accelerated oocyte depletion.

Limitations, reasons for caution: Further studies are needed to explore the role of other meiosis initiation genes in the pathogenesis of POI.

Wider implications of the findings: The present study firstly identified pathogenic *MEIOSIN* variants in patients with POI. Although the causative variation in meiotic initiation genes is rare in POI, our study expanded the spectrum of causative genes and elucidated the different mechanisms in human infertility.

Trial registration number: Not available

Abstract citation ID: dead093.1028

P-706 Non-invasive analysis of mitochondrial DNA mutation for embryo evaluation.

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Study question: Is it possible to amplify whole mitochondrial DNA (mtDNA) and detect all mtDNA mutation in human embryo from spent media after embryo culture?

Summary answer: We were able to amplify whole mtDNA from 43% of spent medium after 24-hour culture. The most of mtDNA mutation in embryo could be detected.

What is known already: Preimplantation genetic testing for aneuploidy (PGT-A) with embryo biopsy is now commonly used to improve pregnancy rate. However, invasive embryo biopsy requires highly trained embryologist and induced higher risk of pre-eclampsia. Therefore, non-invasive methods with cell-free DNA in spent medium have been studied. Our group previously showed euploid or viable embryos at post-implantation stage have lower number of non-synonymous mtDNA mutations and this may help to select the best blastocyst to transfer. Although cell-free DNA is reported to contain mtDNA, it remains unclear whether mtDNA mutations number can be assessed from cell-free DNA.

Study design, size, duration: Donated low grade blastocysts were cultured for additional 24 hours with new media, cell-free mtDNA were obtained from the spent culture medium (non-invasive samples). For comparison, trophectoderm cells were biopsied from each embryo (invasive sample). The mtDNA selective amplifications were done to both samples. We performed mtDNA sequencing by next generation sequencing (NGS) and compared mtDNA mutations between non-invasive and invasive samples to clarify the capability of non-invasive assessment for mtDNA mutation.

Participants/materials, setting, methods: Under the ethical review of Yokohama City University and informed consent with patients, we collected human blastocysts which were discarded because of slow development or morphologically low-grade. We extracted whole DNA from biopsy and medium samples and selectively amplified mtDNA by long-range PCR for two overlapping amplicons. The mtDNA sequencing with NGS was performed to successfully amplified mtDNA. The 90% or more heteroplasmy level non-synonymous mutations were investigated.

Main results and the role of chance: We collected 34 discarded embryos from 13 patients. All embryos were cultured individually for additional 24 hours with 20 μl drop of newly prepared media. Amplified mtDNA were visualized by the presence of 8 kb band following agarose gel-apheresis for two fragments PCR. Both fragments were detected in 41% (14/34) of non-invasive samples and all invasive samples. Only one fragment was detected in 14% (5/34) of non-invasive samples. The completely amplified non-invasive samples and corresponding invasive samples were performed mtDNA sequencing with NGS. In results, 13 non-synonymous mutations were identified in invasive samples. All of these mutations were also detected in non-invasive samples. Although the number and location of mutations completely matched between the invasive and non-invasive samples in 78.5% (11/14) embryos, in other 3 embryos, mtDNA mutation numbers were higher in non-invasive samples even though no mutation existed in invasive samples. This means, if selective mtDNA amplification is completed, mtDNA mutations can be evaluated from the non-invasive samples with a probability of about 80% but include 20% false positive mtDNA mutations. To realize this, we need to improve the mtDNA amplification method.

Limitations, reasons for caution: We can used only clinically discarded embryos because ethical restriction. And it was necessary to additional 24-hours culture to obtain individually cultured spent media because embryos are co-cultured until they are donated. Because of small number of samples available, we keep collecting more samples for more accurate evaluation.

Wider implications of the findings: If the mtDNA mutations can be analyzed from spent medium, high-quality embryos can be selected without invasive embryo biopsy and complicated procedure. If this non-invasive mtDNA analysis can be performed to clinical embryo for embryo transfer, it may contribute to further improvement of pregnancy rate without any invasion to embryo.

Trial registration number: the Japan Society for the Promotion of Science (JSPS) KAKENHI Grant-in-Aid for Scientific Research C (Grant Number JP21K09474) and Early-Career Scientists (Grant Number JP20K18169)

Abstract citation ID: dead093.1029

P-707 Evaluating DualStim/PGTA in poor prognosis patients: Comparison of double ICSI-PGTA vs. oocyte cryopreservation plus single PGTA in luteal phase through laboratory, genetic and clinical results.

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Study question: Which strategy is best for poor-prognosis patients: Two ICSI-PGTA cycles fresh oocytes (follicular plus luteal phase) or one ICSI-PGTA cycle with vitrified and fresh oocytes?"

Summary answer: Laboratory outcomes, ploidy rates and clinical results after euploid blastocyst transfer are similar between the two DualStim/PGTA strategies.

What is known already: Luteal phase ovarian stimulation (LPOS) after follicular phase ovarian stimulation (FPOS) is an effective strategy to obtain more oocytes in a single cycle and reduce time-to-pregnancy. The main goal of controlled ovarian stimulation in PGTA patients is to obtain a sufficient number of oocytes to have at least one euploid blastocyst. The standard treatment for achieving this goal is DuoStim plus oocyte cryopreservation. However, this method may decrease oocyte competence in older patients. To address this, we propose comparing the outcomes of two consecutive fresh ICSI-PGTA cycles to our standard protocol in older patients, as an alternative approach.

Study design, size, duration: This multicentric retrospective study evaluated 91 poor-prognosis PGTA cycles ($\leq$ 5 antral follicle count) using the DualStim approach (182 oocyte pick-ups) in the same menstrual cycle, from January to October 2022. Fifty-three patients (group 1; 746 oocytes retrieved) underwent two consecutive egg retrievals, ICSI, and blastocyst biopsy, after FPOS and LPOS, while 38 patients (group 2; 743 oocytes retrieved) underwent oocyte vitrification after FPOS, followed by LPOS, egg retrieval, oocyte warming, ICSI, and blastocyst biopsy.

Participants/materials, setting, methods: We compared various parameters including oocyte number, maturity, fertilization rates, blastocyst quality, biopsy rates, and genetic results between two groups after both egg

retrievals. Additionally, clinical outcomes after euploid embryo cryotransfer were also compared. Oocytes were microinjected, cultured in Geri® time-lapse incubators, and underwent embryo biopsy on day 5/6. Statistical analysis was conducted using R software (v.4.2.0), including maternal age as a confounding factor. Results were considered significant with a p-value less than 0.05.

Main results and the role of chance: Patient age was found to be significantly different between groups (40.5 ± 2.2 in group-1 vs 38.5 ± 2.7 in group-2; $p < 0.001$) and was used as a confounding factor in statistical comparisons. No significant differences were found in terms of oocytes retrieved per patient (14 ± 7.1 vs 13.6 ± 5.8), maturity rate (79.4 % vs 77.9%), fertilization rate (75.3 % vs 74.6%), or day-5 blastocyst rate (54.8% vs 51.7%). However, a significant difference was observed in the percentage of blastocysts biopsied per group (50.2% group-1 vs 43.0% group-2; $p = 0.023$). After genetic analysis, euploidy rate was found to be lower in group-1 compared to group-2 although not statistically significant (19.8% and 32.6%, respectively, $p = 0.007$/p=0.309 after adjusting for maternal age), and this led to similar average number of euploid embryos per DuoStim cycle (1.1 ± 1.3 group-1 vs 1.3 ± 1.4 group-2). Aneuploidy (73.9% vs 62%) and mosaicism rates (6.3% vs 5.4%) were also comparable between the two groups. No statistical differences were observed between the follicular and luteal phases in each group in terms of laboratory and genetic results. Preliminary clinical outcomes showed similar pregnancy rates with 12 pregnancies achieved in group 1 after 23 euploid embryo transfers (52.2%), and 14/20 in group 2 (70.0%) with no significant difference between groups.

Limitations, reasons for caution: This study is a retrospective analysis and further validation through prospective study is recommended. Additionally, while the average age of the study groups is statistically different due to bias in the clinical prescription of treatment, this difference was statistically corrected.

Wider implications of the findings: Cryopreserving oocytes obtained after FPOS and warming them after the subsequent LPOS does not negatively impact the quality or final results of a DuoStim PGTA cycle. This approach should be considered as it can reduce workload for the IVF laboratory and minimize costs for patients.

Trial registration number: none

Abstract citation ID: dead093.1030
P-708 Establishment of linkage phase, using Oxford Nanopore Technologies, for Preimplantation Genetic Testing of Coffin-Lowry syndrome with a de novo RPS6KA3 mutation

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Study question: There was a challenge to establishing haplotype for PGT in the family with de novo mutation because of the lack of information from affected members.

Summary answer: The ONT platform combined with MARSALA method can be used to perform PGT for DNM patients without the need for other samples as a reference.

What is known already: The proband was a 31-year-old female who was born with mental retardation and language delay. On physical examination, she was found to have distinctive Coffin-Lowry Syndrome features, such as distance between the eyes and low nasal bridge. However, the proband's parents did not show any abnormalities. The couple underwent genetic counseling and whole-exome sequencing (WES). The results showed that a frameshift mutation (c.1496delG, p.Gly499Valfs*25) was identified in the *RPS6KA3* gene, with a true mutation in the proband but no mutations in the parents.

Study design, size, duration: A couple requested genetic counseling at the reproductive center of Zhongshan Boai Hospital and underwent whole-exome sequencing (WES) in 2019. The proband then underwent PGT with Oxford Nanopore Technologies (ONT) and the MARSALA platform to block the transmission of the pathogenic mutation to her offspring in 2022.

Participants/materials, setting, methods: The proband underwent PGT to block the transmission of the pathogenic mutation to her offspring.We used long-read sequencing on the ONT platform to directly detect the mutation and nearby SNPs for construction of the haplotype in PGT. The MARSALA method was used to detect both the SNP-based haplotype and chromosome copy number variations in each blastocyst. Finally, a normal embryo was selected by comparison to the haplotype of the proband and transferred into the uterus.

Main results and the role of chance: Using WES, we found the novel, heterozygous, pathogenic c.1496delG (p.Gly499Valfs*25) mutation of the *RPS6KA3* gene in the proband. The SNP-based haplotype that was linked to the pathogenic mutation site was successfully established in the proband, without the need for other family members to be tested with ONT. Eight blastocysts were biopsied to perform PGT and were assessed with a haplotype linkage analysis (30 SNP sites selected), to give results that were consistent with direct mutation detection using Sanger sequencing. The results of PGT showed that three of the eight blastocysts were normal, without the DNM. Moreover, the patient had a successful pregnancy, after transfer of a normal blastocyst into the uterus, and delivered a healthy baby.

Limitations, reasons for caution: This strategy has only been applied to one case and more cases with de novo mutaition are needed to prove the feasibility of this strategy for PGT-M.

Wider implications of the findings: This study is the first report of a de novo *RPS6KA3* c.1496delG mutation in patient with CLS, which can expands the *RPS6KA3* mutation spectrum and also provides a new strategy based on ONT and MARSALA to perform PGT-M with DNMs without the need for a pedigree sample as a reference.

Trial registration number: Not applicable

Abstract citation ID: dead093.1031
P-709 Predictive heatmap in Preimplantation Genetic Testing for Monogenic disorders (PGT-M) and Structural Rearrangements (PGT-SR)

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Study question: What is the predicted cumulative live birth rate (CLBR) relative to AMH-level for women undergoing preimplantation genetic testing (PGT) for monogenic disorders and structural rearrangements?

Summary answer: Anti-müllerian hormone (AMH) and age allow to give an estimate of the CLBR in PGT and becomes poor when AMH is ≤ 2µg/L and age ≥42y.

What is known already: Ovarian response is an important contributory factor in PGT in order to obtain a sufficient number of embryos for genetic analysis. A proportion of the obtained embryos will be carrier of the disease tested, and another small proportion of the embryos will fail to have a genetic diagnosis hence will not be available for embryo transfer. The number of embryos not suitable for embryo transfer as a result of PGT varies between 25-81% according to the indication for PGT. The combination of female age and AMH are good predictors for CLBR in conventional IVF.

Study design, size, duration: This is a single-centre retrospective cohort study including 1,522 females undergoing 3,130 PGT cycles over a 6-year period (01/01/2015-31/12/2020) at a university-based referral centre for PGT. The principal outcome measure was CLBR per intention to treat (ITT), and the secondary outcome was live birth rate (LBR) per embryo transfer, in couples undergoing PGT-M and PGT-SR either by polymerase chain reaction (PCR), single nucleotide polymorphism (SNP) array, microarray-based comparative genomic hybridization (aCGH), or next-generation sequencing (NGS).

Participants/materials, setting, methods: Mean ± SD was applied for continuous outcomes, whereas (relative) frequency was applied for dichotomous outcomes. Multivariable logistic regression analysis was applied with dependent variable being the CLBR or LBR and the independent variables the AMH, female age, BMI, inheritance mode and PGT-technique. Best-fit (Akaike information criterion(AIC)) versus polynomials were analysed. Non-inferiority testing between different technologies was performed. A 3D-prediction mosaic (including AMH, age and CLBR) was created, which can be used for counseling purposes.

Main results and the role of chance: The mean female age is 32.46 years (SD 4.43), with a mean AMH level of 2.75 μg/L (SD 2.58) and a mean BMI of 24.24 (SD 4.43). Age and AMH significantly affect CLBR irrespective of the inheritance mode or PGT technique used. There is a gradual decline of CLBR from a female age of 25 years onwards reaching a critical threshold of less than 10% CLBR per ITT over the age of 42 with AMH levels ≤2 μg/L. Between cleavage stage biopsy techniques (PCR) and trophectoderm biopsy PGT techniques (SNP array, aCGH, NGS) used there was no significant difference in outcome per ITT, however per embryo transfer there is a significantly higher chance of live birth in SNP array, aCGH and NGS cycles compared to PCR cycles.

Limitations, reasons for caution: Despite the large sample size, the findings are confined by limited confounder adjustment.

Wider implications of the findings: In a PGT-program, couples need to be informed on the limited prognosis, if the combination of female age and AMH is unfavourable, in order to allow them to make other reproductive choices. The prediction mosaic produced in this study can be used as an insightful visual tool in counseling PGT-couples.

Trial registration number: Not applicable

Abstract citation ID: dead093.1032

P-710 Single-cell proteomic analysis of human oocytes reveals a decreased abundance of meiosis and proteostasis regulators with advanced maternal age

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Study question: How does maternal age affect the proteomic content of her oocytes during their final meiotic progression?

Summary answer: Compared to young oocytes, oocytes obtained from women of advanced maternal age (AMA) show a decreased abundance of meiosis and proteostasis regulators.

What is known already: It is well established that AMA leads to a decline in oocyte number and quality, key limiting factors of female reproductive success. Poor oocyte quality is tied to aneuploidies and alterations in cytoplasmic fitness, however the precise mechanisms that underlie oocyte aging remain unknown. Currently knowledge has been largely restricted to transcriptomic studies with no information on the proteome of oocytes obtained from women of AMA. Single-cell proteomic studies may shed light on these mechanisms, as proteins and (post)-translational modifications regulate events unfolding at the oocyte-to-embryo transition in transcriptionally silent oocytes.

Study design, size, duration: A total of 67 oocytes, obtained from 52 women undergoing controlled ovarian stimulation cycles between May 2021 and May 2022, were included in the study. We obtained 18 fresh germinal vesicle oocytes (GVs) and 17 vitrified *in vivo* matured metaphase II oocytes (MIIs) from young women (oocyte donors), and 18 GVs and 14 MIIs from AMA.

Participants/materials, setting, methods: The oocytes were obtained from oocyte donors (n = 27) and AMA patients (n = 25) with a mean age of 24 ± 3.9 years and 39 ± 1.8 years respectively. Single denuded oocytes were snap frozen and their proteomic profiles were generated using the mass spectometry based method plexDIA . Mann-Whitney U test (fold change $>|1.5|$, adjusted p-value <0.05), Spearman correlation ($r_s \geq |0.4|$, p-value <0.05) and ggplot were used in R to compare the different groups.

Main results and the role of chance: A total of 2105 proteins were quantified across oocytes stages and age groups. Most of proteins were present in oocytes independently of their maturation state and related to vital processes, such as cellular respiration and energy metabolism. Fifty-four proteins varied in abundance between immature and mature oocytes, including RS14, YBOX2 and RL10A (translational regulation), CLUS and AHSA1 (post-translational modifications) and proteins involved in meiosis progression (WEE2, AURKA, BUB1B, SPDLY). Notably, we observed a negative correlation between protein levels and maternal age for meiosis regulators (e.g.1433E, CDK1; r_s: -0.7 and -0.5), meiotic spindle proteins (e.g. DYL2, CAPZB, ARP3, GCP3; r_s: -0.6, -0.6, -0.5, -0.5 respectively) and for 5 proteasome subunits (PRS8, PRS6A, PRS10, PSA6, PSMF1; r_s: -0.4 to -0.7). The proteasome is a degradation machinery with multiple roles, including meiosis regulation and proteostasis maintenance. Additional proteins of the proteostasis network were found to decline with age (e.g., TCPH, TCPA; r_s: -0.5, -0.5). Loss of proteostasis is a common feature of aging and diseases. We also observed a positive correlation between mitochondrial and stress response proteins and maternal age (e.g. ATP5L, TMX1; r_s: 0.6, 0.5).

Limitations, reasons for caution: Unlike GVs that were collected fresh, MII oocytes underwent vitrification and warming before being included in the study due to clinical protocols. These procedures may have unknown effects on the proteome.

Wider implications of the findings: We show that a woman's age correlates with changes in her oocyte proteome, which may contribute to the deterioration of oocyte quality through dysregulation of meiosis and disturbance of proteostasis. Proteins showing differential expression in oocytes from women of AMA, such as proteasome, may become targets for improving oocyte quality.

Trial registration number: Not applicable

Abstract citation ID: dead093.1033

P-711 Are there any differences among blastocyst euploid and mosaicism rate (high or low) using fresh or frozen ejaculate sperm in donated oocytes?

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Study question: To compare euploid and mosaicism rate (high or low) among blastocysts derived from fresh and frozen ejaculate sperm in donated oocytes.

Summary answer: No statistically significant differences were found on blastocyst euploid and mosaicism rate between blastocysts derived from fresh versus frozen.

What is known already: Even though 5%-10% autosomal aneuploidies and 5%-100% sex chromosomes aneuploidies are paternally derived, the clinical relevance of paternal contribution to chromosome aneuploidy is frequently overlooked. Besides, male infertility could contribute to the generation of chromosomal abnormalities in the resulting embryos. Sperm cryopreservation is a widely used technique in assisted reproduction for different reasons. However, little is known regarding the potential effect of sperm freezing on embryo aneuploidies.

Study design, size, duration: Retrospective, observational and multicenter study of patients undergoing Preimplantation Genetic Testing for Aneuploidies (PGT-A) from January 2017 to December 2022 in IVIRMA Madrid, Valencia and Barcelona. To elucidate the effect of advance maternal age, 1,780

biopsied blastocysts coming from the egg donation program were analyzed. Autologous sperm samples with less than 2 million spermatozoa per milliliter and male partners with altered karyotype, abnormal spermatozoa fish or any genetic disease were excluded from the study.

Participants/materials, setting, methods: Embryos were cultured in regular incubators, with an atmospheric concentration of 5% O_2, 6.5% CO_2 and a temperature of 37°C. Blastocyst biopsy was performed either on day 5 or day 6 of embryo development and analyzed through Next Generation Sequencing (NGS). Sperm freezing was done following manufacturer's instructions (Sperm freeze, Origio). Continuous variables were compared with a student's t-test and categorical variables with a Chi-square using SPSS statistical program.

Main results and the role of chance: Mean paternal age (44.3 ± 5.20 years old vs 43.2 ± 5.20 years old), sperm concentration (46.67 ± 33.05 mill/ml vs 35.1 ± 32.92 mill/ml) were not significantly different among fresh versus frozen sperm group ($p > 0.05$). A total 1780 blastocysts tested for PGT-A were included: 1267 blastocysts coming from fresh ejaculate sperm (n = 194 patients) and 513 blastocysts from frozen sperm (n = 81 patients). No statistically significant differences were found in blastocyst euploid rate between fresh versus frozen ejaculate sperm (64.5% versus 67.2%, $p = 0.278$;) respectively. Blastocyst mosaicism rate: High mosaicism (>50% of aneuploidy trophectoderm cells) and low mosaicism (30-50% of aneuploidy trophectoderm cells) was comparable between fresh versus frozen sperm (2.7% versus 4.5%; $p = 0.90$) and (3.9% versus 3.7%), respectively.

Limitations, reasons for caution: This study is based on a retrospective data.

Wider implications of the findings: According to our findings, there are no statistically significant differences in the blastocyst euploid and mosaicism rate between fresh and frozen ejaculate sperm.

Trial registration number: Not Applicable

Abstract citation ID: dead093.1034
P-712 Transcriptomic Prediction of Spermatogenesis in the Ejaculates of Nonobstructive Azoospermic Men and Genomic Characterization of their Embryo Developmental Competence

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Study question: Can the genomic assessment of ejaculates from azoospermic men predict successful sperm extraction at testicular biopsy (TESE) and provide information on their reproductive potential?

Summary answer: RNAseq on the ejaculates of NOA men identified transcriptomic predictors of successful TESE outcome, while DNAseq evidenced gene-related mechanistics linked to reproductive performance.

What is known already: The most puzzling form of azoospermia is the testicular type. The chances of successful surgical sperm retrieval for these cases can be unpredictable, even when a benign histopathology has been obtained. The ability to identify individuals with complete loss of spermatogenesis at the seminiferous tubule level would help eliminate anesthesia and surgical risks, as well as financial and emotional distress. We propose to noninvasively assess the ejaculates from these men to predict successful TESE sperm retrieval. We utilize DNAseq to assess the impact of germline mutations on the embryo developmental competence of the surgically retrieved gametes.

Study design, size, duration: Over 4 years, we recruited men with normal peripheral karyotypes and azoospermia (n = 23), confirmed by absence of spermatozoa in extensive semen analyses. We performed epigenetic assessments on their ejaculates to compare transcriptomic profiles according to whether spermatozoa were successfully retrieved after TESE ((+)Sperm,n=11), or not ((-)Sperm,n=12). We then performed DNAseq (IRB 1006101085) on the testicular spermatozoa retrieved, to assess their ability to generate a pregnancy (fertile) or not (infertile), while controlling for maternal age.

Participants/materials, setting, methods: RNA was isolated from seminal fluid for epigenetic assessment (21,855 genes). A > 1 absolute log2fold change and $P<0.0005$ were considered significant. Transcriptomic profiles were compared between the (-)Sperm and (+)Sperm cohorts. For genetic assessment, surgically retrieved spermatozoal DNA from the (+)Sperm and obstructive azoospermic (OA) control (n = 19) cohorts were extracted and amplified. Gene mutation profiles, detected by CLC Genomic Server 9.0, were assessed for the (+)Sperm cohort according to ICSI outcomes, compared to the OA-control.

Main results and the role of chance: Transcriptomic analyses on seminal plasma from the 23 NOA men identified 8 imbalanced genes, involved in spermatogenesis (*TMCO5B,C10orf62,SMKR1,SPZ1*), sperm function (*NEU1,TPTE2*), and testis development (*TRPC1,IGSF11-AS1*). *TPTE2* was almost completely expressed in 81.8%(9/11) of the (+)Sperm cohort (paternal age, 37.1 ± 6yrs), while *IGSF11-AS1* was underexpressed in all 12 (-)Sperm men (paternal age, 34.3 ± 5yrs). Both genes are implicated in spermatogenic defects and are normally highly expressed in testis. Interestingly, *NEU1*, localized on the sperm acrosome and crucial for sperm capacitation, was exclusively underexpressed in (-)Sperm while overexpressed in (+)Sperm cohorts.

Genomic analyses of spermatozoal DNA from the (+)Sperm cohort was compared to a matched control of OA patients (paternal age, 36.8 ± 7yrs). Spermatozoa from the study cohort displayed mutations on genes involved in RNA transcription (*POLR2L*), apoptosis (*AP5M1*), and spermiogenic functions (*AP1S2,AP1G2,APOE*), while these genes were unaffected in the OA-control.

When we compared sperm genetic profiles of the successful TESE cohort (maternal age, 36.8 ± 2yrs, paternal age, 37.1 ± 6yrs) according to ICSI outcome, we found that spermatozoa from the 8 fertile patients, all with term pregnancies, displayed consistent mild mutations on *MPIG6B* (stem cell lineage differentiation) while gametes from their 3 infertile counterparts carried severe mutations on genes (*MBD5,CCAR1,PMEPA1,POLK,REC8,REPIN1,MAPRE3, ARL4C*) involved in early embryonic development, and therefore failed to achieve embryo implantation.

Limitations, reasons for caution: Although we predicted, by transcriptomic analyses of ejaculates from NOA men, the presence of spermatozoa and characterized their embryo developmental competence through genetic profiling, these preliminary results need validation in a larger study cohort. Moreover, although controlled for, a confounding female factor cannot be undoubtfully excluded.

Wider implications of the findings: Epigenetic assessment of seminal fluid from NOA men can noninvasively identify biomarkers capable of predicting complete loss of spermatogenesis, sparing these patients from unnecessary surgery. Gene profiling can identify gametes capable of supporting term pregnancies, laying the groundwork for precision medicine in male infertility screening.

Trial registration number: n/a

Abstract citation ID: dead093.1035
P-713 Preimplantation DNA methylation screening (PIMS) illustrates the outcome of assisted reproductive technology

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Study question: It is unknown whether using DNA methylation can improve the outcome of ART and can exclude the imprinted gene diseases.

Summary answer: Our study provides a criterion on selecting blastocyst according to DNA methylome, which can improve live birth rate and decrease birth defect rate.

What is known already: How to select the embryos is one key factor that can determine the successful rate of ART. As aneuploidy exists in 20-30% of blastocysts, preimplantation genetic screening (PGS) has been widely used, which increases the live birth rate. Unfortunately, the live birth rate remains around 50% even with the help of PGS. DNA methylation is known to play

important function during embryogenesis. Previous study has shown that a large proportion of human embryos have abnormal DNA methylome. However, whether DNA methylation pattern can affect the clinical outcome of ART has not been investigated in clinics.

Study design, size, duration: To determine whether PIMS method can increase live birth rate during ART, the whole genome DNA methylation of 3-5 biopsied cells from trophectoderm were measured. The methylation level and chromosome copy number variation (CNV) were analyzed by using the methylome data. elective single embryo transfer (eSET) will be performed for embryos with euploid chromosome. The live birth rate, pregnant rate and abortion rate will be examined. 182 families including 800 blastocysts were enrolled in PIMT.

Participants/materials, setting, methods: Eligible couples include advanced maternal age, recurrent pregnancy loss with unknown reason, recurrent implantation failure with unknown reason, severe oligoteratozoospermia. Eligible couples must have two or more good-quality blastocysts. We call a blastocyst as good quality if the blastocyst is 4BC or better according to the Gardner morphologic criteria on day 5-6 of embryo culture.

Main results and the role of chance: No one has ever performed clinical trial to investigate the impact of DNA methylation of blastocyst on the outcome of artificial assisted reproductive (ART). Here, we performed PIMS using the biopsied trophectoderm of 800 blastocysts. Chromosome copy number variation and DNA methylation pattern were analyzed by using methylome data. 162 of euploid embryos were transferred. Our data show that significant variation of DNA methylation level of blastocysts can be observed in both younger women (£37 years old) and older women (>37 years). Blastocysts with global methylation level around 0.26 have the highest birth and pregnancy rate, but the lowest abortion rate. The higher the difference methylation value from 0.26 is, the lower the birth rate becomes. Furthermore, by using artificial intelligence method according to different methylated regions between the embryos succeeding in live-birth and those failing in live-birth, we can robustly predict the clinical outcome of blastocysts. PIMS method can find the abnormality of DNA methylation states in the imprinted control regions which can cause the imprinted diseases. Our study provides a criterion on selecting blastocyst according to DNA methylome during ART, which can potentially improve the live birth rate and decrease the birth defect rate.

Limitations, reasons for caution: Because of the low coverage of single cell DNA methylome, PIMS method cannot cover all imprinted control regions.

Wider implications of the findings: PIMS method can potentially find more human diseases which is caused by DNA methylation mutation.

Trial registration number: NCT03642574

Abstract citation ID: dead093.1036
P-714 Clinical utility of FMR1 screening: risk factor or monogenic cause for POI

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Study question: Is there a risk factor association or a monogenic relationship between FMR1 premutation and developing Fragile X-associated primary ovarian insufficiency (FXPOI)?

Summary answer: There is an association between *FMR1* premutation and FXPOI rather than a monogenic relationship, which is highly dependent on ethnicity.

What is known already: Among the 40 genes involved in primary ovarian insufficiency (POI), identified in our recent systematic review (Van Der Kelen *et al.*, 2022), the *FMR1* premutation is considered as the most common cause of POI. A premutation in the *FMR1* gene is defined as a CGG trinucleotide repeat length between 55 and 200 in the 5′ untranslated region. The term FXPOI is used for women with premutation in FMR1 who have a loss of normal function of the ovaries before the age of 40. There is conflicting evidence about the relationship between POI and length of premutation FMR1 alleles.

Study design, size, duration: Curated publications identified in PubMed and Web of Science on genetics of human female infertility and sex development by using the MESH terms, key words and inclusion/exclusion criteria described in our comprehensive systematic review were subjected to screening for "FMR1 gene." The articles included cover a period from 1988 to the 1st of November 2021.

Participants/materials, setting, methods: A total of 161 publications in PubMed and 61 unique publications in Web of Science were identified, and subsequently screened for triplet expansion, primary ovarian insufficiency, and/or early menopause. Of these, 56 papers were selected, excluding publications exclusively on males as well as studies on non-human species, pediatric cases, reviews, and expression studies. The study and control groups repeat numbers, ethnicity, and conclusions in the article are listed.

Main results and the role of chance: Expansion in CGG trinucleotide repeat length happens at female meiosis and the risk for expansion increases with an increasing number of CGG repeats. There is an intermediate zone between 45 and 55 CGG repeats when the allele may be stable or unstable. Among 56 publications, 54 describing FMR1 triplet repeat size in women with POI, 34 were studying only the women with POI (observational) while 20 were including also control group (comparative). 2 publications were meta-analyses in which 13 and 11 case control studies were included respectively, 5 studies being in common.

POI-associated premutations showed a wide range of repeat sizes. Women carrying midsize range repeats (>70-<100) potentially have a higher risk for POI compared to the general population. In recent decades, population-based screenings have indicated that FMR1 premutations are not as prevalent in women with ovarian insufficiency as previous estimates have suggested, but they still represent a substantial cause of POI. When present, the number of AGG interruptions and the size of uninterrupted CGG repeats are directly correlated with the ovarian reserve. No increased risk of POI associated with a premutation was reported among populations of non-European descent, such as the Han Chinese, Indian, and Jordan populations.

Limitations, reasons for caution: Available reports are difficult to compare because the ethnicity and number of patients analyzed, availability of clinical data and the quality of results are different in each study. Additionally, an effect of X-inactivation in POI women with premutation was not studied.

Wider implications of the findings: Owing to the low penetrance and a molecular mechanism that has not yet been fully elucidated, the clinical utility of FMR1 screening in women with reduced ovarian reserve needs further investigation before clinical implication.

Trial registration number: N/A

Abstract citation ID: dead093.1037
P-716 Genetic mutations in women with recurrent assisted reproductive technology failure

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Study question: Are there genetic mutations which may lead to recurrent failures of assisted reproductive technology?

Summary answer: In this study, some genes were found to be candidates for causing repeated ART failures.

What is known already: Although reproductive aging is one of the greatest hindrances to successful pregnancy, many patients remain infertile, enduring recurrent ART failures, and approximately 20% of infertility is idiopathic. At present, the mutation of 8 genes, *PADI6, NLRP2, TLE6, NLRP5, BTG4, KHDC3L, REC114* and *OOEP* has been reported to cause early embryonic arrest, while the mutation of *PATL2* with GV stage arrest; *TUBB8* and *TRIP13* with failure of polar body extrusion; *PANX1* with oocyte death; *ZP1, ZP2, ZP3* with empty follicle syndrome; *WEE2, TLE6* and *CDC20* with fertilization failure have been found.

Study design, size, duration: Between January and October 2021, at Hanabusa Women's Clinic, 25 infertile women, who experienced IVF / ICSI failure more than 5 times, with blastocyst formation rates of less than 10%, were recruited, after informed consent was obtained. 10 staff members of Hanabusa Women's Clinic who conceived and delivered naturally were compared as normal controls.

Participants/materials, setting, methods: Genomic DNA was extracted from whole blood and genomic DNA samples were subjected to whole exome sequencing. High impact variants with allele frequencies of ≦0.20 in the 1000 Genome EAS, which were not found in the control subjects, were selected. Data from the Human Genetic Variation Database (HGVD) was used for the Hardy-Weinberg equation (HWE) to evaluate possible candidates for recurrent ART failures caused by genetic mutations.

Main results and the role of chance: High impact homozygous variants were detected in 57 genes among the study subjects of infertile women, but not among the control subjects. Among these 57 genes, significant differences in the allele frequencies between 8.3KJPN and the patients were detected in *ADAM33* ($p = 0.009$), *CEP89* ($p = 0.012$), *CRIPAK* ($p < 0.001$), *LGALS9B* ($p < 0.001$), *PDZRN3* ($p = 0.001$), *RAET1E* ($p = 0.007$) and *SPATA31A3* ($p = 0.045$). The HWE of each gene was calculated based on the data from HGVD. Among the 57 genes mentioned above, significantly lower HWE was detected in patients as compared to the HGVD in *ADAM33* ($p < 0.001$), *CEP89* ($p = 0.033$), *MICA* ($p < 0.001$), *OR2T29* ($p = 0.003$), *OR52J3* ($p = 0.0497$), *RABL2A* ($p < 0.001$), *RNF17* ($p = 0.0499$), *SPATA31C1* ($p = 0.030$) and *WWTR1* ($p < 0.001$). Having identified one of the piRNA pathway genes, *RNF17*, which had significantly reduced HWE, the localization of RNF17 in the primordial follicle was examined. Immunofluorescent staining showed that RNF17 was sporadically localized in the cytoplasm of primordial follicles.

Limitations, reasons for caution: The sample size of this study was relatively small, and precise information such as age, gender in the database used in this study did not match that of the study patients, thus, the interpretation of the results is limited and further research is required to confirm our findings.

Wider implications of the findings: Among abovementioned genes, one of the piRNA pathway genes, *RNF17*, and one of the Hippo signal pathway genes, *WWER1*, were the most likely causes of repeated ART failures. The findings may provide potential diagnostic markers for patients with recurrent ART failures, helping us understand the genetic basis of female infertility.

Trial registration number: Not applicable

Abstract citation ID: dead093.1038
P-717 Segmental aneuploidies are not related to paternal age in young women

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Study question: Is there an impact of paternal age on embryo segmental aneuploidy rate?

Summary answer: Even when segmental aneuploidies are most linked to paternal origin, results do not show a relationship between paternal age and embryo segmental aneuploidies.

What is known already: Most studies consider maternal age as a risk factor for spontaneous abortion, infertility and genetic defects in the offspring. However, attention has only recently turned to the impact of paternal age on reproductive outcome. In Assisted Reproduction Technology male age has been related to a decrease in sperm quality and clinical outcomes and an increase in sperm DNA damage has been identified among elderly males. Studies assessing the type of chromosomal aneuploidy associated with paternal aging have reported mixed relative risk results for chromosomes 13, 18, 21, and X. Segmental aneuploidies are most linked to paternal origin.

Study design, size, duration: Observational, retrospective and multicentric study. The study was approved by the local institutional review board (IRB). All the patients underwent an IVF cycle with oocytes from egg donors, followed by Preimplantation Genetic Testing for Aneuploidy (PGT-A) at IVI-RMA Madrid, IVI-RMA Valencia and IVI-RMA Barcelona from January 2018 to August 2022. A total of 3412 biopsied embryos obtained from 660 cycles were included in the study.

Participants/materials, setting, methods: Exclusion criteria were the following: altered karyotype, single gene disorder and male factor (sperm concentration lower than 5 million/ ml). Embryo biopsies were performed at blastocyst stage (day 5/ 6). After the trophoectoderm biopsy, all embryos were vitrified. Main outcome was segmental aneuploidy rate among different male age groups (<41 y.o./ ≥40 y.o.). Lineal regression analysis was used for continuous parameters and Chi square was used for categorical variables.

Main results and the role of chance: Descriptive parameters showed no differences, particularly in sperm concentration (43.2 ± 15.6 vs. 40.1 ± 14.3) and sperm motility (27.3 ± 13.7 vs. 29.7 ± 14.8). Lineal regression analysis showed no significant differences in segmental aneuploidy rate ($p = 0.542$). Statistical model showed no significant differences among male age groups (1.28% (0.76-2.01) vs 1.10% (0.69-1.66) $p = 0.542$).

Limitations, reasons for caution: The study has been conducted in three different clinics, there could be some unknown cofounding factors.

Wider implications of the findings: Male age don't have an impact on the embryo segmental aneuploidy rate in young/donors' oocyte

Trial registration number: Not applicable

Abstract citation ID: dead093.1039
P-718 Preimplantation genetic testing in Belgium: recommendations for the genetic centres

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Study question: How do genetic centres in Belgium apply international guidelines on preimplantation genetic testing (PGT)?

Summary answer: We present the national consensus on how to practice PGT, starting from initial patient uptake and embryo selection to follow-up of pregnancies.

What is known already: PGT includes PGT-M for monogenic disorders, PGT-SR for structural rearrangements and PGT-A for aneuploidy screening. The application of whole-genome technologies to trophectoderm biopsies for PGT-M and PGT-SR/A has evidenced the high prevalence of mosaicism due to chromosome segregation errors during the first mitotic divisions, making

biopsy results interpretation and embryo transfer policy more challenging and complex. Guidelines from several international societies have been published in recent years to leverage the standardization of the whole PGT service. Although these are based on expertise and continuously acquiring evidence, it is widely recognized that variations in local and national regulations diversify PGT practices.

Study design, size, duration: Recommendations regarding PGT indications, patient counselling, embryo transfer policy, reporting, quality and pregnancy follow-up have been built to harmonize PGT services nationally. With a special focus on mosaicism, aiming to balance the highest chance for a pregnancy with the lowest risk for a chromosomally abnormal ongoing pregnancy or child.

Participants/materials, setting, methods: The Belgian PGT working group consists of laboratory geneticists, clinical geneticists and IVF specialists from eight nationally accredited centres. The group operates under the BeSHG umbrella and meets quarterly to formulate opinions and statements on PGT practice with the main goal of guaranteeing a high standard of care, exchange knowledge and implement a harmonized workflow. The Belgian social security system provides reimbursement of six genetic testing cycles for all PGT-M/SR patients but not for PGT-A.

Main results and the role of chance: PGT is offered for all monogenic and chromosomal disorders, if the causal genetic variants are known and documented. PGT for HLA-typing is allowed for the therapeutic benefit of an existing child after couple's psychological evaluation. Aneuploidy screening can be offered to specific patient groups to increase IVF success rate. However, social sexing and testing for non-medical or eugenic purposes is prohibited. At intake, the couple is counselled about the genetic aspect of the disease, psychological and financial aspects of PGT, applied methodology, including average success and misdiagnosis rate, and embryo transfer policies. Affected embryos, and embryos without diagnosis for PGT-M/SR are not transferred. For PGT-A (combined or not with PGT-M/SR), embryos are categorized as: (A) Euploid - suitable for transfer, if mosaicism is absent or below the detection threshold. (B) Mosaic transferable - suitable for transfer, but only following ranking and/or counselling and follow up, if mosaicism for any chromosome not described in (C) is detected; (C) Aneuploid or Mosaic - not suitable for transfer, if mosaicism of 8, 9, 13, 16, 18, 21 and X or full aneuploidy of any chromosome is detected. Non-invasive prenatal testing is strongly recommended to all women pregnant after PGT and is nationally reimbursed.

Limitations, reasons for caution: The practical recommendations, which are supplementary to the recently published ESHRE PGT recommendations, are based on expertise and data from the literature available at the time of preparation. As such, they will need regular reconsideration and an update or extension may follow when new solid scientific evidence arises.

Wider implications of the findings: As international recommendations represent a greatest common denominator over many local regulations, this work serves as example for drawing country-specific agreements for genetic centres. The process of consensus expert opinion formation on best practice may be applicable in other countries where PGT service is not overseen by a designated agency.

Trial registration number: Not applicable

Abstract citation ID: dead093.1040
P-719 Evaluation of genetic risk of apparently balanced chromosomal rearrangement carriers by breakpoint characterization

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Study question: What are breakpoint characteristics of ABCR carriers? What are the genetic risks of ABCR carriers caused by chromosome rearrangements?

Summary answer: We analyzed the characteristics of ABCR's breakpoints and found that heterogeneous deletion of some AD disease-related genes may not result in clinical phenotype.

What is known already: ABCRs are common chromosomal abnormalities. People with ABCRs generally lack any visible abnormal features; however, during childbirth, the abnormal pairing and missegregation of homologous chromosomes can lead to the production of unbalanced gametes, resulting in spontaneous abortion or the birth of children with chromosomal disorders. Prenatal diagnosis or preimplantation diagnosis can effectively prevent the birth of children with unbalanced chromosome rearrangements, but no substantial data has been collected to report on the characteristics and potential genetic risks of the chromosome breakage caused by the rearrangement.

Study design, size, duration: This is a retrospective study of 2441 breakpoints in 1219 ABCR carriers who underwent Preimplantation Genetic Testing (PGT) at the Reproductive and Genetic Hospital of Citic-Xiangya from August 1, 2016 to December 31, 2021.

Participants/materials, setting, methods: All patients in this study sought PGT treatment due to primary or secondary infertility caused by ABCRs. To characterize the chromosome breakpoints, the MicroSeq technique, which involves chromosome microdissection and next generation sequencing, was employed. Additionally, gap-junction PCR and Sanger sequencing were utilized to further validate some of the breakpoints.

Main results and the role of chance: In this study, we retrospectively analyzed 2441 chromosome rearrangement breakpoint MicroSeq localization data from 1219 ABCR carriers, including 2364 breakpoint regions in 1182 reciprocal translocations, 68 breakpoint regions in 34 inversions, and 9 breakpoint regions in 3 insertional translocations. We not only identified 8q24.13, 11q11.23, and 22q11.21 as previously reported hotspots of chromosomal balanced rearrangement breaks, but also identified the 10 Mb region of 12q24.13-q24.3 as a possible rare region of balanced rearrangement breaks. the average range of breakpoints identified by MicroSeq technology was only within 3.8 Kb, which clarified 95.82% (2339/2441) of balanced chromosomal rearrangements as to whether they broke genes or not. We identified 960 genes that were interrupted, of which 53 genes with autosomal dominant mode of inheritance (AD) were interrupted. Clinical phenotypic evaluation of these patients carrying interrupted AD genes identified that some of the AD genetic interruptions may not have a clinical phenotype.

Limitations, reasons for caution: MicroSeq cannot precisely pinpoint breakpoints to the gene or single base level. Moreover, carriers of ABCRs were not comprehensively assessed for other clinical characteristics, and the alteration in function of the known Haploinsufficiency gene could not be confirmed. Additionally, the position effect of the breakpoints has not been evaluated.

Wider implications of the findings: This investigation offers a reference for evaluating the pathogenicity and genetic risks of protein-truncation mutations in some AD genes.

Trial registration number: Not applicable

Abstract citation ID: dead093.1041
P-721 Nanopore sequencing for detecting reciprocal translocation carrier status in preimplantation genetic testing

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Study question: Analyze the concordance between nanopore sequencing and MaReCs to validate the feasibility of nanopore sequencing in distinguishing normal embryos from carrier embryos in PGT-SR cycles.

Summary answer: Nanopore sequencing is a powerful strategy for accurately distinguishing nontranslocation embryos from translocation carrier embryos and precisely localizing translocation breakpoints.

What is known already: Balanced reciprocal translocation (BRT) is one of the most common chromosomal abnormalities that causes infertility, recurrent miscarriage, and birth defects. Preimplantation genetic testing (PGT) is widely used to select euploid embryos for BRT carriers to increase the chance of a healthy live birth. Several strategies can be used to distinguish reciprocal translocation carrier embryos from those with a normal karyotype; however, these techniques are time-consuming and difficult to implement in clinical laboratories.

Study design, size, duration: The Nanopore Sequencing was performed on two pregnant patients conducted on Mapping Allele with Resolved Carrier Status" (MaReCs) in PGT-SR cycles to identify translocation breakpoints for a year. Combined conventional MaReCs 'results, amniocentesis was performed to identify karyotypes at 18–20 weeks of gestation to verify the correctness of the Nanopore Sequencing.

Participants/materials, setting, methods: A total of 11 embryos from 2 couple1 and 12 embryos from couple 2 were tested simultaneously in PGT-SR cycles with a combination strategy of mate pair sequencing and PCR breakpoint analysis, SNP array-based comprehensive chromosome screening (CCS), NGS following micro-dissecting junction region (Micro-Seq) to distinguish between normal and carrier embryos. The Nanopore Sequencing based on TGS was performed on 2 couples. Lastly, amniocentesis was performed to test karyotypes.

Main results and the role of chance: The translocation breakpoints in both reciprocal translocation carriers were accurately identifed by nanopore sequencing and were in accordance with the results obtained using MaReCs. More than one euploid non-balanced translocation carrier embryo was identified in both patients. Amniocentesis results revealed normal karyotypes, consistent with the findings by MaReCs and nanopore sequencing.

Limitations, reasons for caution: One limitation is that it is not possible to identify the translocation breakpoints of Robertsonian translocation carriers or those located in the gap regions of the human genome. In addition, high-molecular-weight genomic DNA and large amounts of peripheral blood samples would be a challenge most PGT centers.

Wider implications of the findings: The nanopore sequencing accurately detects translocation breakpoints in BRT carriers and distinguishes translocation-free embryos from balanced diploid embryos in clinical PGT-SR cycles. It is superior to short reads (NGS) for the detection of translocation breakpoints, which shows broad prospects for clinical applications in blocking translocation propagation in the population.

Trial registration number: Not applicable

Abstract citation ID: dead093.1042
P-722 Impact of blastocyst quality and post-thaw embryo culture factors in non-invasive PGT-A informativity on vitrified blastocysts.

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Study question: What are the most important factor/s involved in the informativity of non-invasive PGT-A (niPGT-A) of vitrified blastocysts?

Summary answer: The day of vitrification and post-thaw time in culture of vitrified blastocysts are the most important factors for niPGT-A informativity, without correlation with blastocyst quality.

What is known already: niPGT-A is a technique that allows the non-invasive study of the chromosomal content of blastocysts. The informativity and concordance rates for fresh (Rubio, 2020) and frozen-thawed embryos (Garcia-Pascual, 2022) have been related with the time in culture and the day of media collection. However, there is a need to understand the optimal conditions needed to obtain optimal informativity for frozen-thawed vitrified blastocyst undergoing niPGT-A.

Study design, size, duration: Prospective study including 135 spent blastocyst media (SBM) and the corresponding blastocyst samples from thawed day-5 and day-6 blastocysts from PGT-A (73) and niPGT-A (62) cycles collected from January 2021 until March 2022. Blastocysts were discarded under IRB approval. The embryos were divided into 3 groups depending on the day of development and the time in culture after thawing: group-1, day-5 blastocysts cultured 8h; group-2, day-5 blastocysts cultured 24h and group-3, day-6 blastocysts cultured 8h.

Participants/materials, setting, methods: Media and blastocysts were obtained from IVF patients (29-42 years-old). After thawing, each blastocyst was washed to remove remaining cumulus cells and individually transferred to a 10 μL droplet of fresh media or to an EmbryoScope dish with 20 μL per well. SBM and blastocysts were collected, frozen and analysed by Next Generation Sequencing. Correlation between concordance rates (overall agreement between SBM and blastocyst samples), informativity, embryo quality and time in culture were evaluated.

Main results and the role of chance: All the embryos had a good embryo quality (BB or greater using Gardner Criteria) but different expansion degree. Informativity was significantly lower when day-5 embryos were cultured for only 8 hours (group 1), regardless of the degree of expansion (expansion degree 3, 28.57%; degree 4, 64.29% and degree 5-6, 57.14%, $p < 0.0005$). The concordance rates between SBM and whole blastocysts were very high and no significant different in relation to the expansion degree when comparing day-5 embryos cultured for 8 hours (group 1, 90.5%) with 24 hours (group 2, 93.6%) or with day-6 embryos cultured for 8 hours (group 3, 92.3%). The data suggest that the important factor impacting the informativity rate is the day of vitrification, and the time in culture after thawing, but not the expansion degree of the embryos. Regarding the concordance rates, there is no impact of the time in culture nor the expansion degree.

Limitations, reasons for caution: The main limitation of this study is that all the embryos analysed had fair or good quality but different expansion degrees.

Wider implications of the findings: The high concordance rates observed in frozen blastocysts opens the possibility of expanding the use of niPGT-A to frozen blastocyst without an impact of the expansion degree in the results.

Trial registration number: Not applicable

Abstract citation ID: dead093.1043
P-723 Pre-fertilization genetic testing to select healthy gametes in a mammalian Marfan syndrome model

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Study question: Is it possible to identify disease-free gametes before insemination to prevent vertical transmission of an autosomal dominant disease?

Summary answer: In a mammalian Marfan syndrome model, we successfully generated and identified healthy androgenotes to be utilized as male gametes to produce unaffected conceptuses.

What is known already: In patients with autosomal dominant genetic disorders such as Marfan syndrome, germline heterozygosity poses a risk to offspring health. For these patients, preimplantation genetic testing for monogenic disorders (PGT-M) allows us to select unaffected conceptuses for embryo replacement. However, PGT-M is inherently inefficient and can lead to embryo wastage. This has sparked interest in replicating the genome of individual gametes for (1) pre-fertilization genetic testing and (2) subsequent use as genotyped gamete substitutes during IVF.

Study design, size, duration: For this study, we selected 4 male B6 mice that were heterozygous for the *Fbn1*^{tm1Hcd} mutation and displayed classic manifestations of Marfan syndrome. Spermatozoa from these mice were used to inseminate enucleated wildtype mouse oocytes and generate haploid

androgenetic embryos for (1) *Fbn1*^{tm1Hcd} genotyping and (2) use as unaffected male gamete substitutes.

Participants/materials, setting, methods: Using tail clippings and embryo biopsy specimens, a *Fbn1*^{tm1Hcd} allele-specific PCR primer was developed and validated. Haploid androgenetic embryos were allowed to progress to the 8-cell stage. Subsequently, a single-haploid pseudo-blastomere was isolated for genotyping, while the remainder were individually grafted into activated recipient wildtype oocytes and fused using inactivated Sendai virus to generate biparental conceptuses. Resulting diploid conceptuses were cultured in a time-lapse incubator and then genotyped to confirm the healthy paternal haplotype.

Main results and the role of chance: A total of 175 wildtype B6 oocytes were enucleated yielding 169 ooplasts (96.6% survival rate). The ooplasts were inseminated with spermatozoa from mutant *Fbn1*^{tm1Hcd} mice, yielding 144 (85.2%) monopronucleated haploid androgenetic embryos. Of those, 100 (69.4%) progressed to the 8-cell stage. In 47 (47.0%) of them, genetic analysis by PGT-M on an individual pseudo-blastomere characterized the embryo as unaffected because it harbored the wildtype *Fbn1* allele. The remaining 7 haploid pseudo-blastomeres from 5 *Fbn1* wildtype androgenetic embryos were individually isolated and grafted into 35 recipient oocytes to generate 31 (88.6%) biparental conceptuses with the desired paternal haplotype. These achieved a 61.3% (19/31) blastocyst development rate after 96 hours, displaying normal embryo morphokinesis. A blastocyst biopsy was carried out ($n = 19$), confirming the unaffected paternal haplotype void of the *Fbn1*^{tm1Hcd} mutation.

Limitations, reasons for caution: Although this technique is feasible for pre-fertilization genetic testing, it needs to be reproduced with the same efficiency in humans. A major concern is related to the dynamics and roles of the sperm centrosome in the first mitotic division of human zygotes, which may be missing in the pseudo-gametes.

Wider implications of the findings: This approach to pre-fertilization genetic testing will benefit patients with heritable genetic conditions. In addition, the inherited decondensation of the male genome by the ooplasm may enable genome editing experiments to be performed.

Trial registration number: Not applicable

Abstract citation ID: dead093.1044

P-724 The choice of genetic service provider can have significant implications for the outcome of IVF cycles using preimplantation genetic testing for aneuploidy (PGT-A)

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Study question: Does the choice of PGT-A provider have any impact on clinical results, such as rates of aneuploidy, pregnancy and miscarriage?

Summary answer: The laboratory providing PGT can have a significant impact on various important clinical outcomes. PGT-A providers are not equivalent and should be chosen with care.

What is known already: A growing number of IVF cycles include PGT-A to assist in the identification of euploid embryos for transfer to the uterus. All modern PGT laboratories utilise a method called next generation sequencing (NGS) to predict the copy number of each chromosome in embryo biopsy samples. Given this technical convergence, it is often supposed that genetic results will be similar regardless which PGT laboratory is used, and consequently a clinic choosing a genetics provider need only consider convenience, quality of support and price. Here we consider whether the choice of PGT-A provider might have more profound affects, potentially impacting clinical results.

Study design, size, duration: A large network of IVF clinics switched from PGT-A provider 'A' to provider 'B'. The final 6 months of clinical data using provider A was compared to 6 months of data after the switch to B. There were no changes in any aspect of patient population, treatment protocols, or embryological practice occurred between the two time periods evaluated. Genetic results (aneuploidy/euploidy rates) and clinical outcomes (implantation, miscarriage, ongoing pregnancy/birth) were compared to identify any differences.

Participants/materials, setting, methods: Within the two time periods considered, 9,091 embryos underwent PGT-A with provider A and 10,281 using B. The average female age was 39.0 and 39.3, respectively. Embryos were biopsied at the blastocyst stage and the cells were shipped to the genetics laboratories. Provider A was in the same country as the IVF clinics, while provider B was located overseas. Both PGT-A laboratories used NGS, but employed different methods for DNA amplification, sequencing and data analysis.

Main results and the role of chance: A higher proportion of embryos were classified euploid by provider B in comparison to A (49.3% versus 39.8%; $p < 0.0001$). Differences in reported aneuploidy were particularly striking for younger patients (<38 years; 67.5% euploid according to B versus 52.5% reported by A; $p < 0.0001$). For patients over 37 years, the lower aneuploidy frequency reported by B resulted in fewer cycles with all embryos classified 'abnormal' and more cycles with a transfer (52.2% of 2115 cycles in this age group had a transfer, compared to 48.6% of 1915 cycles for A; $p = 0.02$). Having more embryos classified euploid also benefitted younger patients, providing opportunities to combine morphological selection with genetic evaluation. Improved selection may explain a higher ongoing pregnancy rate observed for patients <38 years (63.2% ongoing after the first embryo transfer for B versus 55.3% for A). Differing miscarriage rates were also observed (18.1% when using A versus 15.1% for B; $p = 0.047$). We speculate that higher rates of euploidy and pregnancy when using provider B might be a consequence of viable embryos being excluded due to incorrect classification as 'abnormal' by A. Additionally, failure to detect some aneuploidies, leading to transfer of abnormal embryos, might explain the higher miscarriage rate associated with A.

Limitations, reasons for caution: There were no detectable differences in the composition of patient populations receiving PGT-A from the two providers, and no apparent changes to protocols used during the two time periods assessed. Nevertheless, it must be acknowledged that the conclusions are based on a retrospective review of data, not a controlled study.

Wider implications of the findings: PGT-A and NGS are umbrella terms encompassing multiple methods with widely varying levels of validation and accuracy. Our results suggest that the choice of PGT-A provider has important implications for clinical results, affecting the number of transferrable embryos, pregnancy, and miscarriage rates. The observed differences likely reflect differing PGT-A accuracies.

Trial registration number: Not applicable

Abstract citation ID: dead093.1045

P-725 Role of Non-Invasive Preimplantation Genetic Testing-Aneuploidy (NIPGT-A) using spent culture media (SCM) and its concordance with Trophoectoderm (TE) biopsy: A Prospective Cohort Study

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Study question: What is the ploidy concordance rate between spent culture media (SCM) and trophectoderm biopsy (TE) using Next Generation Sequencing (NGS) and correlation with clinical outcome?

Summary answer: DNA could be isolated from SCM with successful NGS library sequencing. Ploidy and per-chromosome concordance rate of TE biopsy vs SCM was 68.38% and 85.13%.

What is known already: Many challenges are associated with TE biopsy, and the possibility of NiPGT-A using cell free DNA (cfDNA) from SCM is very intriguing, as it will be simpler, safer and cheaper alternative to the gold standard TE biopsy. The bottlenecks associated with TE biopsy like additional equipment and trained embryologist, can be substantially reduced. The disparities in reported results in various studies comparing SCM vs TE, could be attributed to culture media contamination (maternal, paternal or environment), different laboratory workflow methodologies used for embryo culture, different whole genome amplification methods, library sequencing methods and different algorithms for analysis.

Study design, size, duration: Prospective cohort study was conducted in tertiary care centre from August 2021-June 2022. Ethical Approval was taken from Institute Ethics Committee. Couples with female age $\geq$ 35 years, one or more implantation failures, male factor infertility requiring ICSI, opting for elective single euploid blastocyst transfer, were included in study. Forty four blastocysts were obtained from 14 patients who underwent IVF/ICSI cycles, gave consent for TE biopsy and SCM collection for PGT-A and NiPGT-A respectively.

Participants/materials, setting, methods: Individualised ovarian stimulation was carried out with GnRH antagonist protocol. ICSI was done for fertilization. Embryos were cultured in sequential medium. On day 3, after thorough washing embryo was cultured separately in 10µl micro-droplets. SCM was collected on day-5, before biopsy. DNA extraction, amplification and library preparation from TE biopsy and SCM was done using Veriseq PGS kit (Illumina) and sequencing was done on Illumina MiSeq system. Euploid embryo transfer was done in FET cycle

Main results and the role of chance: Out of 44 blastocysts, 31 were day 5 and 13 were day 6. WGA-DNA from TE biopsy and SCM was 100% successful. Average concentration of amplified DNA obtained from TE biopsy and SCM was 34.88 $\pm$ 9.56 ng/µl, 32.3 $\pm$ 6.84 ng/µl respectively. Quality control parameters for library preparation and sequencing for samples were as per recommended standards. CNV visualization and analysis was done using BlueFuse Multi-Software (Illumina). Ploidy concordance rate could be analysed in 26 embryos, which was 68.38%, per chromosome concordance was 85.13% and sex-chromosome concordance was 73.0%. The sensitivity, specificity, PPV, NPV and diagnostic accuracy of NiPGT-A was 66.6%, 60%, 87.5%, 30% and 65.38% respectively. On comparing day 5 and day 6 blastocysts, day 6 had better concordance rate 72.7% vs 60%, $p > 0.05$. On comparing good (>BB) and poor morphology (<BB)embryos, <BB had better concordance rate 83.33% vs 50%, $p = 0.16$. Per chromosome concordance rate was higher for <BB embryos, 90.5% vs 80.5%,$p = 0.001$. Full maternal cell contamination was suspected when TE was aneuploid/mosaic, XY and SCM was euploid, XX ($n = 4/14$), assessed only in XY embryos. Single euploid blastocyst transfer had 66.6%clinical pregnancy rate. One resulted in healthy live-birth, two on-going pregnancies, 30weeks and 12weeks period of gestation.

Limitations, reasons for caution: Before considering NiPGT-A in routine clinical practice, each embryology lab needs standardization, to implement necessary modifications in routine embryology protocol. Labs should ensure that embryo viability is not affected and should try to minimize chances of maternal or external contamination in SCM to reliably predict concordance rates with high confidence.

Wider implications of the findings: NiPGT-A is useful technique to assess ploidy but presently, NiPGT-A in combination with PGT-A, can help in prioritizing embryos according to their implantation potential. Further studies are required to find out whether NiPGT-A may serve as an alternative to PGT-A and implemented alone in routine clinical practice in future.

Trial registration number: CTRI/2021/04/033121

Abstract citation ID: dead093.1046
P-726 The impact of reporting low-grade mosaicism in PGT cycles according to the number of cycles with at least one embryo for transfer

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Study question: How did the unmasking of low-grade mosaic embryos affect the number of cycles with an embryo for transfer for patients undergoing preimplantation genetic testing?

Summary answer: 5.4% of PGT-A cycles that would have resulted in no embryos for transfer, had one low-grade mosaic embryo that could be considered for transfer.

What is known already: Before the publication of the last practice recommendations for managing mosaic embryos (2022), many fertility clinics requested masking mosaic results in PGT-A reports (mosaic embryos were reported as aneuploid). Recent publications revealed that low-range mosaic embryos had pregnancy rates, live birth rates and miscarriage rates comparable to euploid embryos (Capalbo et al, 2022). New recommendations suggested that it is not appropriate to discard low mosaic embryos, as they had a high chance of resulting in a healthy baby. Therefore, fertility clinics are now asking for the report of mosaic embryos and are developing their own procedures for mosaic embryo transfer.

Study design, size, duration: A cohort of 1696 blastocysts from 553 PGT-A cycles were retrospectively included. The data was collected from PGT-A cases analyzed in the same genetic laboratory between July and December 2022. PGT-A cycles were performed in 17 different fertility clinics from Argentina and all of them, since July 2022, began to request the report of mosaic embryos.

Participants/materials, setting, methods: Trophectoderm biopsies from 1696 day 5, 6 or 7 blastocysts were analyzed by NGS using Ion ReproSeq PGS kit, Ion Chef™, and Ion-S5 System (ThermoFisher Scientific). A customized algorithm was applied for the interpretation of PGT-A results. Mosaic embryos were reported as low mosaic (one or two chromosomes between 30-50% of copy number variation) or high mosaic (one or two chromosomes between 50-70%). Maternal age ranges from 22-44 years. Egg donor cycles were also included.

Main results and the role of chance: From the 1696 blastocysts analyzed, 1630 were informative (96.1%). 49.4% (805/1630) of embryos were euploid. This corresponded to 366 PGT-A cycles (66.2%) for which at least one euploid embryo was available for transfer. From the cycles with no euploid embryos (187/553, 33.8%), 30 patients (5.4%) have at least one low-mosaic embryo for transfer.

The new recommendations for mosaic embryo management made it possible to increase the number of PGT-A cycles with at least one embryo for transfer by 5.4% during this period of time. According to maternal age, these 30 cycles correspond to: 33.3% (10/30) women < 38 y/o and egg donation cycles, 46.7% (14/30) for women between 38-40, and 20% (6/30) for > 40 y/o. The impact was observed mainly in advanced maternal age. For this group of patients, where it could be more difficult to repeat an IVF cycle and obtain euploid embryos, the possibility of transferring a low mosaic embryo could be their only chance. These 30 PGT-A cycles would have resulted in no embryos for transfer if previous policies for masking mosaic embryos (requested by the clinic) would be applied. The possibility for low-mosaic embryo transfer must be then reviewed by the patient and the physician.

Limitations, reasons for caution: Molecular laboratories performing PGT must execute an in-house validation. Our validated algorithm reports low-grade mosaic embryos between 30-50%. However, other platforms could have been validated for different ranges and these results may not be comparable. Five affected pregnancies were reported after transferring mosaic embryos (Viotti, 2021), so caution is required.

Wider implications of the findings: Results from a short period of time (six months) were considered for this analysis and clinical data was not possible to assess. Further analysis is required to evaluate clinical benefits in patients that proceed to mosaic embryo transfers.

Trial registration number: Not applicable

Abstract citation ID: dead093.1047

P-728 Impact of calling mosaicism for a less stringent threshold on clinical pregnancies in more than 6000 PGT-A cycles

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Study question: How does calling mosaicism for a higher threshold affect euploidy, the chance of finding at least one euploid embryo and clinical pregnancy?

Summary answer: Increasing the threshold significantly increased euploidy percentage and the probability of finding at least one euploid embryo, as expected, but also the pregnancy outcomes.

What is known already: NGS technology has shed light on the occurrence of chromosomal mosaicism at the preimplantation stage of development. Evidence regarding clinical outcomes after transfer of embryos with putative mosaic results are accumulating (ESHRE Working Group on Chromosomal Mosaicism, 2022).

A series of studies reporting the birth of healthy babies after the transfer of embryos with a chromosomal mosaic result on PGT-A (Greco et al., 2015; Kahraman et al., 2020; Viotti et al., 2019 and 2021), have been published. Data suggested lower implantation rates and higher miscarriage rates when mosaics were compared with euploid embryo transfers.

Study design, size, duration: This retrospective study covers a period from January 2017 to October 2022 and includes 6063 PGT-A cycles. A total of 18425 blastocysts were biopsied and analyzed with NGS. From January 2017 to December 2020, the cut-off value for reporting mosaicism and euploidy was %20; the rate of which was increased to 30% after January 2021. Chromosomes with PGT-A profiles that deviate from 2 copies by less than 30% were reported as normal.

Participants/materials, setting, methods: A total number of 12751 and 5674 blastocysts were biopsied between January 2017-December 2020 and January 2021-October 2022, respectively. PGT-A was performed on Ion Torrent S5 (Thermo Fisher Scientific).

The mean female age for 20% and 30% cut-off groups were 36.7+-5.1, 35.9+-5.2, respectively. As this age difference was statistically significant, further analysis was performed in age subgroups $<=38$ and >38.

Main results and the role of chance: For PGT-A cycles with female age $<=38$, cycles with at least one euploid embryo in the 30% cut-off group increased from 74.0% to 77.5% whereas cycles with at least one mosaic embryo decreased from 40.8% to 37.0% ($p=0.0212$; $p=0.0274$). Cycles with at least one mosaic embryo without any euploid embryos decreased by 2% (9.2% to 7.2%) ($p=0.0522$). The euploidy percentage out of all tested biopsies was significantly increased from 40.4% to 45.8% ($p=0.0001$).

For cycles with female age >38, ratio of PGT-A cycles with at least one euploid embryo increased from 28.1% to 30.3% in the 30% cut-off group but this was not statistically significant ($p=0.28$). Cycles with at least one mosaic embryo were decreased from 15.1% to 10.8% and cycles with at least one mosaic embryo without any euploid embryo decreased from 9.5% to 5.6% in the 30% cut-off group ($p=0.006$; $p=0.0018$). The euploidy percentage was increased by 2.8% from 15.1% to 18.1% ($p=0.0115$).

The pregnancy outcomes were significantly higher for euploid embryos with the 30% cut-off compared to 20%, as clinical pregnancy was 64.5% vs. 59.1% and clinical pregnancy loss was 7.9% vs. 12.4% ($p=0.0022$; $p=0.0011$). Similarly, ongoing pregnancy was 5.2% higher, but non-significant.

Limitations, reasons for caution: These are the results of a single ART center which is using the same protocols for ovarian stimulation and endometrial preparation in the two periods above mentioned. Although not expected, minor changes may have occurred; similar practices have to be reported.

Wider implications of the findings: Mosaicism is undeniably a true biological phenomenon. However, trophectoderm biopsy and NGS are prone to create an artefactual false mosaicism.

Relaxing the mosaicism calling rate by 10% may have added some better-quality blastocysts to the euploid pool giving the opportunity to embryologists of transferring blastocysts with higher implantation potential.

Trial registration number: Not applicable

Abstract citation ID: dead093.1048

P-729 Factors affecting live birth rates in frozen-thawed single euploid blastocyst transfer cycles

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Study question: What are the factors affecting the rates of live birth in frozen-thawed euploid embryo transfer cycles?

Summary answer: Some demographic characteristics, frozen embryo transfer (FET) cycle parameters, and embryo characteristics of patients affect live birth rates in euploid embryo transfer cycles.

What is known already: The aim of assisted reproductive techniques (ART) is to achieve live birth as soon as possible. Preimplantation genetic testing (PGT) is an embryo selection technique recommended to shorten the live birth time. In addition, PGT reduces the number of failed ART cycles by eliminating embryos with chromosomal abnormalities that will not implant or will cause miscarriage. Despite these, the live birth rate after euploid embryo transfer cycles is still not at the expected level.

Study design, size, duration: This retrospective cohort study was conducted between January 2011 and May 2021 at the IVF and Reproductive Genetics Centre, Memorial Sisli Hospital, Istanbul, Turkey. The study involved a total of 2783 frozen-thawed euploid embryo transfer cycles.

Participants/materials, setting, methods: 2873 euploid FET cycles were analysed in the study to determine factors affecting live birth rates. 30 parameters thought to affect live birth were identified and analysed by logistic regression analysis on the effects of live birth. These parameters include patients' demographics, fresh cycle and FET cycle characteristics, and embryo-related parameters on live birth effects in euploid embryo transfer cycles.

Main results and the role of chance: 53% of 2783 euploid embryo transfer cycles have resulted in a live birth. The birth rate was 0,5 times less in the group with a body mass index (BMI) of higher than 29 compared to the group with a BMI of less than 25 ($p<0.05$). In cycles in which embryos of good-to-moderate-to-poor quality were transferred, the chance of live birth was lower than in cycles in which embryos of top quality were transferred (OR:0.837 OR:0.515 OR:0.528, respectively). The live birth rate was higher in cases where endometrial preparation was performed in natural cycles than in artificial cycles (OR:1.67, $p<0.05$). Embryo transfers without separate blastomeres had a higher live birth rate than embryo transfers with separate blastomeres (OR:1.416, $p<0.006$). The live birth rate was 0.7 times lower in the group that did not undergo embryo re-expansion 4 hours after thawing ($p<0.04$). Embryo transfer on the sixth day was associated with a lower live birth rate than embryo transfer on the fifth day. (OR:0.6, $p<0.02$). The group with a history of recurrent implantation failure (RIF) had a lower live birth rate than the group without a history of RIF (OR:0.5)

Limitations, reasons for caution: This study is a retrospective study.

Wider implications of the findings: In euploid embryo transfer cycles, clinicians and embryologists must know the parameters affecting live birth to guide treatment and select the embryo to be transferred first. In addition, the patient should be informed and guided about these factors.

Trial registration number: Not applicable

Abstract citation ID: dead093.1049

P-730 Detection of mitochondrial reversal following meiotic spindle transfer: a finding of importance for mitochondrial replacement therapies used for the purpose of avoiding mtDNA disease transmission

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Study question: Following transfer of the meiotic spindle from a patient's oocyte into an enucleated donor oocyte, do relative levels of patient and donor mtDNA remain stable?

Summary answer: mtDNA transferred along with the patient's spindle typically remain at a low level. However, disproportionate expansion of the patient's mtDNA can occur in some cases.

What is known already: Mitochondrial DNA disorders are caused by mutations in the mitochondrial genome, disrupting ATP production. They have few treatment options and no cure. All mitochondria are derived from the oocyte, therefore mtDNA disorders are maternally inherited. It has been proposed that disease transmission could be avoided if female mtDNA mutation carriers underwent meiotic spindle transfer (MST), removing the chromosomes (on the spindle) from an affected oocyte and transferring them into the healthy cytoplasm of a donor oocyte. However, research using embryonic stem cells has suggested that the small population of mitochondria transferred along with the spindle can sometimes undergo dramatic expansion.

Study design, size, duration: 25 infertile couples were enrolled in a prospective pilot study evaluating MST as a treatment for infertility. The female participants had a mean age of 37.2, and an average of 6.4 previous unsuccessful IVF cycles (range 3-11) characterised by extremely poor embryo development and without any previous pregnancy. Importantly, none of the patients were carriers of a mtDNA disorder. Oocyte donors with previous successful IVF outcomes were matched with patients according to standard practice.

Participants/materials, setting, methods: Metaphase-II-spindles from patient oocytes were transferred into enucleated donor oocytes. Reconstructed oocytes underwent ICSI and the resulting embryos were transferred at the blastocyst stage. Mitochondrial genomes were sequenced to identify polymorphisms differing between the patient and oocyte donor. These variations were quantified in the embryos (blastocyst biopsies), during pregnancy (amniocentesis), in newborns (cord blood, cord tissue, urine), at 3-6 months and at one year (saliva, urine, blood), revealing the relative amounts of each mitochondrial type.

Main results and the role of chance: This MST pilot study resulted in the birth of six children, indicating that the procedure is compatible with the production of viable embryos, capable of producing healthy live births. The patient's mtDNA was shown to represent <1% of the total in all blastocysts produced, confirming that MST is highly reproducible and that relatively few mitochondria are transferred along with the spindle. For five of the six children, the proportion of the total mtDNA attributable to the patient appeared to be stable, remaining at very low levels in all of the samples from later developmental stages. However, in one child the small quantity of mtDNA transferred along with the spindle increased disproportionately with respect to the mtDNA of the oocyte donor, ultimately representing 30-60% of the total at birth, depending on the tissue tested. The precise timing of the expansion of one type of mtDNA at the expense of the other is not known but occurred sometime between the blastocyst stage and birth. By the time of birth, the levels of donor and patient mtDNA appeared to have stabilised (no further increases were seen at 6 months and one year). All of the children born remain developmentally normal and healthy.

Limitations, reasons for caution: After using MST, several pregnancies were achieved for patients with a long history of unsuccessful IVF attempts, associated with poor oocyte quality and a failure to produce blastocysts. However, this small pilot study lacked the controls necessary for a definitive evaluation of MST as a tool for infertility treatment.

Wider implications of the findings: All children born following MST were healthy. However, our results clearly demonstrate that a substantial degree of mtDNA 'reversal' is possible. Consequently, mitochondrial replacement therapies used for avoidance of mtDNA disorders might not always be successful, even when initial levels of mutant mtDNA in reconstructed oocytes are very low.

Trial registration number: ISRCTN11455145

Abstract citation ID: dead093.1050

P-731 The accuracy of truly non-invasive PGT using spent culture media is insufficient to justify routine clinical use

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Study question: Can non-invasive PGT (niPGT), using a protocol that avoids manipulation of the embryo before spent culture medium is collected, accurately determine embryo chromosomal status?

Summary answer: Accuracy using a truly non-invasive PGT method was low, suggesting this strategy is not appropriate for clinical use and should be restricted to research studies.

What is known already: PGT requires embryo biopsy to obtain small numbers of cells for genetic testing. The procedure requires skilled personnel and specialist equipment, significantly adding to the cost of PGT while also creating training and logistical bottlenecks that reduce patient access to PGT. Additionally, there have been concerns that biopsy could damage embryos. These considerations have stimulated interest in potential non-invasive PGT strategies. Thus far, niPGT methods have mostly focused on cell-free embryonic DNA found in spent culture media, but studies reporting highest accuracy have tended to involve embryo manipulations likely to promote DNA release and therefore cannot be considered truly non-invasive.

Study design, size, duration: Samples of spent culture medium (SCM) associated with 128 embryos were collected. Oocytes had been denuded of cumulus cells, fertilised using ICSI and the resulting embryos thoroughly washed on day-4 before transfer to a fresh drop of medium. SCM samples were collected on day-5 or day-6 of culture. Embryos had not previously been cryopreserved and underwent minimal manipulation prior to SCM collection. Results of SCM analysis were compared to those obtained following conventional 'invasive' PGT.

Participants/materials, setting, methods: Media samples were subjected to whole genome amplification using the PG-Seq Rapid Non-Invasive PGT kit. Concentrations of sequencing libraries were measured, and next-generation sequencing (NGS) was undertaken. The resulting NGS data was analysed with PG-Find Software to predict chromosomal status. In parallel, trophectoderm biopsies from the corresponding embryos were analysed using a well-established and highly validated PGT method, based upon amplification of thousands of sites across the genome, followed by NGS.

Main results and the role of chance: Despite the implementation of rigorous measures to prevent contamination of SCM samples, extraneous female DNA, likely of cumulus cell origin, was frequently observed. Depending on the clinic, 16.7% to 38.1% of media samples associated with male embryos gave a discordant (female) result. This highlights the difficulty of avoiding inadvertent sampling of maternal DNA. There were no instances of male DNA contamination affecting SCM samples from female embryos. We assessed

whether clinic of origin, ploidy status of the embryo, and day of SCM collection influenced the quality of the sequencing libraries (ANOVA). Higher concentrations of libraries and superior sequencing quality scores were observed in SCM collected on day-6 compared to day-5 ($p = 0.011$ and $p = 0.002$, respectively). Chromosomal assessments obtained from SCM were compared to those from standard, trophectoderm-based PGT in a blinded fashion. 50% of SCM samples gave an identical predicted karyotype to their corresponding trophectoderm biopsy (concordant copy number for all chromosomes). When applying a simple aneuploid/euploid classification, 80% of embryos classified chromosomally abnormal using the validated PGT method were also aneuploid according to analysis of the associated SCM sample, but without necessarily having identical karyotypes, while 62% of embryos classified euploid by PGT received the same designation after SCM analysis.

Limitations, reasons for caution: SCM samples were designated 'contaminated' when embryos of one sex (determined using invasive PGT) were incorrectly classified as the opposite sex using niPGT. However, our study was unable to determine when SCM samples were contaminated with DNA of the same sex as the embryo. Consequently, contamination rates could be higher.

Wider implications of the findings: Most niPGT methods are not truly non-invasive, as they include embryo manipulations that increase likelihood of cell death and DNA release. Our results show that obtaining reliable niPGT results is difficult without deviating significantly from optimal embryological protocols. Currently, the accuracy of niPGT seems insufficient to justify routine clinical use.

Trial registration number: Not applicable

Abstract citation ID: dead093.1051

P-732 High degree of concordance between the genetic results of Noninvasive Preimplantation Genetic Testing for Aneuploidies (NIPGT-A) and those obtained with analysis of the whole embryo

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Study question: What is the degree of concordance between Noninvasive Preimplantation Genetic Testing for Aneuploidies (NIPGT-A) versus genetic analysis of the whole embryo?

Summary answer: Cell free DNA ploidy evaluated by NIPGT-A presents very good concordance that of the DNA from whole embryo cells (Cohen's Kappa concordance coefficient: 0.84).

What is known already: After many years of using PGT-A, there are still many concerns, such as risks of invasive action and difficulties in the correct interpretation of mosaicism, which may lead to errors in the interpretation of false-positive and false-negative results. Recently, a new technology (NIPGT-A) has arisen using cell-free DNA present in the spent culture media of human blastocysts. However, there are still few genetic analyses of concordance between NIPGT-A and the whole embryo (gold standard).

Study design, size, duration: This cohort study included a total of 56 blastocysts vitrified on Day-5 that were previously biopsied for PGT-A. All embryos had a diagnosis of aneuploidy and were donated under informed consent by patients following the Human Medical Authority regulations. Blastocysts were thawed and cultured in 15µl drops of culture medium under oil. After their expansion(4-8 hours), the blastocysts and their corresponding spent media were transferred to PCR tubes and stored at -80 °C until analysis.

Participants/materials, setting, methods: DNA in all samples(spent culture medium and whole embryo) was amplified by MALBAC® technology(Yikon Genomics). The DNA concentration of the amplified product was measured using a Qubit 3.0Fluorometer(Thermo Fisher Scientific). The samples were subjected to next-generation sequencing (NGS) using the Illumina MiSeq® system. The ploidy status results obtained from ChromGo™ software(Yikon Genomics) for spent culture medium and the whole embryo were compared to determine the accuracy of NIPGT-A for screening chromosomal abnormalities in each embryo.

Main results and the role of chance: DNA from all 56 spent medium samples and whole embryos were successfully amplified. Comparing the results of NIPGT-A and whole-embryo sequencing (Table 1), the positive predictive value (PPV) was 93.5%, and the false-positive rate (FPR) was 6.5%. Agreement analysis showed very good coefficient concordance (Cohen's Kappa coefficient: 0.84).

Table 1 NIPGT-A X Whole Embryo: results

NIPGT-A	Whole Embryo		
	Aneuploid	Euploid	Total
Aneuploid	43	3	46
Euploid	0	10	10
Total	43	13	56

PPV:93.5% FPR:6.5%
Kappa: 0.84

Limitations, reasons for caution: Although the sample size may be considered small, to the best of our knowledge this is the first comparative analysis between the results of NIPGT-A with whole embryos (gold standard) using the Cohen's Kappa coefficient of agreement.

Wider implications of the findings: NIPGT-A does not require micromanipulation skills, avoiding trophectoderm biopsy trauma, and seems to provide a very good concordance result corresponding to the ploidy status of the whole embryo. Therefore, NIPGT-A should be considered an adequate test for genetic evaluation of an embryo.

Trial registration number: Not applicable.

Abstract citation ID: dead093.1052

P-733 A non-selection study to evaluate non-invasive preimplantation genetic testing for aneuploidy

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Study question: How does non-invasive preimplantation genetic testing for aneuploidy (niPGT-A) perform to predict sustained implantation or live birth (SI/LB)?

Summary answer: Though not comparable to conventional PGT-A, niPGT-A can still be considered an appealing tool for clinical embryo prioritizing given its high negative predictive value (NPV).

What is known already: To omit the need for the invasive trophectoderm biopsy, niPGT-A which screens embryonic ploidy using embryonic cell free DNA released into the spent embryo culture medium (SEM) or blastocoel fluid (BF) was developed. Although the feasibility of niPGT-A has been shown and healthy live births have been reported from embryos screened by niPGT-A, the predictive value of niPGT-A for embryo transfer outcome has not been established yet and evidence is still lacking as to whether embryo selection by niPGT-A is associated with improved pregnancy outcomes. The answers to these are of vital importance to justify niPGT-A for clinical care.

Study design, size, duration: This is a single center, blinded, non-selection study consisting of single frozen embryo transfer cycles with a duration of two years, of which 117 cycles (Arm-1) were completed for 83 patients by transferring an embryo that was only morphologically assessed, and 90 cycles

(Arm-2) were completed for 67 patients by transferring an euploid embryo that was screened by conventional biopsy-based PGT-A.

Participants/materials, setting, methods: Embryos were cultured in 25 μL continuous culture medium until blastocyst stage. The "SEM+BF" samples were ongoingly collected and archived at the end of culture. In Arm-1, after pregnancy outcomes were determined, the corresponding "SEM+BF" samples were pulled out for niPGT-A. In Arm-2, niPGT-A was also performed for some cases that did not end up in an SI/LB. NiPGT-A was completed by NICS and the incorporated ChromGo platform (Yikon Genomics).

Main results and the role of chance: Out of the 117 "SEM+BF" samples in Arm-1, 110 (94%) yielded an informative niPGT-A result. Among these, 46 transferred embryos were determined as "euploid" by niPGT-A and 15 resulted in SI/LB; 32 were determined as "aneuploid" and 27 did not resulted in SI/LB. Therefore, the positive and negative predictive values (PPV and NPV) of niPGT-A were 32.6% (15/46) and 84.4% (27/32), respectively. Another 32 transferred embryos in Arm-1 were determined as "suspected mosaic" and they showed a similar rate of SI/LB per embryo transfer to those "euploid" ones (37.5% vs. 32.6%, P = 0. 8092). Compared to Arm-1 overall, the "transferrable" embryos ("euploid" + "suspected mosaic") determined by niPGT-A resulted in a much higher rate of SI/LB (34.6% vs. 27.6%, P = 0.3399). In Arm-2, 39 out of the 90 transferred euploid embryos resulted in SI/LB. "Euploid" embryos determined by PGT-A resulted in significantly better rate of SI/LB than "euploid" embryos determined by niPGT-A in Arm-1 (43.3% vs. 32.6%, P = 0.0243). Out of the 51 PGT-A "euploid" embryos that did not end up in SI/LB, 25 were also tested by niPGT-A with 6 being "suspected mosaic" and 3 being "abnormal/aneuploid", raising the concern of possible false negative of conventional PGT-A.

Limitations, reasons for caution: The study enrolled a non-selected patient population which resulted in the inclusion of a few patients with a history of repeated implantation failure. Therefore, the PPV of niPGT-A might be underestimated as implantation failure could be attributed to defects in endometrial receptivity rather than in embryo quality.

Wider implications of the findings: This is the first non-selection study to validate niPGT-A in the setting of continuous culture medium. The high informative rate indicates its feasibility for clinics using a similar culture system. It also forms the foundation of a prospective, randomized controlled trial to further assess the benefit of niPGT-A.

Trial registration number: Not applicable

Abstract citation ID: dead093.1053

P-734 Blastocysts derived from tripronuclear zygote including small pronuclear zygote have high diploid and euploid rate with NGS and SNP analysis.

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Study question: What is diploid and euploid frequency of blastocysts derived from tripronuclear zygote (3PN) including a small pronucleus (2.1PN) with NGS and SNP analysis?

Summary answer: Diploid rate of the blastocysts derived from 3PN including 2.1PN was 91.3%, in which euploid, aneuploid and mosaic embryos were 18.7%, 61.3% and 11.3%, respectively.

What is known already: A 3PN zygote is thought to be produced when two sperm enter into the same oocyte during in vitro fertilization (IVF) or whenthe second polar body is not extruded on both IVF and intracytoplasmic sperm injection (ICSI). It has been reported that 3PN is observed around 3-10%, and the embryos are usually discarded by the reason for genetic risks for triploid. However, some studies reported that blastocyst derived from 3PN and/or 2.1PN (defined as small PN < 20μm) contained normal diploid and euploid embryos by the preimplantation genetic testing, and that normal healthy babies were born from these embryos.

Study design, size, duration: We conducted a cross-sectional study between July 2019 and June 2022 in single reproduction center. We included 80 blastocysts in this study which generated from 3PN zygotes including 2.1PN zygotes. Of these, 71 blastocysts were from ICSI and 9 blastocysts were from IVF. These blastocysts were assessed for chromosomal ploidy by next generation sequencing (NGS) and single nucleotide polymorphism (SNP) analysis and embryonic development of these blastocysts were monitored by time-lapse imaging system.

Participants/materials, setting, methods: Average age of women was 39.2 years (22-47 years). The smallest pronuclear diameter who provided blastocysts were 12.7 ± 2.8μm (8.5-19.7μm) in average. The blastocysts were divided between inner cell mass (ICM) and trophectoderm (TE) using laser system under inverted microscope, followed by NGS and SNP. The t-test was performed to analyze differences between triploid and diploid. Statistical significance was defined as $P < 0.05$. All participants provided written informed consent, and Institutional Review Board approval was obtained.

Main results and the role of chance: Diploid rate of the blastocysts derived from 3PN including 2.1PN was 91.3%, in which euploid, aneuploid and mosaic embryos were 18.7%, 61.3% and 11.3%, respectively. Of 80 blastocysts, 64 blastocysts (80%) were able to be divided ICM from TE. In terms of triploid and diploid, the results of ICM and TE were completely concordant, although 15.6% discordancy was observed between ICM and TE. Average ages of the patients with triploid, euploid, aneuploid, and mosaic embryos were 41.1 ± 5.1 years, 36.0 ± 6.3 years, 40.6 ± 3.5 years, and 35.2 ± 3.6 years, respectively. The patients' age with aneuploid embryos was significantly higher than those with euploid and mosaic embryos (P = 0.015 and $P < 0.001$, respectively). Mean diameter of the smallest pronucleus of triploids and diploids were 15.2 ± 4.1μm (8.5-19.7μm) and 12.7 ± 2.8μm (8.5-18.9μm), respectively. Of diploid embryos, euploid, aneuploid, and mosaic were comparable in mean diameter of the smallest pronucleus. There were no differences in insemination methods (IVF and ICSI), frequency of abnormal blastomere division, embryonic developmental speed, and blastocyst grade, between triploid and diploid embryos.

Limitations, reasons for caution: Since this was a cross-sectional study by cytogenetics analysis of blastocysts, causal association between incidence of 3PN (including 2.1PN) and postnatal findings and significance of extra micro pronucleus are unknown. We need to assess longitudinal-study with large sample size of embryo transfer with PGT-A.

Wider implications of the findings: We revealed that blastocyst derived from 3PN including 2.1PN had high diploid and euploid rate by NGS and SNP analysis. Therefore, 3PN including 2.1PN embryos could be used for embryo transfer after chromosome ploidy analyses.

Trial registration number: Not applicable

Abstract citation ID: dead093.1054

P-735 Are there any differences in preimplantation blastocyst chromosomal abnormalities between polycystic ovary syndrome patients and controls? A multi-center retrospective cohort study

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Study question: Are there possible differences in the preimplantation blastocyst chromosome aberrations between polycystic ovary syndrome (PCOS) patients and controls?

Summary answer: PCOS lowers aneuploidy risk but increases mosaicism risk in preimplantation embryos, and PCOS-related insulin resistance should be investigated as a potential cause.

What is known already: PCOS is thought to alter granulosa cell functions and effects on oocyte development, potentially leading to chromosomal abnormalities and increasing the risk of pregnancy loss. However, few studies

have compared the chromosomal status of preimplantation embryos from PCOS patients to those from healthy controls.

Study design, size, duration: This was a multi-center retrospective cohort study conducted at 3 IVF centers. A total of 707 blastocysts from 147 PCOS patients and 3006 blastocysts from 821 control women receiving preimplantation genetic testing (PGT) between 2015 and 2021.

Participants/materials, setting, methods: PGT was performed using next-generation sequencing (NGS). Differences in the chromosome aberrations were compared. Multivariate and subgroup analyses were conducted to control for confounders. Influences of insulin resistance and PCOS phenotype on the risks of specific chromosomal abnormalities were also examined.

Main results and the role of chance: Compared to controls, blastocysts from PCOS patients demonstrated lower aneuploidy rate (14.7% vs. 25.4%, $P < 0.001$) but greater mosaicism rate (16.5% vs. 8.7%, $P < 0.001$). Multivariable analysis adjusting for age, IVF center, body mass index (BMI), total gonadotropin (Gn) dose, and peak serum estradiol (E2) on trigger day, identified PCOS as an independent protective factor against embryonic aneuploidy (adjusted OR = 0.56, 95% CI, 0.42–0.76, $P<0.001$) but a risk factor for embryonic mosaicism (adjusted OR = 2.05, 95% CI 1.48–2.85, $P<0.001$). These differences in chromosomal spectrum were also observed in subgroups stratified by age, IVF center and BMI. Further multivariate analysis suggested that insulin resistance could be responsible for the increased risk of embryonic mosaicism among PCOS patients (OR = 1.89, 95% CI, 1.06–3.34, $P=0.03$).

Limitations, reasons for caution: The main limitation is the retrospective design. Besides, methodological uncertainties still exist in detecting mosaicism in current reproductive medicine. A longer-term, multiple-center prospective study with greater methodological standardization is warranted to confirm the present results.

Wider implications of the findings: These results suggest that PCOS pathology may increase the risk of mitosis errors but not the risk of meiosis errors during oocyte development, and PCOS-related insulin resistance should be investigated as a potential cause.

Trial registration number: 2020[325]

Abstract citation ID: dead093.1055

P-736 Balanced cryptic chromosomal rearrangement carriers detection by optical genome mapping (OGM)

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Study question: To evaluate diagnosis value of optical genome mapping (OGM) for potential preimplantation genetic testing in chromosomal structural rearrangement (PGT-SR) patients with cryptic chromosomal rearrangement.

Summary answer: OGM is an efficient method for cryptic chromosomal rearrangement detection. However, SVs near telomere and centromere regions could hardly be reported with this method.

What is known already: About 5∼10% infertility or sterility people carry balanced chromosomal structural variations (SVs) with high risks of miscarriage and birth defects resulting from imbalanced gametes when producing fetus. However, some balanced cryptic SVs with specific location or small fragments cannot be detected by traditional cytogenetic techniques like G-banding testing or CNV-seq. Currently, several optimized techniques are able to detect typically cryptic structural abnormality. OGM, the molecular genetic detection technique relying on new principle in recent years, have overcome the shortcomings of former techniques and are potentially capable to detect both routine and cryptic SVs well.

Study design, size, duration: From 2019 to 2022, 12 couples in The First Affiliated Hospital of Sun Yat-sen University and The First People's Hospital of Yunnan Province with history of offspring birth defect, recurrent unexplained recurrent miscarriage or unexpected copy number variation in embryos from previous PGT cycles were included for diagnosis or re-analysis.

Participants/materials, setting, methods: Peripheral blood lymphocytes from these patients were collected and detected by OGM or traditional karyotype analysis(G-400 banding), FISH, CNV-seq, third generation sequencing(Nanopore). The results of traditional genetic test and OGM were compared to evaluate the accuracy of OGM detection of chromosomal structure vatiation.

Main results and the role of chance: In the 11 couples bearing adverse pregnancy or childbirth defect with normal karyotype by G-banding, OGM discovered novel chromosome translocations or inversion in 9 couples (81.81%). In 1 couple with t(4:17)(q12;p13) diagnosed by G-banding, OGM detect an additional chromosomal translocation of t(17;19)(q12;p13), which produced the repeated CNVs in chromosome19 observed in embryos from two previous PGT cycles. For small fragment translocation, terminal translocation and inversion, OGM is able to detected balanced structure abnormal of 500kbs. The OGM diagnosis were confirmed by FISH or third generation sequencing (Nanopore) or embryo mutation. The breaking position reported by OGM and Nanopore were comparable. In sum, OGM reported further information of balanced SVs in 83.33% (10/12) potential patients with duplication and depletion in embryos or abortus.

Limitations, reasons for caution: Due to the limitation of principle, the structural abnormalities of some special locations (centromere, secondary constriction, etc.) cannot be detected. OGM has high sensitivity and many mutations, which requires examiners to have strong ability to analyze results and genetic counseling. The price of OGM also limits the promotion and application.

Wider implications of the findings: OGM can provide more precious result for SVs, and PGT-SR can definitely improve the outcome of certain population with cryptic chromosomal rearrangements. The shortcoming of OGM has not been overcame yet, so it is suggested as auxiliary method beside conventional G-binding in certain circumstances in ART population.

Trial registration number: Not applicable

Abstract citation ID: dead093.1056

P-737 Progress in non-invasive embryo assessment with cell-free nucleic acids in blastocoel fluid

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Study question: Can cell-free nucleic acids (cfDNA) from blastocoel fluid (BF) after a trophectoderm (TE) biopsy be used as a non-invasive sample type to assess embryos?

Summary answer: Both BF and spent media carry comparable, highly fragmented cfDNA and cell-free RNA (cfRNA) which may reveal more about the quality of the embryo.

What is known already: Preimplantation genetic testing for aneuploidy (PGT-A) is commonly used by IVF clinics to screen embryos for any chromosomal abnormalities during the IVF process. Noninvasive preimplantation genetic testing (ni-PGT) is an emerging category of tools within the field of assisted reproductive technology. Currently, niPGT products have been developed for embryo aneuploidy detection using cfDNA released by the embryo into the spent media. Embryo spent media (ESM) carries cfDNA, which has the potential to be applied to evaluate the quality of the embryo. Previous studies have demonstrated that cfDNA in ESM is highly fragmented to the nucleosome level.

Study design, size, duration: The quality and quantity of cell free nucleic acid from forty BF-conditioned media (BFCM) samples were analyzed in this study. Embgenix™ Analysis Software was used to analyze and interpret sequencing data. For each sample, the concentration and fragmentation of

cfDNA were examined and compared using generalized linear mixed models. To assess RNA quality at a picogram level, reverse-transcribed and amplified cDNA was used to represent the quality of cfRNA in BFCM.

Participants/materials, setting, methods: Embryos were washed and placed in individual biopsy media droplets for TE biopsy. After biopsy, blastocysts were collapsed, releasing BF into the droplet. The entire droplet was collected as BFCM. The Embgenix ESM Screen Kit was used to prepare sequencing libraries. The SMART-Seq® Single Cell Kit was used to evaluate the RNA quality. The BFCM cfDNA results were also compared to spent media profile that had already been obtained from a different cohort of embryos.

Main results and the role of chance: Amounts of cfDNA fragmentation detected in BFCM samples was comparable to ESM. Whole-genome amplification (WGA) yields correlated to the amount of cfDNA input but not the degree of cfDNA fragmentation. Informative sequencing reads and computed copy number variations (CNV) plot noise, as evaluated by distribution of log2 ratio spread (DLRS), also showed correlation and stable performance down to ~2 pg of cfDNA input. We extracted detectable cfRNA from BFCM and created cDNA for RNA quality analysis. Our model demonstrates a significant correlation between cDNA length and RNA quality metrics, with a low DV200 of cfRNA from BFCM samples.

The quantity and quality of cfDNA released into spent culture media by a developing embryo are comparable to the cfDNA released into BFCM upon TE biopsy, indicating that BFCM has potential as a noninvasive method for assessing the ploidy status of embryos. WGA yield, informative reads, and DLRS of BFCM samples were likewise comparable to data previously acquired using spent media samples. However, the severely fragmented cfRNA in these samples illustrate the inherent instability of RNA and the difficulty of utilizing RNA analysis to determine embryo health. Assays compatible with small RNA or microRNA (miRNA) are more appropriate for RNA-seq of BFCM.

Limitations, reasons for caution: Different clinics use varying methods for embryo culture and biopsy; the data reported here is from a single research center. The characteristic of samples may vary between clinics. Our assay estimates RNA quality using amplified cDNA; nonetheless, discrepancies between fragmented RNA and amplified cDNA may exist.

Wider implications of the findings: In PGT-A, BFCM samples are routinely discarded. Our findings imply BFCM samples contain considerable levels of cell-free nucleic acids which could also be used to screen embryo quality. BFCM samples, therefore, could be analyzed in conjunction with PGT-A to select and/or rank embryos for implantation.

Trial registration number: Not applicable

Abstract citation ID: dead093.1057
P-738 Attitude of BRCA1/2 mutation carriers towards fertility preservation, family planning and preimplantation genetic testing (PGT) for the next-generation primary prevention of breast and ovarian cancer

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Study question: What is BRCA1/2 mutation carriers' attitude toward preimplantation genetic testing (PGT) in attempt to prevent their offspring burden that comes with a known mutation status?

Summary answer: Less than ten percent of BRCA1/2 mutation carriers chose to perform PGT to avoid the birth of a child who is also a carrier.

What is known already: BRCA1/2 mutation carriers encounter many dilemmas during their life such as whether to undergo fertility preservation, when and how to plan their family, when to undergo risk reduction surgeries and whether to perform PGT for the selection of non-carrier embryos. Awareness and attitudes toward PGT are different across countries and has been shown to be associated with the degree of suffering from the knowledge of being a carrier, from personal or familial BRCA1/2 associated breast and ovarian cancer.

Study design, size, duration: This cross-sectional study was conducted by the distribution of an anonymous questionnaire in BRCA1/2 carriers social media platforms and clinics from August 2022 to January 2023.

Participants/materials, setting, methods: Female respondents of the questionnaire with a positive germline BRCA1/2 mutation at any age or marital status.

Main results and the role of chance: The questionnaire was completed by 494 BRCA1/2 mutation carriers. The median age of responders was 43 years old (range 22-79), 90% of them Ashkenazi (full or partial) Jewish ethnicy. 63% of patients were carriers of the BRCA1 mutation, while 37% were carriers of the BRCA2 mutation. 76% of patients became aware of their mutation status following a family history of malignancy. 35% of responders did not have children at the time of mutation discovery. 15% of patients had a fertility issue regardless of their BRCA mutation status. Following mutation discovery, 38% of responders changed their family planning, mostly choosing to have children earlier in life or to have less children than planned. 28% of BRCA carriers have been discussed about their option for fertility preservation and 11% underwent the oocyte or embryo vitrification. 45% of BRCA carriers admitted they have discussed the option of PGT and only 8% underwent the in vitro fertilization and preimplantation genetic testing to select non- carrier embryos. 14% of responders were firmly against the option of PGT. The need for fertility treatment regardless of the mutation status did not significantly change the PGT performance rates ($p = 0.09$).

Limitations, reasons for caution: This study's limitations mostly stem from online distribution of the questionnaire. Responders' bias may cause an overestimation of treatment rates as responders may be those who encounter their physicians more frequently and maintain their follow-up as recommended. Nevertheless, the study represents 494 BRCA1/2 carriers and portrays an important healthcare view.

Wider implications of the findings: Physicians should discuss PGT, family planning and fertility preservation options with BRCA1/2 carriers enabling a personal based decision. It seems that among BRCA carriers, known mutation status largely affects family planning and to a much lesser degree affects fertility preservation and performance of PGT to select non carrier offsprings.

Trial registration number: Not applicable

Abstract citation ID: dead093.1058
P-739 High-throughput Single-cell Survey Reveal a Pathological Niche in Developing Down Syndrome Testis

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Study question: What are the characteristics of developing testes in DS, and how do these alterations affect gamete development and androgen production?

Summary answer: Molecular alterations start accumulating in every cell-type of DS fetal testis. Different cell components, compromised functions, and deviated cellular communications make up a pathological niche.

What is known already: DS is the most common genetic chromosomal disorder. The flat or slightly decreased live birth and the significantly prolonged lifespan contribute to a growing DS population. Their needs for reproductive care come into view. Reproductive health is crucial for spermatogenesis, male characteristics, and even brain behaviors. DS males experience normal puberty, but their sex hormones and associated gonadotropins have altered since the infant, implying testis niche cell dysfunction. Male infertility may result from germ cell and/or niche cell malfunction. A single-cell resolution survey could help thoroughly uncover the heterogeneity of DS by cell type.

Study design, size, duration: We applied high-throughput single-cell RNA sequencing to capture the transcriptomes of DS testis (n = 1). In parallel, testis collected from the fetus reported no genetic disorders were set as control (n = 1). Molecular and histological verifications were conducted with independent samples (3 versus 3). Samples enrolled were around 22 weeks of gestation.

Participants/materials, setting, methods: With ethics approval and informed consent obtainment, DS and control samples were included. DS fetus was confirmed with amniocentesis, while no genetic abnormality was reported during the prenatal examination of the diploid fetus. Single-cell RNA sequencing was conducted with the Micro-well platform, pseudotime trajectory analysis, cellular interaction analysis, and weighted gene co-expression network analysis were performed. Transmission electron microscopy, immunohistochemistry, Westerns, and qPCR were used to verify the main differences.

Main results and the role of chance: A total of 15349 cell transcriptomes, DS and control pooled, were compared and clustered. Major cell types were unabridged in both karyotypes. DS testis contains more Sertoli cells with an atypical expression profile, and more immune cells. DS cells exhibit a much higher level of ribosomal protein (RP genes and a significantlylower level of marker genes for each cell type, such as *INSL3* for DS Leydig cell and *PECAM1* for DS endothelial cell, etc.

Corresponding to cell proportion difference, gene co-expression network and intercellular communication analysis also suggested an immune activation in DS.

Furthermore, pseudotime analysis suggested a relative retardation and malfunction of DS cells.

The main differences in gene expression were further verified. Cell proportion alteration was demonstrated with immunohistochemistry. Overwhelming RPs were shown by transmission electron microscopy. Western and qPCR were used to test the expression difference of RPs, cell-type specific functional genes, etc.

Limitations, reasons for caution: The sample size was limited, although verification experiments were applied with independent samples might increase the credibility. The sample size needs to be extended further. More precise molecular mechanisms of these alterations and clinical dysfunction remains to be elucidate.

Wider implications of the findings: This study described human fetal DS testis at single-cell level. It reveals a pathological niche of altered cell components, compromised cell function, and activated immune response. It provides further targets for complement and/or intervention to rescue the reproductive function and even improve brain behavior in DS patients, soon after birth.

Trial registration number: Not applicable

Abstract citation ID: dead093.1059
P-740 Monogenic and digenic variants in male PLCZ1, ACTL7A and ACTL9 genes cause fertilization failure after ICSI

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Study question: What is the frequency of *PLCZ1, ACTL7A* and *ACTL9* variants in male patients suffering from fertilization failure (FF) after ICSI?

Summary answer: Male patients with FF after ICSI exhibit variants in *PLCZ1* (27,3%), *ACTL7A* (13,6%) and *ACTL9* (4,5%). Some patients present *PLCZ1* variants combined with *ACTL7A/ACTL9* variants.

What is known already: FF after ICSI has been often attributed to phospholipase C zeta (PLCζ) deficiency, and consequently to insufficient calcium release, necessary for fertilization. *PLCZ1* genetic variants have been detected in one-third of males with FF. Recently, actin-like 7A (*ACTL7A*) and 9 (*ACTL9*) variants were also identified, but larger cohort studies are lacking. *ACTL7A/ACTL9* variants alter the acrosome structure, resulting in reduced and abnormally localized PLCζ. Assisted oocyte activation (AOA), by calcium ionophore, restores fertilization in patients with *PLCZ1* variants, and could also benefit patients with *ACTL7A/ACTL9* defects. This study aimed to expand the spectrum of known *PLCZ1, ACTL7A* and *ACTL9* variants.

Study design, size, duration: A prospective study was conducted from June 2020 to December 2022 including 22 male patients (P1-P22) with FF after ICSI. Patients were only recruited when they had a mean fertilization rate ≤33,33% in at least 1 ICSI cycle. All patients donated a saliva sample for genetic screening of *PLCZ1, ACTL7A* and *ACTL9* genes and a sperm sample for the mouse oocyte activating test (MOAT). All couples with FF after ICSI were offered an AOA treatment.

Participants/materials, setting, methods: Genetic screening was performed via next-generation sequencing. Identified variants were classified employing Varsome and confirmed by bidirectional Sanger sequencing. Patients in which uncertain significance (VUS), likely pathogenic or pathogenic variants were found underwent additional diagnostic tests, such as the study of the sperm calcium releasing pattern in mouse (MOCA) and in *in vitro* matured (IVM) human (HOCA) oocytes, immunostaining of PLCζ and ACTL7A, and the analysis of the sperm morphology by transmission electron microscopy (TEM).

Main results and the role of chance: Genetic screening revealed heterozygous *PLCZ1* variants, a novel variant p.Ile379Thr in P10 and the previously published variant p.His233Leu in three patients (P9, P19 and P20). In addition, two novel homozygous *ACTL7A* variants were detected, p.Ser364GlnfsTer9 in P1 and p.Gly214Ser in P22. Interestingly, digenic variants were observed in P6 (*PLCZ1* p.Ile74Thr and *ACTL7A* p.Tyr183His) and in P8 (*PLCZ1* p.His233Leu and *ACTL9* p.Arg271Pro).

All patients with identified variants showed a high oocyte activation rate (>70%) after MOAT, except P1 and P22 which activation rate was <25%, indicating a more detrimental sperm-related deficiency. While MOCA only detected deficient calcium release in P1 (and not for P6, P8, P9 and P10), HOCA revealed absence of calcium oscillations in all patients analyzed (P1, P8, P9 and P10), except for P6. Immunostaining experiments in P1 revealed no ACTL7A signal and decreased PLCζ signal in patient sperm. Moreover, TEM analysis in P1 confirmed acrosome detachment from nuclear membrane.

Overall, AOA increased fertilization rate (from 7,23% to 53,04%) and pregnancy rate (from 7,14% to 46,15%) in patients with identified variants. Specifically, patients with monogenic *PLCZ1* (P9 and P19) and *ACTL7A* (P1) variants, as well as digenic variants (P6 and P8) achieved a live birth after AOA.

Limitations, reasons for caution: Some patients have not undergone all diagnostic tests yet, thus functional data on all the found variants identified is still ongoing. In addition, patient recruitment for this study is continuing. Genetic screening was carried on exonic and flanking intronic regions, which might have missed additional variants in other intronic regions.

Wider implications of the findings: The MOAT/MOCA tests cannot detect some subtle sperm-related activation deficiencies. HOCA represents a more sensitive analysis but requires human oocytes. Therefore, genetic screening of *PLCZ1, ACTL7A* and *ACLTL9* could be a faster and more cost-efficient diagnostic test for FF after ICSI. Furthermore, AOA treatment is very efficient for these patients.

Trial registration number: NA

Abstract citation ID: dead093.1060
P-741 Morphokinetic Goldilocks: assessing the morphokinetic range to identify embryos with the optimal chance of being euploid

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Study question: Can CHLOE-EQ, an AI embryo assessment support tool, automatically identify the optimal time-range of morphokinetic events where chance of euploidy is maximized?

Summary answer: Embryos that are within the normal morphokinetic range have increased chances of being euploid compared to embryos developing at a pace outside the optimal range.

What is known already: The introduction of time-lapse technologies in IVF has revealed quantitative and qualitative morphokinetic parameters that predict embryo viability (ESHRE Workshop group, 2020), but their assessment is time-consuming and subjective. Artificial Intelligence (AI) based tools, such as CHLOE-EQ (Fairtility), are ideally suited to automatically annotate morphokinetics as part of a range of tools to quantify embryo quality and detect abnormalities. There have been several attempts in the literature to predict ploidy with morphokinetics. We postulated that embryos that develop at a normal pace (not too fast and not too slow) would be more likely to be euploid.

Study design, size, duration: Retrospective case-controlled study of 1328 time-lapse videos collected in 2022 from IVF and ICSI embryos from a private single fertility clinic. 142 of those were biopsied and genetically tested by NGS. The embryos were automatically assessed by CHLOE-EQ (Fairtility), an AI embryologist support tool.

Participants/materials, setting, methods: Time-lapse videos were automatically annotated using CHLOE-EQ(Fairtility) for morphokinetics, number of pronucleates and anomalies. The frequency distribution for each morphokinetic parameter was compared between euploid and aneuploid embryos to establish ranges for optimal euploidy rate. The ranges between optimal (maximum euploidy rate) and sub-optimal (outside optimal range) were compared (t-test). Efficacy of blastocyst, utilisation and ploidy prediction by CHLOE blast score at 68hpi and CHLOE-EQ score were assessed using the area under the curve (AUC).

Main results and the role of chance: For each morphokinetic event, an optimal range for identification of euploids was identified (**tPNf**:21.37-25.78; **t2**:24.01-28.6; **t3**:34.07-39.20; **t4**:35.5-40.64; **t5**:46.12-53.92; **t6**:48.77-55.63; **t7**:50.22-57.45; **t8:**52-60.21; **t9:**67.35-75.55; **tM:**78.49-89.08; **tsB**:92.20-102.39; **tB:**99.54-109.83; **tEB**:106.42-120.38). Optimal range of euploid embryos was smaller than the total range for all embryos ($p < 0.001$): **tPNf** (0.27 vs 152.36), **t2**(5.52 vs 158.96), **t3**(22.7 vs 159.29), **t4**(30.38 vs 167.96), **t5**(32.02 vs 168.29), **t6**(35.58 vs 155.44), **t7**(41.04 vs 157.65), **t8**(41.37 vs 158.06), **t9**(48.85 vs 158.39), **tM**(56.4 vs 163.89), **tSB**(84.74 vs 173.26), **tB**(93.01 vs 168.62); **tEB**(95.96 vs 164). Embryos with optimal ranges across morphokinetic events had a higher euploidy rate than embryos with suboptimal ranges [50% (11/20), 35.35% (35/99), NS].

CHLOE-BLAST Score at 68hpi was predictive of blastulation (AUC=0.86), whilst CHLOE-EQ Score was predictive of utilisation (AUC 0.88) and euploidy (AUC=0.64) and CHLOE Ranking was predictive of utilisation (AUC=0.91) and selection for transfer (AUC=0.80).

Limitations, reasons for caution: This is a single-center, retrospective study, where only the blastocysts deemed suitable for biopsy were assessed for ploidy. Therefore, the ploidy rate of non-blastocysts or inferior quality embryos is unknown, creating a potential bias regarding the lower cutoff threshold for optimal ranges.

Wider implications of the findings: CHLOE-EQ can identify the optimal morphokinetic time range to maximise the chance of an embryo being euploid, a potentially valuable biomarker for embryo assessment, selection, managing patients expectations down to individual embryos, and helping reduce the chance of viable embryos being discarded.

Trial registration number: Not applicable

Abstract citation ID: dead093.1061

P-744 Reporting chromosomal mosaicism reduces the overall accuracy of preimplantation genetic testing for aneuploidies: results from the extended in vitro culture of 230 human embryos

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Study question: What is the accuracy of reporting chromosomal mosaicism in the context of next generation sequencing (NGS)-based preimplantation genetic testing for aneuploidies (PGT-A)?

Summary answer: Extended in vitro culture of human embryos demonstrated that reporting mosaicism increased the risk of false positives, reducing the overall accuracy of PGT-A to 70%.

What is known already: PGT-A has been introduced into IVF practice to improve clinical outcomes. However, diagnostic accuracy remains controversial, particularly regarding chromosomal mosaicism. While NGS allows the detection and reporting of mosaicism from a trophectoderm (TE) biopsy, the inability to discern biological variability from technical artefacts continues to limit the interpretation of PGT-A results. As reports of mosaicism often have a major impact on patient management, achieving an appropriate level of standardization will be vital to avoid overestimation. Extended in vitro culture of human embryos up to 12 days post fertilization (D12) delivers an important, clinically relevant platform for the validation of PGT-A.

Study design, size, duration: A total of 230 clinically unsuitable blastocysts were included in the study. Embryos were selected based on their original diagnosis following PGT-A as: uniformly abnormal (n = 158), aneuploid and mosaic (n = 21), mosaic only (n = 36) and euploid (n = 15) but carrying a monogenic disease mutation in homozygosis. Blastocysts were originally classified as mosaic if they contained 30-70% abnormal cells, with <50% mosaicism defined as low level and >50% considered high level mosaicism.

Participants/materials, setting, methods: Vitrified blastocysts were warmed and cultured to D12 using established protocols to generate embryo outgrowths. A total of 90 outgrowths were selected for NGS. Outgrowths were collected whole (n = 64) or separated into 2-4 portions (n = 26), which were then analyzed individually. We correlated the original PGT-A diagnoses to developmental outcomes and the chromosomal status of the embryo outgrowths. Fisher's Exact test (two-sided) was used for evaluating associations. All p-values <0.05 were considered significant.

Main results and the role of chance: Following extended culture, 49.1% (113/230) of embryos remained viable and attached, while 50.9% (117/230) degenerated and detached. As previously observed in our model, euploid blastocysts and embryos diagnosed with trisomies, duplications or chromosomal mosaicism were significantly more likely to attach throughout the extended culture, while monosomies, deletions and complex chromosomal constitutions impaired in vitro development (total attached, 78.3% vs. 24.8%, $p < 0.0001$). When compared to the original biopsy, we established 100% concordance when reporting euploidy and uniform whole chromosome aneuploidies, demonstrating an overall high sensitivity of PGT-A. Of the blastocysts diagnosed as mosaic only, 80.6% (29/36) remained viable throughout the extended culture. These were sequenced either whole (n = 11) or in parts (n = 18). At D12, 69.0% (20/29) of these embryos were uniformly euploid, while 27.6% (8/29) were uniformly aneuploid across all outgrowth samples. Notably, the latter were all originally diagnosed as high level mosaics. One embryo (3.4%) revealed a true mosaic configuration, with evidence of a reciprocal event across the different outgrowth samples. Ultimately, the proportion of embryos accurately diagnosed as euploid was 25.0% (8/35), with an overall false positive error rate of 77.1%. False positives were largely attributed to the diagnosis of mosaicism, 85.2% (23/27) or segmental aberrations, 14.8% (4/27).

Limitations, reasons for caution: This study was performed using a single PGT-A assay and cannot be extrapolated to other platforms. To evaluate the predictive value of chromosomal mosaicism, we selected embryos based on their chromosomal profiles. This may underestimate the accuracy of PGT-A, as sampling of embryos was not performed randomly.

Wider implications of the findings: We confirm the high sensitivity of PGT-A, as euploidy and uniform whole chromosome abnormalities were detected with high accuracy. However, predictive value is considerably reduced when diagnosing mosaicism. Given the high false positive rate, reporting mosaicism remains difficult to justify, as potentially viable embryos continue to be excluded for transfer.

Trial registration number: NA

Abstract citation ID: dead093.1062
P-745 Follicular fluid exposure may activate gene expression in mature human sperm before fertilization

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Study question: Does follicular fluid (FF) exposure shape human sperm RNA content and are these changes acquired through genomic or nongenomic manner?

Summary answer: FF exposure altered sperm RNA content potentially via activation of sperm RNA transcription machinery and caused selective intake of FF RNA transcripts in sperm.

What is known already: Mature sperm have been widely recognized as transcriptionally inert. Therefore, sperm RNAs have been regarded as the sole remnants of the gene expression process that occurs during spermatogenesis. Supporting this view, most sperm histones are replaced by protamines in developing spermatids to enable tighter packaging of the genetic material, which should prevent mature sperm cells to express their genes. However, in humans, approximately 15% of the sperm DNA remains associated with histones, raising the possibility that these genome regions could be available for *de novo* RNA transcription under an appropriate stimulus.

Study design, size, duration: Fresh, mature sperm were divided into three aliquots. One aliquot was used as an untreated control sample, the second and third aliquots were treated with either PureSperm® Wash (SW) solution or an FF pool of five females for three hours. RNA sequencing (RNA-seq) data was collected from FF and sperm samples. Results were complemented with Chromatin Immunoprecipitation Sequencing (ChIP-seq) data from human mature sperm (GEO: GSE15690 and GSE49624) and nascent RNA capture followed by PCR.

Participants/materials, setting, methods: All male and female participants were of reproductive age, non-smoking, healthy individuals who reported no history of infertility. Differentially expressed genes (DEG) were identified based on RNA-seq data (adjusted p-value < 0.05, logFC $< |0.75|$). The histone status of the genomic loci of DEGs was evaluated using ChIP-seq data. DEGs were also subjected to Gene Ontology analysis. Activated RNA transcription was confirmed by the Click-iT™ Nascent RNA Capture kit and PCR.

Main results and the role of chance: RNA-seq showed 193 DEGs between untreated and FF-treated samples of which 30 genes were upregulated and 163 were downregulated. Furthermore, 18 DEGs were detected between SW- and FF-treated sperm of which 17 genes were upregulated and one downregulated. Finally, 137 DEGs (all downregulated) were found between untreated and SW-treated samples. Among upregulated genes, chemokine-signaling, ion transport, and steroidogenesis-related biological functions were enriched whereas RNA processing, translation, and protein targeting-related biological processes were enriched among downregulated genes (untreated vs. FF-treated). ChIP-seq data revealed that 93% of upregulated DEGs are located within histone-bound chromatin and most of them bear both active and inactive histone markers. Finally, we confirmed that the upregulation of at least two genes (*CXCL3* and *CXCL8*) resulted from active RNA transcription by sperm, whereas the upregulation of *IGF2* was due to the selective internalization of mRNA from FF. Additionally, we identified 1396 protein-coding mRNAs present in human FF that were associated with gene expression-related biological processes such as the regulation of transcription, processing of RNA subtypes, and translation.

Limitations, reasons for caution: Due to the limited sample size, the generalizability of our results should be validated in the future. Furthermore, molecular mechanisms behind observed nascent RNA production needs to be clarified in more detail, including potential FF-mediated remodeling of sperm histone markers involved in the activation of gene expression.

Wider implications of the findings: Our findings demonstrate that FF exposure changes sperm RNA content, which seems to be partly caused by the active transcription of mature sperm, which has been widely believed to be non-existent. Therefore, our results can have many important implications for a deeper understanding of fertilization, early embryogenesis, and infertility.

Trial registration number: Not applicable

Abstract citation ID: dead093.1063
P-746 Carrier Screening analysis in more than 20.000 patients. Is complete analysis important vs genotyping analysis?

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Study question: How comprehensive gene sequencing analysis impacts on carrier screening (CS) results and couple risk reduction?

Summary answer: Comprehensive genetic sequencing analysis facilitates more accurate carrier rates and provides better information for final panel design and partner risk reduction.

What is known already: Since CS began in the 1970s, it has helped millions of couples at risk of affected offspring affected by genetic diseases.From the first panels for Ashkenazi Jewish to today's pan-ethnic CS panels, genetic testing has improved from analysis of a few known pathogenic variants to full sequence analysis. The American College of Medical Genetics and Genomics (ACMG) published Standards and Guidelines for the Interpretation of Sequence Variants in 2015, providing an important tool for full sequence analysis for CS. CS analyses can report only already known mutations (genotyping) or screen for all variants with ACMG-guidelines and laboratory experience (full sequencing).

Study design, size, duration: This study includes 20774 patients screened with an expanded carrier screening panel of 301 genes, including autosomal recessive (AR) and X-linked (XL) disorders by next-generation sequencing (NGS). ACMG guidelines for variant interpretation were followed in 13954 patients and only pathogenic and likely pathogenic variants were reported. 6820 patients were analyzed with genotyping analysis. 15758 matching analyses were performed for couples and gamete donors. The study has been conducted between August 2018 and December 2022.

Participants/materials, setting, methods: DNA was extracted from saliva and blood samples and sequenced. The data obtained were processed by means of computer tools. The carrier frequency (CFR) was obtained from the full sequencing analyses. The observed CFRs was compared with the expected CFRs obtained from databases and literature.To define its similarity a range of +-25% was accepted as similar CFR. For matching analyses, high at-risk couples were divided for autosomal recessive diseases(both carriers) and X-linked diseases (female carriers).

Main results and the role of chance: For the 301 genes analyzed, 34%(103) had a similar CF than the expected one and, more importantly, 65%(198) showed different CFR. In some cases this difference is due to the lack of complete information on the CFR on bibliography and databases like the CFR associated a diseases with more than one gene associated or genes related with more than one disease, some of which with low clinical implications, usually underdiagnosed. One of the genes on which the CFR was not similar to the expected was *SMN1*. For this well-known gene, related to spinal muscular atrophy, the CFR was higher than expected, probably due to the analysis of the variants c.*3+80T>G(p.?),c.*211_*212del(p.?) that indicate a

higher risk of 0 + 2 configuration. The patients with these variants and 2 *SMN1*-exon8 copies are at higher risk to be silent carriers, but they may not be carriers.

For the 15758 performed, if one or both patients were analyzed by genotyping analysis, 3,6% (432/12055 matchings) identified had high risk of affected offspring for autosomal or X-Linked recessive diseases. When both patients were analyzed by sequencing, 6% (223/3703 matchings) were identified at high risk of affected offspring of the diseases analyzed allowing a higher identification of couples at risk.

Limitations, reasons for caution: The analysis was performed on 301 genes, in order to have a better understanding of CFR for panel designs or patients genetic counseling, a larger cohort of genes should be taken into account, these results show the importance of complete analysis to assess the carrier risks.

Wider implications of the findings: It is important to have wider genetic databases for CFR but also for variant pathogenicity in order to have better results on CFR analyses and panel designs, in order to provide proper genetic counseling to patients looking to improve the chances of healthy offspring.

Trial registration number: Not applicable

Abstract citation ID: dead093.1064

P-747 Chromosomal mosaic characteristics among women under IVF treatment: multicentric study of 2282 mosaic embryos diagnosed by preimplantation genetic testing with trophectoderm biopsy

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Study question: Which chromosomal mosaic features do women under IVF treatment show?

Summary answer: Single aneuploidies were the most represented among mosaic embryos and their frequency as well as the level of mosaicism increased in older women.

What is known already: Chromosomal mosaic embryos are characterised by the presence of chromosomally different cell lines within the same embryo. We previously demonstrated that reproductive potential of mosaic embryos is affected by the complexity of and the number of aneuploid cells present in trophectoderm (TE) biopsy. Although with the introduction of next generation sequencing (NGS) chromosomal constitution of human embryos have been elucidated, only limited number of mosaic have been characterised. Therefore, we performed a larger-scale multicenter study on mosaic embryos to examine the patterns and prevalence of chromosome specific mosaicisms in TE samples.

Study design, size, duration: This is a retrospective cohort study from May 2019 to May 2021 of 20200 embryos obtained from 5770 women under IVF treatment. From this cohort, 2280 mosaic embryos were analysed. All embryos were cultured to blastocyst stage; TE biopsy was performed on Day-5 of development or on Day-6/7 for slow growing embryos.

Participants/materials, setting, methods: TE biopsies underwent comprehensive chromosome screening (CCS) utilizing validated NGS. TE biopsies were classified as mosaic if they had 20%-80% of abnormal cells. For statistical analysis, embryos were divided in: single whole-chromosome trisomy, double whole-chromosome trisomies, single whole-chromosome monosomy, double whole-chromosome monosomies and complex aneuploidy (more than two different aneuploidies) groups. In addition, the frequency of single, double and complex segmental aneuploidies was evaluated. For each group, the trend related to maternal age was examined.

Main results and the role of chance: Among 2282 mosaic embryos single (whole-chromosome and segmental) aneuploidies were the most represented(52%) followed by complex(36%) and double ones(14%). When embryos were divided based on different types of aneuploidies, complex aneuploidies were the most frequent(34%) followed by segmental(31%), single monosomies(14%), single trisomy(12%). The more affected chromosomes within trisomies were 16(7%), 19 (7%), 21 and 22(6%). Monosomy involved mainly chromosomes 7(8%), 21 and 22(7%). Chromosomes 1, 5(9%), and 9(8%) were the most targeted by segmental duplication while segmental deletion mostly involved chromosomes 1(11%), 2(13%) and X(8%). The frequency of single aneuploidies as well as the percentage of mosaicism increased in older women.

Limitations, reasons for caution: This study was retrospective and observational, demonstrating the relative frequency of mosaicisms but not offering any direct insight into the clinical relevance of the findings. More clinical data must be obtained before this approach can be evaluated as an additional tool to choose mosaic embryos for the transfer.

Wider implications of the findings: This study provides a large dataset of mosaic embryos and offers detailed information on the distribution of different types of mosaicism among a general population of women under IVF treatment.

Trial registration number: Not applicable

POSTER VIEWING
REPRODUCTIVE SURGERY

Abstract citation ID: dead093.1065

P-749 Increased accuracy in infertility workup by the additional use of new modern diagnostic methods ("diagnostic TRIO"): results from a large retrospective analysis

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Study question: Is there any positive effect of adding new modalities to the traditional infertility work-up, like 3D sonography, office hysteroscopy and endometrial biopsy?

Summary answer: During infertility workup conventional diagnostic tests should be combined with new approaches (*3D-TVS*, *OHSC* and *endometrial biopsy) to achieve* more accurate and less invasive diagnostics.

What is known already: Female infertility can be explained by functional or organic abnormalities affecting the reproductive system. The most common organic causes include abnormalities within the uterine cavity (e.g. endometrial polyp, submucosal fibroid, intrauterine adhesion), morphological disorders (e.g. dysmorphic uterus, uterine septum) and abnormalities of the endometrium (e.g. chronic endometritis).

Study design, size, duration: In our retrospective study, we examined patients with primary and secondary infertility. All the patients were assessed by *3D-TVS* and *OHSC* to detect morphological and intrauterine disorders, and in special cases (repeated implantation failure – RIF), *endometrial biopsies* were carried out to detect endometrial disorders.

Participants/materials, setting, methods: Data of 606 patients examined between 2018 and 2022 were analyzed retrospectively.

Main results and the role of chance: In 606 cases 3D-TVS and OHSC were performed in patients (65.51 % primary, 34.49 % secondary infertility), who had unknown reason to infertility. By the combination of 3D-TVS and

OHSC we could verify uterine disorders in 39.93 % of cases. Together these two diagnostic methods found the probable infertility causing lesion almost 40 % of our patients. In the subgroup of 40 repeated implantation failure (RIF) patients, the "diagnostic TRIO" confirmed a disorder in 57.50 % of the cases.

Limitations, reasons for caution: Number of patients should be more to conclude more accurate data.

Wider implications of the findings: The proper condition of the uterine milieu, through correction of uterine cavity if necessary, endometrial receptivity are of paramount importance for the success of the treatment; as a result, intrauterine surgery as a new subspecialty has been developing.

Trial registration number: DE RKEB/IKEB: H.0250-2020

Abstract citation ID: dead093.1066
P-750 A systematic review of vasovasostomy (VV) and vasoepididymostomy (VE) techniques for Vasectomy Reversal: How far have we come?

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Study question: A systematic review of current literature evaluating different techniques in performing vasovasostomy (VV) and vasoepididymostomy (VE) including robotic-assisted and minimally invasive techniques and their outcomes

Summary answer: Macrosurgical, microsurgical and robot-assisted techniques have evidence for use in vasectomy reversal. The use of robotic assistance in may have benefit over standard techniques.

What is known already: While vasectomy is a common procedure worldwide, six percent of these patients request a reversal procedure. Reasons for this include change of mind, a new relationship, death of a child or wanting more children. While sperm harvest combined with in-vitro fertilisation (IVF) is an alternative, factors including timing of patency and durability of patency need to be considered prior to deciding between vasectomy reversal or sperm harvesting with IVF. In 1977, microsurgical techniques for vasovasostomy were described by Silber and Owen, and microsurgical vasectomy reversal procedures became more common following this. The evidence for differing approaches is lacking.

Study design, size, duration: A systematic literature review was performed using databases Medline and PubMed was performed, comprising studies between 1979 to July 30 2022. Systematic review and review articles of vasovasostomy and vasoepididymostomy were included. Original studies on robotic-assisted techniques and minimally invasive approaches were also included. Exclusion criteria included animal studies and non-English. Level of evidence was evaluated.

Participants/materials, setting, methods: From the eligible articles identified from the above criteria, data was extracted from review articles including techniques used for vasovasostomy and vasoepididymostomy. Surgical outcomes including pregnancy rates and postoperative patency were considered surgical outcomes and data was extracted regarding this from above articles. Data from original studies on minimally invasive techniques were also extracted from the above articles.

Main results and the role of chance: For vasovasostomy, techniques described in the literature include microscopic vasovasostomy, mini-incision microscopic vasovasostomy (MVV), mini incision MVV using Moon's clamp, and robotic assisted vasovasostomy.

Described by surgeons as the "most technically challenging procedure", the early approach to vasoepididymostomy comprised a 3-4cm scrotal incision for delivery of testis to provide adequate exposure to the entire epididymis and vas deferens.

When vasoepididymostomy was compared to vasovasostomy, prolonged recovery periods, increased pain and swelling were identified.

The techniques described for vasovasostomy comprise end-to end, end-to side, three-suture triangulation intussusception (TIVE), two-suture longitudinal intussusception (LIVE), deferential vessel-sparing LIVE, mini-incision vasoepididymostomy, robot-assisted vasoepididymostomy (RAVE).

Retrospective reviews of microsurgical vasovasostomy using a 2-layer anastomosis report patency rate of 85%, similar to reports from single layer repairs. Microsurgical techniques allow accurate apposition of narrow lumen ends, allowing unobstructed semen flow and low level evidence suggests high patency rates. Macrosurgical vasovasostomy may be more simple, less expensive and a quicker alternative. Elzanaty et al. found no clear difference between microsurgical and microsurgical vasovasostomy in terms of vasal patency. Both techniques appeared to be effective when performed by an experienced surgeon. Preliminary evidence for robotic assistance suggests a role in reduced morbidity and accuracy of repair.

Limitations, reasons for caution: With low level evidence for each technique, formal clinical guidelines are not forthcoming. Further evaluation and longer follow-up is required to assess clinical usage and true cost-benefit ratio. There is also a need for a standardised measurement tool to provide accurate and objective outcome measures to compare techniques.

Wider implications of the findings: This systematic review provides evidence for available techniques in vasovasostomy and vasoepididymostomy for vasectomy reversal. Outcome measurement tools are presented, highlighting the lack of standardised definitions of patency and failure. The current review enables surgeons performing vasectomy reversal to determine the most effective, efficient and cost-effective technique for their practice.

Trial registration number: 'Not applicable'

Abstract citation ID: dead093.1067
P-752 Cervical cerclage versus vaginal progesterone in the prevention of preterm birth in singleton pregnancies among women with a short cervical length: A retrospective cohort study

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Study question: To compare the maternal and neonatal effects of cervical cerclage and vaginal progesterone in preventing singleton preterm births in women with a short cervical length.

Summary answer: Cervical cerclage showed no benefit in preventing preterm birth; however, it prolonged gestation by 39 days compared to vaginal progesterone treatment.

What is known already: Clinically, there may be a bias toward the choice of cervical cerclage if the cervix is short (<10 mm) and, similarly, toward vaginal progesterone use if the cervix is long (>30 mm). However, there are no clear treatment guidelines for the best options for singleton pregnant women with a CL between 10 and 30 mm. Furthermore, there is insufficient evidence to determine whether one treatment is superior to the other in preventing PTB and decreasing adverse neonatal outcomes in singleton pregnancies in such cases.

Study design, size, duration: Asymptomatic pregnant women having singleton pregnancies, with a CL between 10 and 30 mm, measured using transvaginal ultrasound at 12–26 weeks of gestation, who delivered at our hospital between January 2009 and August 2020, were included in our study. Based on the inclusion and exclusion criteria, 267 singleton pregnant women, of whom 116 were treated with cervical cerclage and 151 with vaginal progesterone, were finally included.

Participants/materials, setting, methods: Independent sample *t*-tests and differential analysis were used to analyze the data based on their distribution. The Cox semi-parametric regression model was used to analyze the gestation weeks at delivery and neonatal outcomes. The decision regarding cesarean delivery was based on a binary logistic regression model.

Main results and the role of chance: In the initial analysis, the number of preterm births was significantly higher in the cerclage group than in the vaginal progesterone group; however, after multivariate adjustment for confounding factors, the latency period from diagnosis to delivery was found to be significantly prolonged in the cerclage group.

Limitations, reasons for caution: There are currently no uniform criteria for determining whether study subjects are eligible for cervical cerclage or

vaginal progesterone treatment, which may present some cases of noncompliance.

Wider implications of the findings: In our study, we found that cervical cerclage did not have significant advantages over vaginal progesterone treatment in preventing PTB. However, upon adjustment for confounding factors, it was shown to prolong the gestational age by 39 days compared to vaginal progesterone treatment.

Trial registration number: non-clinical trials

Abstract citation ID: dead093.1068

P-753 Preferences in subfertile women for hysterosalpingography or transvaginal hydrolaparoscopy in the fertility work-up: a labelled discrete choice experiment.

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Study question: What aspects do subfertile women prefer for hysterosalpingography (HSG) or transvaginal hydrolaparoscopy (THL) to assess their Fallopian tubes?

Summary answer: The chance of a false negative result, failure rate, and waiting time are attributes that impact women's preference for tubal patency testing strategy.

What is known already: THL and HSG are commonly used diagnostic tests for tubal patency testing, both with different features. HSG is a radiological procedure whit contrast to evaluate tubal patency, whereas THL is a procedure where access to the pouch of Douglas is obtained with an endoscope and the tubes are tested with methylene blue. THL was found non-inferior to HSG as a first-line test in terms of conception leading to live birth.

Until now, limited research has been performed to study the values and preferences of subfertile women in the diagnostic work-up.

Study design, size, duration: We conducted a labelled discrete choice experiment (DCE), which is an attribute-based survey method for measuring preferences. This DCE consisted of 2 questionnaires with each 12 different choice sets. Women with an indication for tubal patency testing were included in the study between September 2021 and January 2023 in two Dutch hospitals. They were randomly assigned for questionnaire 1 or 2.

Participants/materials, setting, methods: Attributes were defined based on literature review, structured patient interviews and expert focus groups. This resulted in five final attributes: the chance of having a "false negative" result, complication rate, failure rate, subsequent management after a failed procedure and waiting time. Women were asked to choose between choice sets with hypothetical scenarios of two tubal patency tests with different levels of the attributes. Data were analysed by using multinomial logistic regression.

Main results and the role of chance: The questionnaire was returned by 75 out of 80 women. Mean age was 31.8 years and mean duration of subfertility was 23.5 months. 65 women experienced primary subfertility vs 10 who experienced secondary subfertility.

For THL women preferred a lower chance of a false negative result ($p < 0.001$), a lower failure rate ($p = 0.008$) and a shorter waiting time ($p = 0.003$). Outcomes for chance of complications and management after a failed procedure were not significant. Only for the group of women with primary subfertility, if a THL is not conclusive or if there is a failure to reach the pouch of Douglas, women prefer to have a conventional laparoscopy over expectant management ($p = 0.01$).

Women choosing HSG preferred a lower chance of a false negative result ($p < 0.001$), a shorter waiting time ($p = 0.02$) and a lower chance of complications ($p = 0.001$).

Because of the labelled design we performed, we saw that women chose for THL significantly more than for HSG; 83% vs 17% ($P = 0.03$). This may be caused by way of counselling and the fact that these medical centres are one of the few centres in the Netherlands that perform the THL.

Limitations, reasons for caution: Our analysis with 78 women, showed significant outcomes in terms of preference on specific attributes, but it is possible that we would have found stronger associations with a larger sample size.

Wider implications of the findings: These results provide more insight in the perspective of subfertile women about the aspects of tubal patency testing in the fertility work-up and they enable informed decision making. Further research is needed to compare our findings to the other forms of tubal patency testing in the fertility work-up.

Trial registration number: N20.009

Abstract citation ID: dead093.1069

P-754 Chronic poor healing wounds of post cesarean scar diverticulum: altered angiogenesis and immunobiology

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Study question: To detect the influences of cesarean section scar diverticula (CSD) on the pregnancy outcomes in ART and explore the mechanisms.

Summary answer: In the CSD group, the cumulative live birth rate (CLBR) was significantly reduced, accompanied by excessive proliferation of fibroblasts and expansion of vascular proliferation.

What is known already: Diverticulum can reduce the chances of embryo implantation and may lead to spontaneous miscarriages if the implantation is close to or in the CSD. The accumulation of bloody fluid can be detected in approximately 42% of women with large CSD. The trapped blood is slowly released and could affect cervical mucus, sperm transport, and implantation. The previously data have revealed that disrupted uterus microbiota is closely associated with local inflammatory cytokines which could also be a clue for impaired fecundity in CSD.

Study design, size, duration: This retrospective cohort study was conducted using data from a single, large university-affiliated fertility center. Data was retrospective analyzed among women underwent ART procedure from May 2010 to March 2021. Follow-up basic experiments to explore relevant mechanisms.

Participants/materials, setting, methods: Data was retrospective analyzed among 31375 women underwent ART procedure in the Sixth affiliated Hospital of Sun Yat-sen University. To optimize the precision of the study, patients in the CSD group and the NCS group were matched using propensity score matching method. Perform hysteroscopy and obtain cervical swab and endometrial tissue of lower segment. To explore the difference of uterine cavity environment between CSD group and vaginal birth (VB) group by histopathological and immunological analysis.

Main results and the role of chance: The CS group had significantly lower CLBR compared to NCS group. Histopathological analysis showed that the higher prevalent of chronic endometritis is accompanied by excessive fibroblast proliferation at the lower segment of uterus and significantly exaggerated vascular proliferation in situ. Intrauterine inflammatory cytokines including IL-1α, IL-1β, IL-6, IL-8, TNF-α and SDF-1α were also increased in CSD group.

Limitations, reasons for caution: The main limitation of our study is its retrospective nature and single center, which might have underestimated the total number of women with CSD and could lead to a bias.

Wider implications of the findings: Lay emphasis on anti-infective therapy, modulate the imbalance of reproductive tract flora and enhance early lesion healing post-operation are of great necessity. Future studies exploring the preventive and therapeutic strategies of CSD should focus on immune inflammatory response and angiogenesis.

Trial registration number: Not applicable

Abstract citation ID: dead093.1070

P-756 Change of anti-Müllerian hormone (AMH) value for ovarian reserve after minimal invasive benign ovarian cystectomy: Da Vinci robotic system (Xi and SP) and laparoscopic system.

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Study question: To investigate impact on ovarian reserve after minimal invasive ovarian cystectomy using two platforms; Da Vinci robotic system (Xi and SP) and laparoscopic system.

Summary answer: Benign ovarian cystectomy using Da Vinci robotic system takes a long time, but it is an effective minimally invasive method to preserve ovarian function.

What is known already: With the development of minimally invasive surgical methods such as laparoscopic and robotic system, patient satisfaction has increased not only in terms of pain relief but also cosmetic aspects such as smaller scars. Protection of ovarian function during surgery is important in terms of fertility preservation, and this should be considered first in minimally invasive surgery. Serum anti-Müllerian hormone (AMH) is a widely used index to evaluate ovarian reserve.

Study design, size, duration: This study included patients who underwent laparoscopic or Da Vinci robotic (Xi or SP) ovarian cystectomy for benign ovarian cysts between January 1, 2018 and September 30, 2022 at a single institution. A retrospective study was conducted through electronic medical chart review.

Participants/materials, setting, methods: A total of 128 patients were enrolled. Among them, 71 patients underwent laparoscopic surgery and 58 patients underwent robotic surgery. The preoperative AMH value was determined as the value within 4 weeks before surgery, and the postoperative AMH value was determined as the value from 1 month after surgery to within 1 year after surgery. The AMH change value (ΔAMH) was expressed as a percentage value; (postAMH – preAMH) x 100 / preAMH

Main results and the role of chance: There was no significant difference in preoperative age, BMI, parity, cyst size, and cyst position ratio. Estimated blood loss during operation, Hb drop, length of hospital day, adhesion detachment rate, and cyst rupture rate also showed no difference. However, the operation time was significantly shorter in the laparoscopic group. (68.51 ± 30.99 minutes vs. 105.17 ± 38.87 minutes, $p < 0.01$)

The mean preoperative AMH was significantly higher in Da Vinci robotic system than laparoscopic system. (5.89 ± 4.81 ng/mL vs. 4.02 ± 3.61 ng/mL, $p = 0.02$) The mean postoperative AMH was also higher in Da Vinci robotic system. (4.31 ± 3.34 ng/mL vs. 3.02 ± 2.64 ng/mL, $p = 0.02$) But, the mean ΔAMH was not significantly different between two groups. (-19.55 ± 40.67 % in laparoscopic system vs. -19.95 ± 38.79 % in robotic system, $p = 0.96$) When the robot groups were divided into Xi system ($N = 21$) and SP system ($N = 37$) and compared, but ΔAMH did not show significant differences among the three groups. (-19.55 ± 40.67 % in laparoscopic system vs. -14.63 ± 47.80 % in Xi system vs. -22.97 ± 32.97 % in SP system, $p = 0.75$)

Even in the patient group with preoperative AMH below 2, ΔAMH was -9.50 ± 57.58 % in the laparoscopic system ($N = 20$) and -11.72 ± 60.92 % in the robotic system ($N = 11$), showing no significant difference between the two groups. ($p = 0.92$)

Limitations, reasons for caution: A limitation of this study is that the measurement period of AMH was set within a wide range within 1 year after surgery. In addition, the small sample size when divided into two systems, SP and Xi, is also a limitation.

Wider implications of the findings: Compared to the existing laparoscopic system, the robotic system does not show a significant difference in protection of the ovarian reserve, so it will be widely selected as an option for minimally invasive surgery.

Trial registration number: Not applicable

POSTER VIEWING

SAFETY AND QUALITY IN ART

Abstract citation ID: dead093.1071

P-758 Impact of serum estradiol levels prior to progesterone administration in artificial frozen embryo transfer cycles:a retrospective study over 26,000 cycles

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Study question: To investigate whether serum estradiol (E2) levels prior to progesterone administration in the FET-cycle affect the Clinical pregnancy rate (CPR) and live birth rate (LBR).

Summary answer: High serum E2 levels prior to progesterone administration in Artificial endometrial preparation are associated with a decreased live birth rate and clinical pregnancy rate .

What is known already: Although the use of FET has progressively increased, it is still unknown whether a specific endometrial preparation protocol should be favored over another. Owing to their minimal need for clinic visits and a larger scheduling flexibility, artificial cycles for FET are widely used. Additional to the ultrasonographic endometrial thickness check prior to FET, the need for endocrine monitoring in artificial cycles remains unclear. Specifically, the value of proliferative phase serum hormone measurements remains a point of discussion.

Study design, size, duration: A total of 26,194 artificial FETs performed in a Tertiary-care academic medical centre between 2016 and 2021 were subdivided into 3 groups according to the following late-proliferative serum E2 level percentiles: ≤p33.3 (E2 ≤118 pg/ml; n = 8784), p33.4–p66.6 (E2 from 118.1 to 231.9 pg/ml; n = 8662) and ≥p66.7 (E2 ≥232 pg/ml; n = 8748).

Participants/materials, setting, methods: A retrospective study over 26,194 cycles,from 2016 to 2021 were included.Univariable and multivariable logistic regression analysis was performed.

Main results and the role of chance: In 26,194 cycles, there were significant differences in CPR and LBR among the tri-sectional quantiles groups with E2 levels. After stratifying the analysis by age, BMI, infertility diagnosis, type of embryo transferred, number of transferred embryos, infertility factors, method of fertilization, and endometrial thickness on transfer day, there were still significant differences in CPR and LBR among the tri-sectional quantiles group with E2 levels. Multivariate regression analysis showed that female age, thin endometrium, high serum E2 level, tubal factor infertility were risk factors for CPR and LBR, ICSI fertilization, blastocyst transfer, the number of transferred embryos, ovulation disorder infertility and male factor infertility were protective factors for CPR and LBR, BMI was only a risk factor for LBR.

Limitations, reasons for caution: •Different routes of administration affect serum estrogen levels,and the relationship of serum estrogen levels and endometrial estrogen concentrations is unclear

Wider implications of the findings: High miscarriage rate following FET has been a point of debate in literature.Future research should definitely focus on the the usefulness of serum E2 evaluation to optimize the success rate.

Trial registration number: Not applicable

Abstract citation ID: dead093.1072

P-759 Discard, or not discard, that is the question. An international survey among 117 embryologists about their decision on 50 poor-quality/day7 blastocysts cultured in time-lapse incubators.

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Study question: Are decisions on day7 and/or poor-quality blastocysts' (PQB; <Gardner's BB) use consistent among embryologists of diverse European countries and what factors influence them?

Summary answer: "Discard" choice prevalence was ≈40%, however inter-rater agreement was just fair (Fleiss-k=0.26). Country of practice, maternal age and number of sibling embryos influence each decision.

What is known already: Blastocyst morphokinetics and morphological quality are associated with competence and used to make clinical decisions, even though evaluations are poorly reproducible across embryologists. Moreover, the increasing utilization of PQB and adoption of culture beyond 144 hours-post-insemination is further challenging the decision-making process. Previous surveys reported limited concordance among embryologists in the "discard"/"use" choices made for day7 and/or PQB based on single 2D-pictures without cycle/patient information. However, for instance whole embryo preimplantation development, reproductive history, number of sibling embryos, can influence each decision. Different regulations and mindsets across countries may further influence these choices and require investigation.

Study design, size, duration: Anonymous survey in Microsoft-Forms run within a large IVF-network. Each laboratory-director shared a link to the survey with his/her colleagues via 3 e-mails sent weekly between 22/12/2023 and 12/1/2023. 117 embryologists participated representing 6 European Countries, 9 IVF-groups, and 28 centers. Raters inspected EmbryoScope videos of 50 PQBs and/or day7 blastocysts' whole preimplantation development and decided whether to "use" ("transfer-fresh"/"cryopreserve"/"biopsy and cryopreserve") or "discard" each of them. Inter-rater agreement was measured with Fleiss-k.

Participants/materials, setting, methods: Couples' information (age, infertility diagnosis, previous attempts, sperm analysis, number of sibling blastocysts obtained) were provided, and participants were asked their years of experience, center location, average number of cycles and average maternal age, number of colleagues, presence of time-lapse-incubators. The AI-score (CHLOE-EQ, Fairtility and iDAScore v1.0, Vitrolife), chromosomal diagnosis and clinical outcomes were known for all embryos by one embryologist not involved in the survey, but the participants were blinded to them.

Main results and the role of chance: Participants were Italian (40%,N=47), Spanish (24%,N=28), Portuguese (5%,N=6), Czech (5%,N=6), Swedish (23%,N=27) and Icelandic (3%,N=3). 2263(38.7%) "discard" and 3587(61.3%) "use" decisions were recorded. Czech, Portuguese, and Italian embryologists expressed lower (17 ± 7%,23 ± 14% and 27 ± 18%), Spanish intermediate (37 ± 16%), and Nordic higher (67 ± 11%) "discard" decision rates.The prevalence of "discard" responses per embryo was 37.4 ± 24.2%(2-87). The larger the number of sibling blastocysts the higher the embryologists' propensity to "discard" PQBs/day7 (unstandardized coefficient-B:+4.4%,95%CI:+0.9 to + 7.9,p=0.015). The most prevalent "use" choice was "biopsy and cryopreserve" (33.1 ± 21.7%,2-78). Maternal age (unstandardized coefficient-B:+2.2%,95%CI:+1.3 to + 3.8,p<0.01) and number of sibling blastocysts (unstandardized coefficient-B:-9.2%,95%CI:-15.2% to -3.3%,p<0.01) influenced this rate.Overall, inter-rater agreement was 0.26 (standard-error:0.003;"fair"), with "moderate" agreement for Czech and Portuguese (0.41,standard-error:0.037), "fair" for Spanish (0.38,standard-error:0.007), Italian and Nordic embryologists (0.28,standard-error:0.006 and 0.009).CHLOE-EQ and iDAScore v1.0 were not associated with embryologists' decisions. No difference was shown for AI-scores in euploid versus aneuploid blastocysts (CHLOE-EQ: 0.2 ± 0.32,0-0.9 versus 0.2 ± 0.29,0-0.93; iDAScore v1.0: 4.2 ± 1.6,2.2-6.7 versus 4.3 ± 1.5,2.1-9), while the embryologists more often chose "discard" among the latter group (28.3 ± 21%,9-71 versus 41.6 ± 24.8%,2-87). Five blastocysts were transferred and 3 implanted (CHLOE-EQ: 0.001,0.416 and 0.003; iDAScore v1.0: 2.6,5.0 and 6.6; "discard" choice rates: 19%,15% and 10%).

Limitations, reasons for caution: All embryologists were asked to decide regardless of the regulation enforced in their countries (e.g., PGT-A forbidden, discarding viable embryos not allowed). Nonetheless, the legislative, social, and clinical background might still have affected their choice. The survey included only private IVF clinics located in Europe.

Wider implications of the findings: European embryologists' decision-making regarding PQB/day-7 embryos is inconsistent. Mostly, legislative/clinical background and cycles' characteristics influence each decision. However, this practice has clinical implications, especially in poor-prognosis patients. Artificial-Intelligence may improve the reproducibility by removing intrinsic human subjectivity. However, it is still currently underpowered to assess these outlier embryos

Trial registration number: Not applicable

Abstract citation ID: dead093.1073

P-760 Effect of relaxin on human endometrial stromal cell characteristics

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Study question: Does relaxin (RLX) regulate decidualization and pro- and antiangiogenic factors in primary human endometrial stromal cells (hESC), primary decidual cells (pD) and hTERT-immortalized hESC (T hESC)?

Summary answer: RLX treatment enhances decidualization and affects the mRNA expression levels of pro- and antiangiogenic factors.

What is known already: Patients undergoing frozen embryo transfer in an artificial cycle (AC-FET) are at a 2-fold increased risk for preeclampsia compared to embryo transfer in a natural cycle. This may be related to the absence of the corpus luteum in an artificial FET cycle, which produces important hormones such as estrogen and progesterone in early pregnancy. These hormones are supplied during AC-FET, whereas other products of the corpus luteum, such as RLX, are not. Low maternal RLX levels were found in women who developed preeclampsia, but the direct relationship between RLX and the occurrence of preeclampsia remains to be elucidated.

Study design, size, duration: Primary hESC were isolated from biopsies of women without endometrial pathology, pD cells from biopsies of the maternal side of the placenta of uncomplicated pregnancies and T hESC purchased from American Type Culture Collection. Cells were decidualized with either 0.5 mM cyclic adenosine monophosphate (cAMP) or a cocktail of 10 nM estradiol, 1 µM progesterone and 0.5 mM cAMP (EPC) and additionally treated with 0, 0.3 or 1 ng/ml RLX for twelve days.

Participants/materials, setting, methods: RNA was isolated on day zero and day twelve from all three cell types. mRNA expression level of decidualization markers (prolactin (PRL), insulin like growth factor binding protein 1 (IGFBP1)) as well as pro- and antiangiogenic factors (vascular endothelial growth factor (VEGF); placenta like growth factor (PlGF); soluble Fms-like tyrosine kinase-1 (sFlt-1); and endoglin (ENG)) were evaluated by quantitative real time polymerase chain reaction (qRT-PCR), N = 3 biological replicates.

Main results and the role of chance: Decidualization treatment of hESC with cAMP caused more noticeable changes to cell morphology than

treatment with EPC. While there was no rise of decidualization markers PRL ($p = 0.99$) and IGFBP1 ($p = 0.95$) in the cAMP treatment group, hESC after EPC treatment showed a significant increase in both PRL ($p = 0.0003$) and IGFBP1 ($p = 0.0001$). RLX treatment in the EPC treated hESC and pD significantly increased the mRNA expression levels of PRL and IGFBP1 (both $p < 0.05$, respectively), as well as the expression of the proangiogenic marker VEGF ($P = 0.05$). A comparative analysis with T hESC showed a moderate effect on VEGF and PlGF. No effect of RLX was detected for IGFBP1 and PRL mRNA expression levels. The mRNA expression level of the antiangiogenic marker ENG increased in the EPC group in both, primary hESC ($P = 0.04$) and T hESC ($P = 0.01$) after treatment with 0.3 ng/ml RLX compared to control. ENG mRNA expression level increased in a dose dependent manner in pD but not in hESC and T hESC. RLX treatment with 1ng/ml of cAMP decidualized hESC significantly enhanced mRNA expression levels of VEGF ($P = 0.002$) and PlGF ($P = 0.02$) only in hESC but not in T hESC or pD.

Limitations, reasons for caution: Our pilot study indicates that RLX treatment improves decidualization and affects pro- and antiangiogenic factors in three different human endometrial stromal cell models. A wider approach is needed to investigate the effects of RLX in the decidualization process and pro- and antiangiogenic factors.

Wider implications of the findings: Our results provide the basis for further studies elucidating the relationship between low maternal RLX levels and the occurrence of preeclampsia. This could lead to the development of improved prediction models and new treatment options in assisted reproduction that reduce the risk of preeclampsia, e.g. in AC-FET.

Trial registration number: Not applicable

Abstract citation ID: dead093.1074
P-761 Live-birth and neonatal outcomes from BEYOND, a randomised controlled trial comparing efficacy and safety of individualised follitropin delta dosing in GnRH agonist versus antagonist protocols

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Study question: What are the live birth and neonatal outcomes following an individualised follitropin delta dosing regimen in either a long GnRH agonist or GnRH antagonist protocol?

Summary answer: Live birth rates and neonatal outcomes were similar, supporting the efficacy and safety of follitropin delta in both GnRH agonist and antagonist protocols.

What is known already: Follitropin delta is used for ovarian stimulation and is administered as a fixed daily dose, individualised based on bodyweight and anti-Müllerian (AMH) levels. Registrational trials for follitropin delta used a GnRH antagonist protocol. Preliminary study data shows that individualized follitropin delta is efficacious when used with a long GnRH agonist protocol (RAINBOW trial); however, there is no comparative data for the effects on live birth rates and neonatal outcomes with individualised follitropin delta in a long GnRH agonist versus an antagonist protocol.

Study design, size, duration: This RCT compared the efficacy and safety of individualised follitropin delta dosing using a long GnRH agonist versus an antagonist protocol and was conducted between May 2019 and February 2022. The trial was designed to describe potential differences in the number of oocytes retrieved between the two GnRH analogue protocols (reported separately). A total of 437 participants were randomised, with a post-trial follow-up period to 4 weeks after birth to record birth and neonatal outcomes.

Participants/materials, setting, methods: Participants were 18–40 years old with AMH ≤35 pmol/L, undergoing their first ovarian stimulation cycle for IVF/ICSI at specialist reproductive health clinics in Austria, Denmark, Israel, Italy, the Netherlands, Norway and Switzerland.Live birth rates were compared using a logistic regression model with age and AMH at screening as factors. Multiple imputation was used for randomised subjects withdrawing before start of stimulation. Subjects with transfer cancellation due to COVID-19 related reasons were excluded.

Main results and the role of chance: All participants had a single blastocyst transferred, except two participants ≥38 years in the agonist group who received a double transfer resulting in one dichorionic diamniotic pregnancy for one subject, complicated by maternal hypertension and preterm birth at 33 weeks. All 133 participants with ongoing pregnancies (138 fetuses) were included in this post-trial follow-up analysis. Late pregnancy losses (after confirmed ongoing pregnancy) were similar in the agonist and antagonist groups (2.7% and 1.7%). There were 130 live births (agonist group: 75 neonates [67 singletons; 4 sets of twins]; antagonist group: 60 neonates [58 singletons; 1 set of twins]). Estimated live-birth rates were 35.8% and 28.7% in the agonist and antagonist groups, respectively, per started cycle (treatment difference 7.15%; 95% CI: –2.02; 16.31; $p = 0.1265$). There were two neonatal deaths in the agonist group – a set of monochorionic twins born at gestational age 24 weeks + 2 days died shortly after birth due to prematurity. The two treatment groups were comparable with respect to neonatal health data for singletons and twins, and incidence of congenital malformations (2.7% and 3.3%, respectively). Neonatal admissions to intensive care units were similar for both groups (10 and six neonates, respectively).

Limitations, reasons for caution: Double blastocyst transfer was permitted for participants ≥38 years with no good-quality blastocysts. Neonatal health is dependent on singleton/twin status. Outcomes of transfers with cryopreserved blastocysts were not followed up – the cumulative live birth rates and neonatal outcomes after cryo-transfer are not known.

Wider implications of the findings: The safety profile of individualised follitropin delta dosing was similar in GnRH agonist and antagonist protocols. Live birth rates following individualised follitropin delta were also similar for both GnRH protocols. There were no safety concerns with respect to the neonatal health after ovarian stimulation with follitropin delta.

Trial registration number: ClinicalTrials.gov identifier: NCT03809429; EudraCT number: 2017-002783-40

Abstract citation ID: dead093.1075
P-762 Exploring the Efficacy and Beneficial Population of Preimplantation Genetic Testing for Aneuploidy Start from Oocyte Retrieval Cycle: A Real-World Study

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Study question: Does preimplantation genetic testing for aneuploidy (PGT-A) increase chance of live birth among women undergoing IVF? What kind of women are most likely to benefit?

Summary answer: PGT-A is effective in people with specific indications, including maternal aged ≥38 years, diagnosed with recurrent pregnancy loss or intrauterine adhesions.

What is known already: As an embryo-selection technique, PGT-A is extensively used in in-vitro fertilization (IVF), but its efficacy and potential beneficial population is unclear.

Study design, size, duration: This was a retrospective cohort study. We analyzed data from women who underwent assisted reproduction treatment in the Reproductive and Genetic Hospital of CITIC-Xiangya. We included all first oocyte retrieval cycles from January 1, 2016, to

November 31, 2019, and related fresh- and thawed-embryo transfer cycles up to November 31, 2020.

Participants/materials, setting, methods: The study cohort included 60,580 women. We used 1:3 propensity score matching to balance baseline data between women with PGT-A and without PGT-A. Stratified analyses according to age, body mass index, ovarian reserve/response, and potential indications were predesigned to explore benefits in sub-populations. The main outcome was cumulative live birth rate (CLBR) and the other observed outcomes including live birth rate (LBR), pregnancy loss, clinical pregnancy, pregnancy complications, newborn birth weight and neonatal malformation.

Main results and the role of chance: A total of 4,195 (95.1%) PGT-A users' first oocyte retrieval cycles were matched to 10,140 non-users' oocyte retrieval cycles. We found that women utilizing PGT-A had significantly lower CLBRs (27.5% vs. 31.1%; odds ratio (OR)=0.84, 95% confidence interval (CI), 0.78–0.91; $P<.001$) following the first oocyte retrieval cycle than did women not utilizing it. However, first transfers among women utilizing PGT-A had significantly higher pregnancy (63.9% vs. 46.9%; OR = 2.01, 95% CI, 1.81–2.23; $P<.001$) and live birth rates (LBR; 52.6% vs. 34.2%, OR = 2.13, 95% CI, 1.92–2.36; $P<.001$), as well as lower early miscarriage (12.8% vs. 20.2%; OR = 0.58, 95% CI, 0.48–0.70; $P<.001$), preterm birth (8.6% vs 17.3%; $P<.001$), and low birth weight rates (4.9% vs. 19.3%; $P<.001$). Moreover, subgroup analyses showed patients with maternal aged ≥38 years, diagnosed with recurrent pregnancy loss or intrauterine adhesions benefit from PGT-A, exhibiting significantly increasing LBRs following first transfer, without reducing CLBRs.

Limitations, reasons for caution: The retrospective design introduces inevitable bias. And the data originated from a single IVF center, thus, extrapolation of our findings may be limited, multi-center large data studies are needed to further confirm these findings.

Wider implications of the findings: Although PGT-A use does not increase and may decrease CLBR per oocyte retrieval cycle, it is effective in people with specific indications. Careful selection of suitable populations and proper clinical management for mosaic embryo transfer are important for effective PGT-A implementation.

Trial registration number: NA

Abstract citation ID: dead093.1076
P-763 Intrauterine insemination following controlled ovarian stimulation with gonadotropins: how to lower the multiple pregnancy rate without decreasing the live birth rate ?

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Study question: What cancelation policy in controlled ovarian stimulation-intrauterine insemination (COS-IUI) cycles allows to lower the multiple pregnancy rate (MPR) without decreasing the live birth rate (LBR)?

Summary answer: An algorithm based on the woman's age, serum Estradiol level and number of follicles ≥14 mm on trigger day reduces the MPR without impacting LBR.

What is known already: While the MPR in IVF cycles has significantly decreased in the past decades, it has remained stable and relatively high in COS-IUI cycles, at around 10-15%. The main reason behind this continuously high MPR in COS-IUI cycles has been the relative inability to lower it without significantly decreasing the overall pregnancy rates. Several risk factors are associated with MP in COS-IUI cycles, and recommendations have varied between the different scientific societies, but to date, there is no consensus on the best strategy to decrease the risk of MP in COS-IUI cycles without compromising the pregnancy and live birth rates.

Study design, size, duration: A bicentric observational cohort study at the Angers University Hospital (group A) and the Besançon University Hospital (group B) between January 2011 to December 2019. Approximately 350-400 IUI cycles are performed yearly in each center. All patients who had a clinical pregnancy following COS-IUI during the study period were included. Our main outcome measure was the MPR and our secondary outcome measures were the clinical pregnancy (CP), miscarriage and LBR.

Participants/materials, setting, methods: In group A, the starting gonadotropin dose was 50-100 IU/day, and the algorithm for cycle cancelation was based on the woman's age, serum Estradiol (E_2) level, and number of follicles ≥14 mm on trigger day. In group B, the starting gonadotropin dose was 100-150 IU/day and the cancelation policy was case-by-case and physician dependent, based on the woman's age, number of follicles ≥15 mm, and number of previous failed COS-IUI cycles, without predefined cut-offs.

Main results and the role of chance: We included 6582 COS-IUI cycles (3387 in group A and 3195 in group B) that resulted in 884 clinical pregnancies (790 singletons, 86 twins and 8 triplets). The MPR was significantly lower in group A compared to group B (8.1% vs 13.3%, p = 0.01). The CPR (13.4% vs 13.4%, p = 0.99), the miscarriage rate (14.5% vs 15.6%, p = 0.64) and the LBR (10.8% vs 11.9%, p = 0.16) were comparable between groups A and B. Univariate analysis showed the following factors to be predictive of the risk of MP: the treatment center (OR = 1.73 [1.12-2.68]), the number of follicles ≥10 mm (OR = 1.22 [1.11-1.36]) and ≥14 mm on trigger day (OR = 1.43[1.20-1.70]). Multivariate analysis also showed the following factors to be predictive of the MP risk: the treatment center (aOR=1.63 [1.02-2.60]), the number of follicles ≥ 10 mm (aOR=1.20 [1.07-1.34]) and ≥14 mm on trigger day (aOR=1.39 [1.16-1.66]). The cycle cancelation rate was comparable between groups A and B (7.2% vs 7.2%, p = 0.93), while cycle cancelation rate for excessive response to COS was significantly lower in group A compared to group B (19.3% vs 35.9%, p < 0.001). The rate of divergence from cancelation protocol was significantly lower in group A compared to group B (0.09% vs 1.1% p < 0.001)

Limitations, reasons for caution: The main limitation of our study is the retrospective design. The algorithm needs to be tested in other populations for further validation.

Wider implications of the findings: The use of low starting doses of gonadotropins (50-100 IU/day), and the application of a strict algorithm that takes into account the woman's age, serum E_2 level and number of follicles ≥14 mm on trigger day allows to optimize the success rates of COS-IUI cycles

Trial registration number: Not applicable

Abstract citation ID: dead093.1077
P-764 Mode of conception and future neurologic morbidity of the offspring: a sibling analysis

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Study question: To investigate the risk of long-term neurological morbidity among children born following fertility treatments while employing sibling matched analysis to maximize confounder control.

Summary answer: Fertility treatments appear to be an independent risk factor for long-term neurological morbidity of the offspring up to 18 years of age.

What is known already: Controversy exists regarding the association between fertility treatments and long-term morbidity of the offspring.

Study design, size, duration: A retrospective population-based cohort analysis was performed, including all sibling deliveries occurring between 1991 and 2021 at a tertiary medical center. Offspring were followed-up until the age of 18 years.

Participants/materials, setting, methods: The study population included 10,810 siblings of women who had at least one spontaneous pregnancy and

at least one pregnancy following fertility treatments - in vitro fertilization (IVF) and ovulation induction (OI). A Kaplan–Meier survival curve was used to compare the cumulative neurologic morbidity incidence and a multivariable Cox survival hazards regression model was used to control for confounders.

Main results and the role of chance: There were 5,935 siblings (54.9%) conceived following fertility treatment and 4875 spontaneously conceived siblings (45.1%). Gestational diabetes mellitus and hypertensive disorders were more common in the fertility treatment group (9.9% vs. 5%, and 7.7% vs. 4.7%, respectively, $p < 0.001$). Likewise, the rates of Cesarean delivery (33.6% vs. 2.3%, $p < 0.001$), preterm delivery (11.9% vs 7.6%, $p < 0.001$) and low birth weight (12.1% vs.7.2%, $p < 0.001$) were higher in the fertility treatments group, as compared with the spontaneous pregnancy group.

Neurological morbidity was significantly higher among siblings born following fertility treatments as compared with those conceived spontaneously (10.2% vs. 8.2%; $p < 0.001$). In particular, psychiatric emotional disorders were more common in the fertility treatment group (4.4% vs. 3.3% $p = 0.002$). Using a Kaplan-Meier survival curve, siblings conceived following fertility treatments had higher cumulative incidence rates of neurological morbidity (Log-Rank, $p = 0.012$). After controlling for confounders, such as maternal age, diabetes mellitus, preterm delivery and hypertensive disorders, using a Cox regression model, being born following fertility treatments was associated with long term neurological morbidity (adjusted HR = 1.2, 95% CI 1.02-1.32, $p = 0.027$).

Limitations, reasons for caution: This retrospective cohort study can only provide evidence of association but not causation. Also, our study results are hospital-based diagnoses which most likely represent acute and severe disease.

Wider implications of the findings: In this large retrospective cohort study of siblings, a significant and independent association was found between fertility treatments and long-term neurological morbidities.

Trial registration number: N/A

Abstract citation ID: dead093.1078
P-765 Consequences of not complying with human resources guidelines in in vitro fertilization laboratories.

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Study question: The objective was to analyze the consequences on the health of Clinical Embryologists for not complying with the scientific societies' human resources guidelines in Spain.

Summary answer: Not complying with the recommendations of the human resources guidelines lead to a worse quality of working life and worse physical health of Clinical Embryologists.

What is known already: Few previous studies show that Clinical Embryologists are one of the health professionals who suffers more stress at work, especially women (López-Lería et al, 2014). Many scientific societies (ASEBIR, ESHRE, etc) have made several human resources guidelines trying to ensure proper functioning of the in vitro fertilization laboratories. In Spain the Quality Interest Group of ASEBIR, made a calculator (Cassandra) to help calculate the best number of Clinical Embryologists per cycle is need in a clinic.

They established that the correct proportion is one Embryologist per 102 cycles.

Study design, size, duration: The study was carried out between March and November 2021. We asked about sociodemographic variables, physical health and we used the CVP 35 test to analyze the quality of work life. Regarding physical health we asked about lower limb pain, upper limb pain, neck pain and back pain. In relation with the CVP35 test, it includes 3 dimensions: workload, manager's support and intrinsic motivation.

Participants/materials, setting, methods: A survey was sent to 870 active workers (Clinical Embryologists), members of ASEBIR. The survey was completed by 184 subjects. The data was separated into two groups: clinics that had at least one Embryologist per 102 cycles and the clinics that did not. The data was analyzed using statistical program SPSS.20. Most of the study variables did not follow a normal distribution (Kolmogorov-Smirnov) and for the comparison of means, the U-Mann-Whitney test was used.

Main results and the role of chance: In order to have a better comparison, all data were normalized from 0 to 100 (Arbitrary Units). Regarding to the physical health, the data reflects a mean of 27.52 AU of lower limb pain, 35.78 AU of upper limb pain, 59.08 AU of neck pain and 62.39 AU of back pain. In relation to the CVP35 results, the mean of workload was 65.1 NU, the mean of manager's support was 53.69 AU and the mean of intrinsic motivation was 78.14 AU.

Only the 41.7% ($n = 75$) of the clinics complied with ASEBIR's recommendations. We observed that the workers of the clinics that did not comply with ASEBIR's recommendations had lower physical health. They had increased lower limb pain ($p = 0.002$), upper limb pain ($p = 0.036$), neck pain ($p = 0.02$) and back pain ($p = 0.036$).

Regarding quality of work life, we observed that the workers of the clinics that did not comply with the recommendations had lower quality of work life. They had decreased manageŕs support ($p = 0.047$) and increased workload ($p = 0.004$), despite having similar intrinsic motivation ($p > 0{,}050$).

Limitations, reasons for caution: The surveyed subjects belong to Spain and their legal and labor conditions. We must be cautious when extrapolating the results to other countries.

Wider implications of the findings: Not complying with the recommendations of human resources guidelines leads to worse physical health and worse quality of work life. It would be advisable to adopt actions in order to force the clinics to comply with the recommendations to avoid a bad professional quality of life in the IVF laboratories.

Trial registration number: Not applicable

Abstract citation ID: dead093.1079
P-766 Re-assessment of the Belgian legislation on embryo transfer in a blastocyst transfer policy

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Study question: How does a multiple fresh blastocyst transfer policy impacts live birth rate (LBR) and multiple live birth rates (MLBR) in relation to maternal age ?

Summary answer: Fresh transferring of ≥ 2 blastocysts only increases LBR in women above the age of 36 and substantially increases risk of multiples in all age categories.

What is known already: Multiple pregnancies after ART are recognized as a major complication as it significantly contributes to extreme preterm and very low-birthweight births. Even in the presence of a strict Belgian embryo transfer law in some subgroups in which double embryo transfer (DET) is allowed (women aged <36 years in cycle rank 3 up to 6 and in women aged 36-40 years in cycle rank 2), twining rates remain above 10%. A study by Tannus *et al.*, (2017) concluded that double blastocyst transfer (DBT) is associated with both higher LBR and higher twin birth rates compared to elective single blastocyst transfer (eSBT).

Study design, size, duration: A single center retrospective, observational cohort study was conducted between July 2010 and December 2020. Both fresh IVF and ICSI cycles were included. A total of 8,402 fresh transfers were performed (2,948 on day 3 and 5,454 on day 5) over the course of the study period. SET was performed in 6,108 cases, DET in 2,077 cases, triple embryo transfer (TET) in 202 cases and multiple embryo transfers (MET) in 15 cases.

Participants/materials, setting, methods: The primary outcomes of this study were LBR per transfer and twin pregnancy rate. Generalized estimating

equations (GEE) with the patient as subject was applied to account for clustering of cycles within women. An interaction analysis between the number of embryos transferred and age categories in the day 5 group was performed. A P-value of < 0.05 was considered statistically significant.

Main results and the role of chance: In women aged <36 years, chances of a live birth were not significantly higher when two or more vs. a single blastocyst were transferred (OR: 1.014; 95%% CI 0.791 - 1.299; $p = 0.91$). However, transferring more than one blastocyst in women aged 36-40 years and >40 years did result in a higher chance of live birth compared to SBT (OR: 1.631; 95% CI: 1.218 - 2.185; $p = 0.0008$ and OR: 1.634; CI: 1.219-2.190; $p = 0.0008$, respectively).`

Women aged 36-40 years and > 40 years had an estimated 19.3% and 66.3% lower odds respectively of a live birth after the transfer of a day 5 embryo, vs. women aged <36 years (OR: 0.800, 95% CI: 0.687-0.931; $p = 0.0034$ and OR: 0.337, CI: 0.268-0.424; $p < 0.0001$, respectively). Overall, live births were 28.5% higher when 2 or more blastocysts were transferred compared to an SBT (OR: 1.285 95% CI 1.1.077 – 1.533) ($p = 0.0046$). The estimated odds for a twin pregnancy were 17.8 times higher for DBT/MBT compared to SBT (OR 17.814; 95% CI: 10.74 - 29.55) ($p < 0.0001$).

Limitations, reasons for caution: This study is limited by its retrospective design and no interaction analysis between number of twin pregnancies and age categories was possible due to the low number of twins.

Wider implications of the findings: The data indicates that in women younger than 36 years only one blastocyst should be transferred regardless the embryo quality or the cycle rank. Only for patients older than 40 years, transfer of two blastocyst(s) could be considered.

Trial registration number: Not applicable

Abstract citation ID: dead093.1080

P-768 A network meta-analysis yields new insights on the efficacy of different progestogen regimens for luteal support in fresh embryo in vitro fertilization

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Study question: How do seven progestogen regimens rank in their efficacy for achieving clinical pregnancy when used as luteal support in women undergoing fresh embryo transfer?

Summary answer: Given as luteal support and based on clinical pregnancy rate, intramuscular progesterone was most effective, followed by oral dydrogesterone and then micronized vaginal progesterone gel.

What is known already: A large number of randomized controlled trials (RCTs) have compared fresh in vitro fertilisation cycle luteal support with progestogens. A number of systematic reviews and conventional meta-analyses have likewise been published. A network meta-analysis integrates direct and indirect comparisons between treatments in a single analysis, and allows the ranking of treatments and the estimation of heterogeneity in both the effect of any given treatment and the inconsistency ('incoherence') in the evidence from different pairs of treatments. Network meta-analyses can thus increase the precision and robustness of inferences from the literature on treatment effect estimates.

Study design, size, duration: A literature search was performed using BIOSIS, Embase, and MEDLINE and supplemented with manual searches to identify RCTs reporting the efficacy of progestogen regimens in luteal support during fresh embryo transfer. A single network meta-analysis was used to compare progestogen regimens, combining both direct and indirect evidence across the selected studies using the R package 'netmeta' and fixed and random effects models. The analysis was repeated and confirmed using SAS.

Participants/materials, setting, methods: Peer-reviewed full publications that compared individual progestogens (either with placebo or another progestogen) were included. Studies were excluded if they included frozen embryo transfers, used human chorionic gonadotropin or formulations not currently available for the indication, or if they did not report at least clinical pregnancy rate. Two authors (EK, QW) individually reviewed papers to collect data. Where doses differed between studies for an individual progestogen the doses were noted and the results combined.

Main results and the role of chance: Among studies from 1987–2022, 35 RCTs met the inclusion criteria, comprising 17,955 observations of 7 treatments plus placebo and resulting in 43 pairwise comparisons for the network meta-analysis of clinical pregnancy rate (the primary outcome). Based on the random effects model, the top three regimens versus placebo were intramuscular (IM) progesterone, Duphaston® (oral dydrogesterone), and Crinone® (micronized progesterone vaginal gel) (see Figure). Compared with placebo, only the top 3 ranking compounds had confidence intervals that excluded zero, with a risk difference to placebo (95% confidence interval) of 0.08 (0.01-0.14) for IM progesterone and 0.07 (0.01-0.13) for both Duphaston® and Crinone®. The P-score (fixed, random) ranking for the compounds was: IM progesterone 0.77, 0.79; Duphaston® 0.78, 0.69; Crinone® 0.65, 0.65; Cyclogest® (vaginal micronized progesterone) 0.58, 0.60; Endometrin® (vaginal micronized progesterone) 0.52, 0.54; Prolutex® (subcutaneous progesterone) 0.31, 0.36; Utrogestan® (vaginal micronized progesterone) 0.34, 0.31; and placebo 0.05, 0.06. Ranking was confirmed by SUCRA score (surface under the cumulative ranking curve). Studies were thoroughly reviewed to ensure similarity assumption. Homogeneity and consistency of the network were investigated and fulfilled. Exclusion of placebo-controlled studies did not impact the overall results or rankings.

Limitations, reasons for caution: Treatment duration was assumed identical across arms in each study and therefore was not included in this analysis. Most studies were single-centre with a small sample size. Methodological rigor was heterogenous between trials. Direct comparisons with placebo were only available for the top three compounds.

Wider implications of the findings: Three progestogen regimens showed a positive effect on clinical pregnancy rate versus placebo. Frequently used vaginal preparations do not lead the efficacy league table when the available direct and indirect comparative evidence is collated. Relevant efficacy differences likely exist between the available progesterone regimens.

Trial registration number: n/a

Abstract citation ID: dead093.1081

P-769 fertility treatments and the risk for long-term respiratory morbidity of the offspring; a sibling analysis

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Study question: Is the risk of long-term respiratory morbidity increased among children born following fertility treatments (in-vitro fertilization and ovulation induction)? Sibling analysis to maximize confounder control.

Summary answer: Fertility treatments do not appear to be an independent risk factor for long-term respiratory morbidity of the offspring up to 18 years of age.

What is known already: Studies have found an association between fertility treatments and long-term respiratory morbidity of the offspring, specifically obstructive sleep apnea (OSA).

Study design, size, duration: A retrospective population-based cohort analysis was performed, including all sibling deliveries occurring between 1991 and 2021 at a tertiary medical center. Offspring were followed-up until the age of 18 years.

Participants/materials, setting, methods: The study population included 10,691 siblings of women who had at least one spontaneous pregnancy and at least one pregnancy following fertility treatments. A Kaplan–Meier survival curve was used to compare the cumulative respiratory morbidity incidence, and a multivariable Cox survival hazards regression model was used to control for confounders.

Main results and the role of chance: There were 5,869 siblings (54.9%) conceived following fertility treatment and 4,822 spontaneously conceived siblings (45.1%). Using a univariable analysis, respiratory morbidity was higher among siblings born following fertility treatments as compared with those conceived spontaneously (9.6% vs. 8.4%; $p = 0.023$), in particular, OSA (2.6% vs. 1.8%; $p = 0.005$). However, using a Kaplan-Meier survival curve, being born following fertility treatments did not have a higher cumulative incidence rate of long-term respiratory morbidity (Log-Rank, $p = 0.339$). Likewise, when using a Cox regression model, controlling for confounders such as maternal age, diabetes mellitus, preterm delivery, and hypertensive disorders, the association between being born following fertility treatments was no longer associated with long-term respiratory morbidity (adjusted HR = 1.0, 95% CI 0.88-1.14, $p = 0.957$).

Limitations, reasons for caution: This retrospective cohort study can only provide evidence of association but not causation. Also, our study results are hospital-based diagnoses of respiratory morbidity which most likely represent acute and severe disease.

Wider implications of the findings: In this large retrospective cohort study of siblings, we showed that, in contrary to current literary findings, and after matching for siblings, fertility treatments are not associated with long-term respiratory morbidity of the offspring. These findings raise questions about the existence of certain maternal predispositions that may associate the two.

Trial registration number: Not applicable

Abstract citation ID: dead093.1082
P-770 Risk factors for placenta accreta spectrum among pregnant women conceived with assisted reproductive technology

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Study question: What are the risk factors for placenta accreta spectrum (PAS) among women conceived with assisted reproductive technology (ART)?

Summary answer: The risk of PAS was associated with elevated estradiol and progesterone levels during COH, and HRT protocol, and abnormal endometrial thickness when preforming FET.

What is known already: Increasing evidence suggests an association between ART and PAS. Some studies have reported a high rate of diagnosis of PAS after embryo transfer compared to those with spontaneous conception. Despite this, little is known about the detailed risk factors for PAS during the procedures of ART.

Study design, size, duration: This retrospective case-control study included 1541 pregnant women with live birth conceived ART between 2013 and 2019 in a university hospital.

Participants/materials, setting, methods: Pregnant women were categorized into case and control group according to the postpartum diagnosis of PAS. The associations between PAS and ART procedures were estimated using multivariate logistic regression, and represented as adjusted odds ratio (aOR) and 95% confidential interval (CI).

Main results and the role of chance: Among all pregnant women in this study, 118 were delivered with PAS, and 1423 were delivered without PAS. During the procedures of COH and oocytes retrieval, elevated levels of estradiol (aOR=1.03, 95% CI: 1.01-1.06) and progesterone (aOR=1.02, 95% CI: 1.00-1.04) were found to be associated with the risk of PAS. The number of oocytes retrieved over 10 were also found to have an association with PAS (aOR=1.58, 95% CI: 1.05-2.37). Additionally, FET was another risk factor forPAS (aOR=1.93, 95% CI: 1.06-3.51). Among pregnant women with FET, preparing the endometrium using HRT protocol increased the risk of PAS (aOR=2.46, 95% CI: 1.57-3.84). Notably, PAS was also found to have an association with abnormal endometrial thickness after preparing the endometrium, whether too thin (<7mm: aOR=15.90, 95% CI: 4.42-57.21) or too thick (>14mm: aOR=6.56, 95% CI: 1.84-23.45).

Limitations, reasons for caution: As a hospital-based study, several confounders related to medical procedures have been taken into consideration. However, it is not possible to rule out unknown confounders.

Wider implications of the findings: Caution is warranted when preforming COH, and endometrial preparation before FET due to the increased risk of PAS after deliveries.

Trial registration number: NA

Abstract citation ID: dead093.1083
P-771 Prospective comparison of different needle sizes (17G vs 18G) and aspiration pressures (110 vs 220 Hgmm) for egg collection in oocyte donors: randomized, pilot study

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Study question: How does higher aspiration pressure (220Hgmm) and thinner oocyte retrieval needle size (18G) affect egg retrieval rate, intervention duration, and post-operative pain in oocyte donors?

Summary answer: Egg retrieval rate and developmental potential did not vary according to needle thickness or aspiration pressure, whereas with higher aspiration pressure the intervention was shorter.

What is known already: Although transvaginal ultrasound guided egg collection has been successfully used for more than two decades, technical parameters of the egg collection are still not standardized and are often based on the manufacturer's recommendations which have not been evaluated in a clinical setting. Only a handful of studies evaluated the use of thinner oocyte retrieval needle aiming at reducing ovarian trauma (Wikland 2011, Kushnir, 2013).

Study design, size, duration: After obtaining informed consent consecutive oocyte donors ($n = 105$) were prospectively assigned on the day of egg retrieval scheduling to three equal-sized groups (group 2: 17G needle, 220 Hgmm, group 1; 18G needle, 220 Hgmm, group 3: 17G needle, 110 Hgmm) using two different oocyte retrieval needles (Kitazato, Japan) and two different aspiration pressures (Labotect, Germany). Oocyte donor age, BMI, baseline AMH, ≥14 mm and ≥10 mm follicular counts were not significantly different among groups.

Participants/materials, setting, methods: Oocyte donors were stimulated with GnRH antagonist protocol and oocyte maturation was triggered with GnRH agonist. Pre- and postoperative pain measured by visual analogue score (VAS:0-10) and duration of each egg collection performed under sedation was recorded. Retrieved mature eggs were assigned to previously matched recipients or electively vitrified for use in our centre's egg bank. The developmental potential of retrieved eggs was evaluated by the fertilization and blastocyst formation rate of assigned, fresh oocytes.

Main results and the role of chance: As aspiration pressure and needle size decreased, the average duration of egg collection became longer (08:02, 09:36, 10:50 minutes, $p = 0.002$). Although postoperative pain scores were

uniformly low, there was a marginally lower postoperative pain score in group 3, where a lower aspiration pressure was used (1.6 ± 1.4, 1.9 ± 1.9, 1 ± 1.1, $p < 0.0001$). No oocyte donor experienced any significant postoperative complication. The number of retrieved eggs (27.3 ± 12, 26.8 ± 13.6, 26.1 ± 12), oocyte retrieval rate per number of ≥ 14 mm follicles (104, 103, 104%), and the proportion of damaged eggs with ruptured zona pellucida (3.8, 2.7, 3.7%) were not significantly different among groups. Fertilization (72, 73, 73%) and blastocyst formation (51, 53, 46%) rates of injected fresh mature oocytes were not significantly different.

Limitations, reasons for caution: Due to the lack of similar studies, the size of each study group (n = 35) was determined by convenience instead of a previous sample size calculation.

Wider implications of the findings: Precise calibration of technical parameters of egg collection merits further attention and continuous monitoring, especially in hyper-stimulated oocyte donors where an excellent oocyte yield should be balanced against a shorter intervention, maximally reduced postoperative pain, and the lowest possible rate of complications.

Trial registration number: Not applicable

Abstract citation ID: dead093.1084

P-772 Three-dimensional folliculometry (SonoAVC) provides a better estimation of total and mature oocyte yields compared to conventional two-dimensional assessment in oocyte donors: a prospective, cohort study

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Study question: Does three-dimensional folliculometry (using SonoAVC) provide a better estimate of the number of retrieved and mature oocytes in egg donors compared to conventional two-dimensional assessment?

Summary answer: Three-dimensional folliculometry was superior to the two-dimensional ultrasound assessment and provided an excellent prediction (+/-20%) of total oocyte yield in almost two-thirds of participants.

What is known already: When used for monitoring ovarian stimulation, 3-D folliculometry has been shown to reduce inter-, and intra-observer variability and was significantly faster than conventional, two-dimensional ultrasound assessment (Vandekerchove F, 2014, Rei 2019). Although so far, no study has demonstrated improved clinical outcomes in IVF patients, the impact of 3D-folliculometry was not yet thoroughly evaluated in oocyte donation programmes, where expected total and mature oocyte yield is a crucial variable.

Study design, size, duration: Ninety-one consecutive oocyte donors were prospectively evaluated using three-dimensional folliculometry with SonoAVC software (E8, General Electric). A single operator (DB) performed all 3D ultrasound measurements immediately before starting oocyte retrieval and the oocyte retrieval procedure too. Two-dimensional measurement of follicles (average of two perpendicular diameters) was performed two days before egg retrieval by a single operator (NR) (Mindray DC7). Oocyte donors' age (26.2 ± 3.7 years), BMI (23.8 ± 3.9), and baseline AMH (4.6 ± 2.3 ng/mL) were also registered.

Participants/materials, setting, methods: Voluntary, anonymous oocyte donors were stimulated with a GnRH antagonist protocol and triggered with a GnRH agonist. Good, moderate, and poor prediction by ultrasound were defined as an oocyte/follicle count ratio of 80-120% (+/-0-20%), 60-79% or 121-140% (+/- 21-40%), and less than 60% or more than 140% (+/- more than 40%), respectively. For total oocyte yield, a threshold of all follicles 13 mm or above was used, both for the two- and three-dimensional assessment.

Main results and the role of chance: The number of total and mature oocytes retrieved was 22.2 ± 9.4 and 19.0 ± 8.3, respectively with an excellent average maturity rate of 86% (range: 44-100%). No donors were excluded from the study due to suboptimal visualization of ovaries with SonoAVC. For total oocyte yield; good, moderate, and poor prediction was achieved in 37, 44, 19% of donors with two-dimensional and in 63, 29, 8% of participants with three-dimensional folliculometry, respectively ($p = 0.002$). Significant underestimation of total oocyte yield (defined as more than 6 „missing" total oocytes) was less frequent with 3D assessment (12 versus 25% $p = 0.022$). For total oocyte yield, linear regression analysis showed a significantly higher r-square value with three-dimensional assessment (0.676 vs 0.278). For mature oocytes, the prediction was less precise than for total oocyte yield. Good, moderate, and poor prediction was achieved in 40, 30, 30% of donors with two-dimensional and in 55, 30, 15% of participants with three-dimensional folliculometry, respectively ($p = 0.031$). For mature oocytes, linear regression analysis showed a significantly higher r-square value with three-dimensional assessment (0.664 vs 0.282). The best SonoAVC threshold to predict total and mature oocyte yields was the sum of all follicles equal or above 13 mm and total follicle count between 24-13 mm, respectively.

Limitations, reasons for caution: Due to practical reasons, three- and two-dimensional measurements were not performed on the same day or with the same ultrasound equipment. In future studies, 3D-folliculometry could be used at the egg collection scheduling (2-3 days before the procedure) so that predictive information on oocyte yield would be available earlier.

Wider implications of the findings: Apart from reducing inter-observer variability and diminishing discomfort (faster examination time), 3D-folliculometry might have a significant role in the management of OD programmes by improving the prediction of total/mature oocyte yields. 3D-measurements performed before the procedure could also play a role in the quality control of the egg collection procedure.

Trial registration number: Not applicable

Abstract citation ID: dead093.1085

P-773 No impact of cleavage-stage or blastocyst-stage embryo biopsy on growth and health in children up to 2 years of age

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Study question: Does embryo biopsy for preimplantation genetic testing (PGT) followed by fresh or vitrified-warmed embryo transfer affect children's health up to 2 years of age?

Summary answer: Cleavage- or blastocyst-stage embryo biopsy followed by fresh or vitrified-warmed-embryo transfer had neither impact on anthropometry at birth, infancy or childhood nor on health outcomes.

What is known already: Literature data regarding neonatal outcomes after embryo biopsy for PGT show mixed results due to small sample sizes and/or the heterogeneity in terms of embryo biopsy (cleavage- or blastocyst-stage) and type of embryo transfer (fresh or frozen-thawed). Even fewer data exist on the impact of embryo biopsy on children's health beyond infancy, including growth.

Study design, size, duration: This single-center cohort-study compared outcomes in singletons conceived after cleavage- or blastocyst-stage embryo biopsy either in a fresh or vitrified-warmed transfer cycle with results after non-biopsied embryo transfer between 2014 and 2018. Pregnancies after IVM, oocyte vitrification or oocyte/embryo donation were excluded. Eligible singletons living in Belgium were invited for examination at 3-6 months and 2 years. Anthropometric measurements at birth, infancy and childhood and health outcomes including surgeries, medication use and hospitalisations are reported.

Participants/materials, setting, methods: Birth characteristics were available for 630 and 222 children after cleavage-stage and blastocyst-stage embryo biopsy and for 1532 children after transfer of a non-biopsied embryo. Follow-up data were available for 426, 131 and 662 children, respectively.

The impact on children's health following embryo biopsy in vitrification and fresh cycles was the primary outcome. Other outcomes were the impact of timing of biopsy and of vitrification. Subgroup analysis according to infertility background was additionally performed.

Main results and the role of chance: Regarding the impact of embryo biopsy, either at the cleavage or blastocyst stage, no differences in anthropometrics were found at birth, infancy or childhood in vitrified-warmed transfer cycles compared to outcomes in non-biopsied vitrified-warmed transfer cycles, even after adjustment for neonatal, treatment and maternal characteristics. Likewise, no impact of embryo biopsy (cleavage stage) was found in fresh transfer cycles.

The timing (day 3 or day 5/6) of embryo biopsy did not affect anthropometrics at birth, infancy or childhood. However, children born after cleavage-stage embryo biopsy followed by a vitrified-warmed transfer cycle had larger birth sizes than children born after cleavage-stage biopsy followed by a fresh transfer.

Weight and height gain from birth to infancy and from infancy to early childhood were comparable in all biopsied and non-biopsied groups.

Reassuringly, comparable rates of major congenital malformations, (severe) developmental problems, hospital admissions, surgical interventions and of chronic medication use up to the age of 2 years were observed in biopsied and non-biopsied groups.

Subgroup analysis in children born to parents with an infertility diagnosis showed that birth parameters were not different in children born after embryo biopsy.

Limitations, reasons for caution: As the majority of the PGT cycles in our center are performed for a genetic indication only, the number of children born after embryo biopsy in infertile parents is rather limited and the results should therefore be interpreted cautiously.

Wider implications of the findings: Our findings add reassurance that embryo biopsy does not adversely affect the health of offspring up to 2 years of age. This is of particular importance given the widespread shift to blastocyst-stage embryo biopsy in PGT.

Trial registration number: Not applicable

Abstract citation ID: dead093.1086
P-774 Congenital Heart Disease in Children Born after Assisted Reproductive Technology: A Nordic Cohort Study.

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Study question: Do children conceived after assisted reproductive technology (ART) have a higher risk of Congenital Heart Disease (CHD) in comparison with children born after spontaneous conception?

Summary answer: Children born after ART have a higher risk of a major CHD compared with children born after spontaneous conception.

What is known already: It is well-known that children born after ART have higher risk of birth defects compared to children born after spontaneous conception. Congenital heart disease (CHD) is the most common birth defect, accounting for almost 50% of all major birth defects. Several cohort studies and systematic reviews have also found an increased risk of CHD in children born after ART, a recent review included 41 studies and 25 856 ART children covering CHDs in ART children. Conflicting results have been reported for specific CHDs.

Study design, size, duration: This Nordic registry-based cohort study from CoNARTaS (Committee of Nordic ART and Safety) included 171 774 live births born after use of ART and 7 772 474 live births born after spontaneous conception during a study period of up to three decades (Denmark 1994-2014, Finland 1990-2014, Norway 1984-2015, and Sweden 1985-2015). National data from ART registries, medical birth registries, malformation registries, patient registries, cause of death registries, and population registries were crosslinked.

Participants/materials, setting, methods: International Classification of Diseases (ICD) versions 8, 9, 10 were used for CHD codes at birth and up to one year of age. Major CHDs were defined according to European Concerted Action on Congenital Anomalies and Twins (EUROCAT). According to EUROCAT, 26 of the major CHDs were considered as severe. All major CHDs were further classified into six different groups in a hierarchical order. Minor CHDs as well as stillbirths and pregnancy terminations were excluded.

Main results and the role of chance: Data from Sweden included a total of 3 228 526 singletons and multiples; 62 243 children conceived after use of ART and 3 166 283 children after spontaneous conception. Among ART children, 2.27% (n = 1411) were diagnosed with any major non-chromosomal CHD during their first year of life, corresponding figures for children born after spontaneous conception was 1.43% (n = 45 310) (Odds Ratio [OR] 1.60; 95% Confidence Interval (CI) 1.51 to 1.69, $p < 0.0001$). Severe CHD occurred in 0.40% (n = 249) in the ART group and 0.31% (n = 9 795) in the spontaneous conception group (OR 1.29; 95% CI 1.14 to 1.47, $p < 0.0001$). Ventricular septal defect was the most common CHD, 0.97% (n = 603) in the ART vs 0.64% (n = 20 343) in the spontaneous conception group (OR 1.51; 95% CI 1.39 to 1.64, $p < 0.0001$), followed by atrial septal defect; 0.84% (n = 525) in the ART and 0.42% (n = 13 363) in the spontaneous conception group (OR 2.00; 95% CI 1.83 to 2.19, $p < 0.0001$).

Limitations, reasons for caution: This analysis included only non-adjusted Swedish data. Further analysis will include Danish, Finnish, and Norwegian data with adjustments, and subgroup analysis on singletons and multiples. No data are available on prenatal diagnosis, or pregnancies not ending to a live birth. A skewed distribution in these outcomes might introduce a bias.

Wider implications of the findings: Pregnancies conceived after use of ART may benefit from screening with fetal echocardiography. Although most children with CHD reach adulthood, the mortality rate is still high for this group during the first years of life. Early detection with antenatal diagnosis and pre/postnatal intervention may be beneficial and improve the outcome.

Trial registration number: Not applicable

Abstract citation ID: dead093.1087
P-775 Responsible Artificial Intelligence in Clinical Decision Making: Anomaly Detection in Trigger Day Optimization

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Study question: Is there a benefit of using outlier detection when developing an AI algorithm for predicting the number of oocytes retrieved in different trigger days?

Summary answer: Using an outlier detection as a pre-step for other algorithms helps to determine if a prediction\decision should be made for a specific scenario.

What is known already: Outlier detection is a process that helps in identifying unusual or unexpected observations in the data. Outliers can arise for a variety of reasons, such as: measurement errors, data entry mistakes, genuine rare events or data the model did not observe when training. In recent years multiple AI algorithms have been developed across a variety of areas in reproductive health (embryos, sperm, etc.), however not many publications have been made on the importance of implementing outlier detection when assisting doctors in clinical decisions.

Study design, size, duration: The data used for developing this algorithm consists of 9,618 antagonist protocol cycles performed in a large center serving over 50 physicians, between August 2017 and November 2022. The data was divided into three subsets, representing cases in which the physician performed the trigger 0, 1 or 2 days after a blood and ultrasound test day.

Participants/materials, setting, methods: Three outlier detection models were developed for a pre-developed trigger day selection algorithm. Each outlier model was developed for a different trigger day subset. Those models were built using Local Outlier Factor algorithm, an unsupervised method. Each model was applied to its specific data set that had been standardized and preprocessed using principal component analysis (PCA). Additionally, A rule-based outlier detection method was implemented, based on thresholds for different hormones.

Main results and the role of chance: The three outlier detection models were evaluated using train, validation, and test sets, identifying approximately 1.5%, 1.5%, and 2% of the data as outliers, respectively. Additionally, each model was evaluated using other datasets, for example, the "trigger today" outlier detection model was evaluated using "trigger tomorrow" and "trigger in two days" datasets, which resulted in ∼12% and ∼87% of the data being identified as outliers. To further assess the models, additional subsets were created for different trigger times, such as in 3 days, 4 days, 5 days, and 6 or more days. The results of the "trigger today" outlier detection model were highly accurate in identifying outliers, with at least 99.5% of the data being detected as outliers in all of the additional subsets. Instances with a closer relative trigger time will result in similar instances, therefore, detecting a lower percentage of outliers.

Limitations, reasons for caution: The outlier detection models were developed specifically for antagonist protocol cycles. Additionally, there may be rare cycles that occur infrequently and may not be well represented in the data, resulting in them being incorrectly identified as outliers, even though they are a legitimate scenario.

Wider implications of the findings: Outlier detection is a crucial aspect of AI algorithms to ensure accurate results. It recognizes cases that are outside of the model's training set as well as abnormal values and typos, thus avoiding predictions on untested cases. This is especially important when first deploying algorithms on diverse populations.

Trial registration number: Not applicable

Abstract citation ID: dead093.1088
P-776 Impact of a poor-quality euploid blastocyst on the implantation of a good-quality one in double embryo transfers

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Study question: Does the transfer of a poor-quality blastocyst along with a good-quality blastocyst improve clinical outcomes compared to single good-quality blastocyst transfer in euploid-only transfer cycles?

Summary answer: Transferring a poor-quality with a good-quality blastocyst improved clinical pregnancy rates but live birth rates were not significantly increased compared to single good-quality blastocyst transfer.

What is known already: Single embryo transfers (SET) are often preferred when using euploid blastocysts with good morphological characteristics. However, co-transfer of a euploid blastocyst with poor morphological characteristics along with a good-quality euploid blastocyst is still practiced. Studies on embryos with unknown ploidy status suggested that poor-quality blastocysts may hinder the outcomes of good-quality blastocysts when transferred together. Controversial results imply that co-transfer of a poor-quality along with a good-quality blastocyst is associated with similar clinical pregnancy rates and significantly increases multiple pregnancy rates. Outcomes of such double embryo transfers (DET) compared to SETs are yet to be determined in euploid-only blastocyst transfers.

Study design, size, duration: Retrospective cohort of two centers including 500 SETs and 718 DETs using only euploid blastocysts in FET autologous cycles between March 2017 to January 2021. Transferred blastocysts were graded ≥BL3CC (Gardner criteria) and underwent trophectoderm biopsy on days 5-7 for aneuploidy testing with Next Generation Sequencing. Classification of blastocyst quality (good vs poor) was based on inner cell mass, trophectoderm grades, and the day of blastocyst biopsy.

Participants/materials, setting, methods: Patients underwent SET of good-quality or DET of good and poor-quality euploid-blastocysts. Endometrial preparation of frozen embryo transfer cycles was achieved with hormonal replacement or within a natural cycle. Reported outcomes included clinical pregnancy (CP), live birth (LB), and high-order pregnancy rates. For comparison of expected and observed LB rates, theoretical rates were estimated with Monte-Carlo simulations using binomial density function and SET data, under the assumption of independency for success rates of individual embryos.

Main results and the role of chance: After adjusting for Age, AMH, BMI, and cycle preparation method (artificial vs natural), DET of good-quality and poor-quality blastocysts was associated with higher CP rates compared to SET of a good-quality blastocyst (73.1% vs 63.8%, OR: 1.67, 95% CI: 1.12-2.55, $P = 0.014$). After adjusting for confounders, DET of good-quality and poor-quality blastocysts was not associated with significantly higher LB rates compared to SET of a good-quality blastocyst (56.8% vs 52.8%, OR: 1.25, 95% CI: 0.87-1.83, $P = 0.234$). Moreover, high-order pregnancy rates were significantly higher in the DET group compared to SET (40.4% vs 1.1%, $P < 0.001$).

Using a model based on SET data, we estimated theoretical LB rates that should be achieved if the LB chance of co-transferred embryos were independent. The model showed that the observed LB rates of DET good and poor-quality blastocysts were significantly lower than the expected average (observed 56.8% vs expected 66.4% [95% CI: 59.0 to 73.8%], $P = 0.0154$).

Limitations, reasons for caution: This study has a retrospective design, and estimates are susceptible to residual confounding from unobserved or unaccounted variables. Theoretical rates were estimated under the assumption of independency of transferred embryos.

Wider implications of the findings: Despite higher CP, DET of poor and good-quality blastocysts did not increase LB compared to SET of good-quality blastocyst and was associated with higher multiple-pregnancy rates. Lower than expected LB rates imply DET to be detrimental to cumulative-LB, and sequential-SET may be preferable, regardless of morphological quality of euploid blastocysts.

Trial registration number: Not applicable

Abstract citation ID: dead093.1089

P-777 Can endometrial compaction predict live birth/ongoing pregnancy rates in assisted reproductive technology cycles? A systematic review and meta-analysis

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Study question: Can endometrial compaction (EC) predict live birth/ongoing pregnancy rates (LBR/OPR) in assisted reproductive technology (ART) cycles?

Summary answer: Our meta-analysis shows that LBR/OPRs are similar in cycles with or without EC. Analysis of studies with euploid embryo transfers (ETs) also yield similar LBR/OPR.

What is known already: EC is a novel concept, defined as a change in endometrial thickness (EMT) between the end of follicular phase and ET day. Early studies implied that EC can be a predictor for improved LBR/OPRs in ART. However, subsequent studies presented conflicting results. The role of EC in predicting ART success remains undetermined and there is paucity of data whether a subgroup of women exists for whom EC may be predictive for ART success.

Study design, size, duration: We performed a systematic review and meta-analysis of studies reporting LBR/OP with regard to EC. Two independent authors searched electronic databases from the date of inception until January 29, 2023. Primary outcome was LBR/OPR per ET. Secondary outcomes were clinical pregnancy (CP) and miscarriage rates per ET. For dichotomous outcome measures, Mantel-Haenszel risk ratios (RRs); with 95% CIs are presented. A fixed or random effects model was used based on heterogeneity of the data.

Participants/materials, setting, methods: Initial search yielded 4816 studies. After exclusion through titles, 81 studies were assessed by reading their abstracts. Full text of 22 studies were evaluated. Finally, 16 studies involving a total of 13976 cycles were included in the meta-analysis. Included studies were further scrutinized based on the definitions for EC, fresh or frozen-thawed ET, endometrial preparation methods and utilization of preimplantation genetic diagnosis. Data for primary and secondary outcomes were extracted by two authors independently.

Main results and the role of chance: In our meta-analysis of studies using a cut-off value of 5% change in EMT to define EC, LBR/OPR was similar in cycles with and without EC [RR = 1.10, 95%CI = 0.97 to 1.24; 11 studies, 6157 cycles]. When no cut-off was implemented and the minimum EMT changes were taken into account for each study, LBR/OPR rates were comparable between cycles showing EC or not [RR = 1.04, 95%CI = 0.96 to 1.12; 16 studies, 13976 cycles]. In concordance, analysis of the six studies with 2710 cycles reporting only euploid embryo transfers yielded similar LBR/OPR for cycles with and without EC [RR = 1.01, 95%CI = 0.90 to 1.12]. LBR/OPR was comparable for cycles demonstrating EC or not when only cycles with artificial hormonal preparation for frozen-thawed ET were analyzed [RR = 1.09, 95%CI = 0.97 to 1.21; 11 studies, 5811 cycles]. Regarding the secondary outcome parameters, with a cut-off of 5% change in EMT, clinical pregnancy rates [RR = 0.98, 95%CI = 0.90 to 1.07; 8 studies, 5578 cycles] and miscarriage rates [RR = 0.91, 95%CI = 0.69 to 1.19; 8 studies, 3413 cycles] were similar between compaction and no-compaction cycles.

Limitations, reasons for caution: Around 3/4 of the studies are retrospective. While most studies define EC as ≥ 5% change in EMT, some used 0% or 10%. Moreover, some studies presented data as "compaction" and "no compaction" while others preferred "compaction", "same" and "expansion". We combined "no change" and "expansion" groups as the ''non-compaction'' group.

Wider implications of the findings: Once a promising predictor for ART success, EC does not seem to be helpful in predicting LBR/OPR and calculating EC may be obsolete. Moreover, analysis on euploid ETs imply that embryo, not EC, determines the outcome. Further studies, preferably on euploid ETs, with a homogeneous definition of EC are needed.

Trial registration number: Not applicablez

Abstract citation ID: dead093.1090

P-778 Same mother-different Oocyte: Comparison of obstetric and perinatal outcomes between pregnancies achieved through oocyte donation versus those achieved through spontaneous conception for the same mother

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Study question: Is there a difference in obstetrical and perinatal outcomes between pregnancies achieved through oocyte donation versus those achieved through spontaneous conception for the same mother

Summary answer: OD pregnancies show higher incidences of preterm labor and pregnancy-induced hypertension, and lower incidences of small for gestational age, compared to spontaneous pregnancies

What is known already: Oocyte donation (OD) is an integral part of modern assisted reproductive care and has been used for over three decades. OD pregnancies have an increased rate of hypertension, cesarean section, and preterm labor. Yet most of these complications are attributed to the mothers going through OD, not to the biological effect of different oocyte sources (autologous Vs. donation).

Study design, size, duration: This study is a retrospective cohort study that utilizes electronic data from Maccabi Healthcare Services. This 2.5-million-patient integrated care organization represents 25% of the pregnant population in the country. The data used in this study were collected from 2000 through 2018.

Participants/materials, setting, methods: The study included mothers who experienced both a spontaneous conception (SP) pregnancy and later an oocyte donation (OD) pregnancy. The study compared obstetric and perinatal outcomes, including the incidence of preterm labor (PTL) and small for gestational age (SGA). Mother's obstetric outcomes such as pregnancy induced hypertension (PIH), gestational diabetes, malpresentation, and postpartum hemorrhage were also analyzed. A paired t-test was performed to compare each outcome for the same mother

Main results and the role of chance: The cohort included 194 mothers who had a first spontaneous pregnancy and a second pregnancy with oocyte donation.

The mean age of the mother during her OD pregnancy was statistically older than the OD pregnancy (mean difference 2.2 years, $p = 0.0028$). No difference was found between pregnancies regarding the BMI ($p = 0.216$). A trend toward a higher prevalence of PIH was found in the OD pregnancy compared to the SP (mean difference 2.5, $p = 0.058$). No difference was found between pregnancies regarding gestational diabetes ($p = 0.7$), malpresentation ($p = 1$), retained placenta ($p = 0.318$), and Cesarean section ($p = 0.565$).

Perinatal outcomes show a higher prevalence of premature labor before the 34th week and 32nd week in the OD pregnancies compared to SC pregnancies (mean difference 3.6, $p = 0.034$ and mean difference 3, $p = 0.33$ respectively). No difference was found between pregnancies regarding labor before the 37th week ($p = 1$). A statistically lower prevalence of SGA was found in the OD pregnancy compared to the SP ($p = 0.0057$).

Limitations, reasons for caution: Due to its retrospective character, some information is lacking. Information regarding indications leading to OD/AO are missing which might have elaborated our findings

Wider implications of the findings: OD pregnancies should be considered high-risk, primarily due to the increased risk of PTL and PIH. Adequate recommendations should be considered, including cervical length surveillance, frequent blood pressure measurements, and perhaps prophylactic low-dose aspirin to reduce the risk of preeclampsia.

Trial registration number: 0046-18-BBL

Abstract citation ID: dead093.1091
P-779 School performance of children conceived by assisted reproductive technology

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Study question: Does elementary school performance differ between children conceived through assisted reproductive technology (ART) compared to children born to subfertile couples not conceived by ART?

Summary answer: Children born after ART perform equally well at age 12 (end of elementary school) compared to children born to subfertile couples not conceived by ART.

What is known already: It is well established that pregnancies conceived by ART are at higher risk of adverse outcomes, such as pre-term birth and a lower birth weight, also when restricted to singleton pregnancies. However, whether ART also affects outcomes in later life, such as school performance, is less clear. Although some studies indicate that there might be subtle differences in the school performance of ART and spontaneously conceived children, it remains unclear whether this is due to the ART treatment, parental factors or a combination of both.

Study design, size, duration: Data were used from the OMEGA-cohort, a historical nationwide cohort with prospective follow-up in the Netherlands. The cohort comprises all offspring of women who were treated in one of the 13 IVF clinics or 2 regional fertility centers in 1983-2010. Of 89,249 live-born children, 54,417 were ART-conceived and 37,832 were not ART-conceived (conceived naturally with or without ovarian stimulation) by subfertile women.

Participants/materials, setting, methods: Data on type of fertility treatment and maternal risk factors were available from medical records of the mothers and through the national perinatal registry. The OMEGA-cohort was linked to the education dataset of Statistics Netherlands, including school performance data for the period 2006-2017, leaving 24,806 children in the analytical cohort. The overall test Z-score and Z-scores on the separate domains (Dutch language and mathematics) between ART and non-ART children were compared using multivariable linear regression.

Main results and the role of chance: The cohort comprises 14,958 ART-conceived children and 9,848 non-ART children with test scores around age 12 in 2006-2017. The mean overall score was 536,5 (SD = 9.7), with scores of 536.4 (SD = 9.9) in ART-conceived children and 536.6 (SD = 9.5) in non-ART children.

After adjustment for maternal age and maternal and paternal educational level, in a multivariable linear regression model, the overall test Z-score was also not different among ART-children compared to non-ART children from subfertile parents (β=-0.02, 95% CI=-0.04-0.01). Overall test Z-scores were not different according to different ART treatment modalities, IVF (β=-0.02, 95%CI=-0.05-0.01), ICSI (β=-0.01, 95% CI=-0.05-0.02) and frozen embryo transfer (β = 0.07, 95%CI=-0.01-0.14) when compared to children born to subfertile couples not using ART. ART children performed equally well on mathematics and Dutch language compared to children born to subfertile couples not conceived by ART.

Limitations, reasons for caution: Although the current analyses include a large proportion of the ART children in the Netherlands with data on school performance and our study had sufficient power to address the research question, it is not clear to which extent our results are generalizable to other countries.

Wider implications of the findings: Reassuringly, based on first results, children born after ART had comparable test scores at the end of elementary school compared to children from subfertile couples not conceived by ART.

Trial registration number: Not applicable

Abstract citation ID: dead093.1092
P-780 Oocyte proteome and cumulus cell transcriptome analysis reveal oocytes live in a hypoxic microenvironment underlie the arrest of human early embryos in vitro culture

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Study question: Can cumulus cell transcriptome analysis combined with oocyte single-cell proteomics uncover the cause of human early embryos arrest during assisted reproductive treatment?

Summary answer: Oocytes living in a hypoxic microenvironment underlie the arrest of human early embryos at cleavage stage in vitro culture.

What is known already: Preimplantation embryo developmental potential largely depends on gamete quality in assisted reproduction treatment. Human embryonic genome major activation has occurred by the eight-cell stage, up to 3 days after fertilization. Therefore, to a large extent, the development competence of embryo at cleavage stage relies on oocytes quality, which acquire during follicle development. Cumulus cell (CC) plays an essential role in transmitting signals within the ovary and supporting oocyte growth and maturation. We used whole transcriptome analysis of cumulus cells (CC) and single-cell proteomics of oocyte to gain insights into the molecular mechanisms of embryo development arrest at cleavage stage.

Study design, size, duration: Fifty CCs samples and eleven oocyte samples were obtained from fifty-eight couples who underwent IVF/intracytoplasmic sperm injection (ICSI) treatment. The total CCs average age was 30.18 ± 4.51(control group) and 32.59 ± 3.43 (DA group). The total oocyte average age was 33.13 ± 4.83. Donated CCs were recruited between January 2018 and December 2019. Donated oocytes were recruited between September 2022 and October 2022.

Participants/materials, setting, methods: CCs samples were obtained from 33 patients whose rate of good-quality embryos is above 90% (as control) and 17 patients with more than 90% embryos fail to reach six-cell stage (Grade II) on day 3 (as development arrest, DA). Oocytes at GV and MI stage respectively were collected for proteomics. Differentially expressed genes were analyzed using the R package DESeq2 or DEP (filtered with q-value≤0.05 and Foldchange≥1.5). EGSEA package was used to perform pathway analysis.

Main results and the role of chance: RNA sequencing identified 18458 genes expressed in CCs of both groups, 31 of which were differentially expressed. CCs from patients whose embryos development arrested, contains 36 differentially expressed genes (DEGs) up-regulated and 4 DEGs down regulated. Gene ontology showed that up-regulated genes were significantly enriched with annotations related to oxidoreductase activity, mitochondrial respiratory chain complex IV and response to hypoxia. TF enrichment analysis found out that up-regulated DEGs was enriched in hypoxia-inducible factor 1 (*HIF-1*) induced transcription. It has been reported that HIF-1-mediated gene expression can be elicited by hypoxia, which could restrict mitochondrial biogenesis and ATP-dependent cellular processes. So, we supposed that oocytes live in an ultra-hypoxic microenvironment underlie human early embryos arrest. In consideration of the difficulty in detection of the oxygen level in antral follicles cavity, the protein level changes of oocytes in DA group were used to reflect oocyte status. There were 47 proteins up-regulated and 105 proteins down-regulated at GV stage; 55 gene up-regulated and 48 genes down-regulated at MI stage. Among these, overlap with up-regulated genes in both stages included tuberous sclerosis complex1(TSC1) and Neutral amino

acid transporter A (ASCT1), which play key roles in cellular response to oxygen-glucose and hypoxia stress response.

Limitations, reasons for caution: Because of the lack of credible data reflecting accurately the dissolved O_2 concentration adjacent to an oocyte. Determination of dissolved O_2 within antral follicles is fraught with technical difficulties. We can just verify our hypothesis by single-cell proteomics of oocyte and Ultra-Low-oxygen treatment *in vitro*.

Wider implications of the findings: We provide a new hypothesis that hypoxic follicular harmed oocyte quality, causing human early embryos arrest. The DEGs we detected in CCs and oocyte could be the biomarker candidates for embryo developmental potential. Ovarian stimulation protocols for embryo development arrested patients could consider diminishing oocyte exposure time in hypoxic follicles.

Trial registration number: Not applicable

Abstract citation ID: dead093.1093
P-783 Obstetric and neonatal outcomes after single vitrified-thawed vs. fresh blastocyst transfer: a population-based linkage study

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Study question: Does the transfer of a single vitrified-thawed blastocyst optimise obstetric and neonatal outcomes compared to a single fresh blastocyst transfer?

Summary answer: A single vitrified-thawed blastocyst transfer is associated with a lower risk of preterm delivery and small-for-gestational-age babies but a higher risk of large-for-gestational-age babies.

What is known already: Several studies have been performed comparing the outcomes of ART pregnancies after fresh or frozen embryo transfers. Nevertheless, a closer examination of the available studies indicates a significant heterogeneity in terms of the clinical protocols they compare, predominantly in regards to the developmental stage of the embryos (cleavage vs. blastocyst), the number of embryos transferred (single vs. multiple) and the cryopreservation method used (slow-freezing vs. vitrification). The use of single blastocyst transfer and vitrification is currently considered the gold standard of care but relevant studies on the safety of these technologies regarding the obstetric and neonatal outcomes are lacking.

Study design, size, duration: Autologous ART cycles where a single Day 5/6 embryo was transferred were identified (n = 188,730) in the Australian ART registry (ANZARD) and these were linked with 20,214 birth records (deliveries >20 weeks of gestation or with a weight of > 400gr of the Perinatal Data Collection which had taken place in two Australian States between 1st of January of 2009 and 31st of December of 2016. Cycles including preimplantation genetic testing or assisted hatching were excluded.

Participants/materials, setting, methods: Two cohorts were formed, the Fresh group, containing 89,299 ART treatment cycles linked to 11,138 PDC birth records, and the Vitrified-Thawed (VT) group, containing 75,596 ART treatment cycles linked to 7,518 PDC birth records. Comparisons of obstetric and neonatal outcomes between these groups were performed using generalized estimating equations to account for the clustered nature of data while also accounting for multiple confounders. Significance level was set at 0.01 to correct for multiplicity of testing.

Main results and the role of chance: The risk of preterm delivery was significantly lower in the VT group compared to the fresh group (adjusted relative risk-aRR: 0.78, 99% CI 0.69-0.88). This persisted in a sensitivity analysis (accounting for the embryo ranking within each cohort) by comparing pregnancies occurring after the first vitrified-thawed embryo transfer following a freeze-all cycle (VTFA) with the fresh ET group (aRR: 0.71, 99% CI: 0.54-0.94). The probability of pregnancy-induced hypertension was not significantly different between the two groups (aRR: 1.15, 99% CI: 0.99-1.34). There was also no indication of an increased risk of PIH when the VTFA group was compared to the fresh ET group (aRR: 0.94, 99% CI: 0.68-1.30). The probability of SGA was 35% lower in the VT group compared to the fresh ET group (aRR: 0.65, 99% CI: 0.56-0.74). When only VTFA babies were compared with the fresh ET group, then the probability of SGA was 20% lower, but this was not statistically significant. For LGA babies, the risk was significantly higher (~45%) in the VT group compared to the fresh ET group (aRR: 1.46, 99% CI: 1.30-1.65). The LGA risk remained significantly higher in the VTFA compared to the fresh ET group (aRR: 1.34, 99% CI: 1.04-1.72).

Limitations, reasons for caution: This is a population-based study using linked administrative datasets; therefore not all confounders could be accounted for. Furthermore, the smaller sample size of the sensitivity analysis combined with the strict level of significance adopted might have rendered some sensitivity analyses underpowered particularly for rare outcomes

Wider implications of the findings: This analysis shows the differences in obstetric and neonatal outcomes between fresh and frozen embryo transfers studying vitrification and single embryo transfer exclusively. For certain outcomes, it appears that the effects are modified when the embryo ranking within each cohort is accounted for.

Trial registration number: Not applicable

Abstract citation ID: dead093.1094
P-784 First trimester screening for preterm preeclampsia after ART: high risk, high gain?

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Study question: What is the preterm preeclampsia (pPE) incidence in selected high-risk ART patient populations when applying a first trimester screening approach to recommend prevention via aspirin?

Summary answer: One in five high-risk ART patients had a calculated risk >1/100 and despite aspirin prophylaxis still 22.2% of them developed pPE.

What is known already: Preeclampsia (PE) is an obstetrical complication defined by new-onset hypertension after 20 weeks' gestation with proteinuria, end-organ (including placental) dysfunction or both. First trimester pPE screening is gaining ground in prenatal care since a study on approximately 60.000 singleton pregnancies reported an algorithm able to detect 76% of pPE and 38% of term PE (tPE). A RCT showed that aspirin, prescribed upon positive screening, led to an effective (62%) reduction of pPE incidence. This screening/prevention strategy has not been validated in the context of ART pregnancies, while some ART patient groups are known to be at higher risk for PE.

Study design, size, duration: This is a retrospective, single-center, observational cohort study, including 242 patients between 18 and 48 years old, pregnant after ART and considered at higher risk of PE because of one of the following characteristics: diagnosed with polycystic ovary syndrome (PCOS), being an oocyte recipient (OR) or pregnant from a frozen embryo transfer in an artificially prepared cycle (HRT-FET). Patients were included from May 2021 until July 2022.

Participants/materials, setting, methods: Screening was systematically offered between 11-14 weeks' gestation, according to the Fetal Medicine Foundation (FMF) algorithm including maternal factors, mean arterial pressure, uterine arteries pulsatility index and serum placental growth factor. If the risk of developing PE was >1/100, administration of 160mg aspirin was started until 36 weeks' gestation. Primary outcome was the incidence of pPE(<37 weeks). Secondary outcomes were the incidence of tPE(≥37 weeks) and the incidence of positive first trimester screening (risk >1/100).

Main results and the role of chance: A total of 242 unique patients were screened: 58 oocyte recipients (ORs), 89 PCOS and 95 HRT-FET patients (the latter having no PCOS and using autologous oocytes).

The overall positive screening incidence was 21.9%(53/242), with the highest positive screening rate in ORs (27.6%, 16/58). In PCOS and HRT-FET patients, we observed a positive screening test in 20.2%(18/89) and

20.0%(19/95), respectively. *For comparison, the positive screening rate reported for the general population was 10.5%.*

Despite the prophylactic aspirin regimen started upon positive screening, in the current study an overall pPE incidence of 22.6%(12/53) was observed *while the reported pPE incidence in the general population in this setting was only 1.6%.* More specifically, 31.2%(5/16) ORs with positive screening developed pPE although they used aspirin. For PCOS and HRT-FET patients, this was 11.1%(2/18) and 21.1% (4/19), respectively. tPE occurred in 7.5%(4/53) of women with positive screening and aspirin intake (1/16 in ORs, 1/18 in PCOS patients and 2/19 following HRT-FET). *For comparison, in the general population this was 6.7%.*

Following a negative first trimester screening test, 2.1%(4/189) of patients developed pPE and 3.7%(7/189) tPE. *In a non-selected population, these incidences have been reported to be 0.2% and 1.1%, respectively*

Limitations, reasons for caution: This study is limited by its retrospective study design, small sample size and observational nature. Nevertheless, it is of major importance to communicate the strikingly high pPE incidences in specific subtypes of ART patients despite first trimester screening and aspirin prophylaxis.

Wider implications of the findings: Our findings suggest that current screening algorithms are not optimized for women pregnant following ART. In pregnancies from oocyte donation, aspirin might be less effective. Future research should aim at finetuning PE screening methods specifically for ART patients and differential mechanisms of PE origin need to be further investigated.

Trial registration number: Not applicable

Abstract citation ID: dead093.1095

P-785 Promising Reproductive Outcomes In Patients With Refractory Thin Endometrium After Autologous Bone Marrow Regenerative Cell Therapy (ABM-RCT) – A Case Series

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Study question: Does Autologous Bone Marrow Regenerative Cell Therapy (ABM-RCT) improve endometrial thickness and receptivity and restoration of fertility in refractory cases of thin endometrium?

Summary answer: Selective Autologous Bone Marrow Regenerative Cell Therapy from Seragen (ABM-RCT) improves endometrial thickness, pregnancy, and live birth rate in refractory cases of thin endometrium.

What is known already: The impaired endometrial function can limit implantation due to insufficient tissue regeneration. Clinical investigation has proved that stem cells existing in the endometrium might originate from bone marrow and provides evidence that bone marrow-derived cells have a non-hematopoietic physiologic contribution to decidual stroma and play a vital role in implantation and pregnancy maintenance and non-hematopoietic BMSCs are able to impact decidual molecular milieu and overcome implantation defects. ABM-RCT is the most commonly used cell therapy and its safety and efficacy are well-established and documented. This forms the basis for exploring and standardizing ABM-RCT in the management of refractory thin endometrium.

Study design, size, duration: Eleven patients (from age 29 to 43 with refractory thin endometrium resistant to conventional treatment modalities were recruited and obtained informed consent with detailed explanation that the therapy is still experimental and the risk of failure was given to the couple as part of gynec-oncology treatment. Five patients had asherman's syndrome, two patients had genital tuberculosis, two patients had thin endometrium hypo-responsive/unresponsive to estrogens, with RIF and two patients underwent chemo and radiotherapy

Participants/materials, setting, methods: BM aspiration was performed under local anesthesia, from the iliac crest using a disposable BM aspiration needle (Jamshidi, 11 G) and collected in heparinized syringes. Progenitor cell enrichment was done by Seragen's Selective Enrichment Protocol. Peripheral blood was collected to enrich growth factor concentrate. A 2.9mm Hysteroscope was used with an operating channel and egg pickup needle attached to it and cells were implanted in subendometrial zone in all four walls of the uterine cavity.

Main results and the role of chance: All patients presented to us with refractory thin endometrium in HRT cycle despite giving all possible medications. Hence the decision was taken for Autologous Bone Marrow Cell Concentrate Therapy.Post ABM-RCT, endometrial thickness showed increase 100% (11/11) of more than 8 mm in all cases on day of embryo transfer with average endometrial thickness improvement was 1.8 mm than previous cycles, with uniform triple layer pattern.10 out of 11 (91%) patients conceived after autologous stem cell injection. 9 Out of 10 (90%) patients conceived had delivered healthy babies and 1 patient (9%) had a miscarriage at 12th week of pregnancy. 1 patient did not conceive will be assessed for immunoprofiling and one more rejuvenation cycle.

Limitations, reasons for caution: Our study outcomes are consistent with several previous studies and the feasibility of treatment at an IVF setup and encouraging results. Well-planned study with more patients is warranted to evaluate safety, effectiveness, and cost of this modality before it becomes integrated in treatment of this frustrating condition during IVF procedures

Wider implications of the findings: Our results demonstrates selectively enriched ABM-RCT enhances endometrial thickness, tissue remodeling, uneventful gestation, improved pregnancy and live birth rate. Need for Surrogacy in Non-Responsive Thinendometrium May Be Re-Evaluated. Role of hematopoetic stem cell research and knowledge generated is representing reliable strategy in IVF that may be routinely used in clinicalpractice.

Trial registration number: Not applicable

Abstract citation ID: dead093.1096

P-787 Ovarian morphology and length of follicular phase differ between immediate and postponed modified natural-cycle frozen embryo transfer (mNC-FET) cycles – Sub-study of an ongoing RCT

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Study question: Does ovarian morphology and length of the follicular phase differ between immediate and postponed mNC-FET cycles?

Summary answer: More cystic follicular residue after oocyte pick-up was observed at cycle day 2-5 in immediate vs postponed mNC-FET. The immediate follicular phase was longer.

What is known already: Whether the optimal timing for treatment with mNC-FET is in the cycle immediately following ovarian stimulation (OS) and oocyte pick-up, or in a subsequent cycle, has been much debated. Recent evidence suggests that reproductive outcomes after immediate vs postponed FET are comparable or even better in programmed-cycle FET. Due to concerns about suboptimal ovarian and endocrinological conditions in the natural cycle immediately following an OS/IVF cycle, postponed FET has become the standard treatment in most settings. However, studies describing attributes of the immediate NC-FET are lacking and little is known about cycle characteristics and ovarian morphology shortly after oocyte pick-up.

Study design, size, duration: The present descriptive sub-study is based on data from an ongoing Danish, multicentre, randomised controlled trial (RCT), investigating if mNC-FET can be performed in the cycle immediately following OS and oocyte pick-up, without compromising pregnancy and live birth rates. Participants were randomized 1:1 to mNC-FET in the immediate

vs a subsequent cycle. The first 102 participants were included in the present sub-study. Data was collected between April 2021 and December 2022.

Participants/materials, setting, methods: Women with a regular menstrual cycle, aged 18-40 years, undergoing single blastocyst mNC-FET were eligible for inclusion. Ovarian morphology and cycle length were compared between immediate and postponed mNC-FET using Chi-squared test for categorical variables, and independent sample T-test or Mann Whitney U-test for continuous variables. Categorical variables were reported as numbers and percentages, continuous variables as mean and standard deviation or median and range.

Main results and the role of chance: Background characteristics including age, BMI, AMH and normal cycle length were similar for women in the immediate and postponed group, apart from a lower rate of elective freeze all-transfers (30.2% vs 55.1%, $p = 0.011$) in the OS cycle preceding FET, in the immediate vs the postponed group. The total number of cystic follicular structures (hypo- and non-hypodense) >10 mm (2 (range 0-11) vs 0 (range0-3), $p = <0.001$) were higher in the immediate vs the postponed group on cycle day (CD) 2-5 of the treatment cycle. On the day of hCG-trigger, there was no significant difference in the total number of cystic follicular structures between the groups, but a higher number of non-hypodense structures was found in the immediate group ($p = 0.021$). Endometrial thickness was greater in the immediate vs postponed group (8.6 vs 7.8 mm, $p = 0.031$) while the mean size of the dominant follicle was similar 17.1 vs 17.3 mm between groups ($p = 0.410$). The average day of hCG-trigger was CD15 (range 9-24) in the immediate group compared to CD12 (range 5-28) in the postponed group ($p = 0.001$). More ultrasound scans of follicular development were needed in the immediate vs postponed group (3 vs 2, $p = 0.012$).

Limitations, reasons for caution: The proportion of elective freeze-all in the OS cycle preceding FET differed between the immediate and postponed group which may bias the results. The sample size limits stratified analyses.

Wider implications of the findings: The findings of this study indicate that cystic follicular ovarian structures shortly after oocyte pick-up are commonly occurring. However, most of these structures seems to regress before the time of ovulation. The follicular phase was longer in immediate cycles, and whether this effects pregnancy outcomes is yet unknown.

Trial registration number: NCT04748874

Abstract citation ID: dead093.1097
P-788 Embryo vitrification affects the epigenome of preimplantation embryos and the effects are inherited by the female offsprings.

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Study question: How does embryo vitrification regulates the transcriptome of preimplantation embryos through epigenetic regulation and how are the effects transmitted to the female offsprings?

Summary answer: Embryo vitrification changed the DNA methylation level of preimplantation embryo and disturbed glycolipid metabolism of female offsprings in mice.

What is known already: Vitrification is a type of embryo cryopreservation increasingly frequently used clinically. The effect of embryo freezing on the health of offspring has been widely concerned. Some clinical studies have shown that offspring obtained from frozen embryo transplantation has higher birth weight and may be at higher risk of certain birth defects, increased risk of type 1 diabetes and cancer. But not all studies agree. The offspring safety of vitrification needs further follow-up research. Mouse model is a helpful tool for us to carry up follow-up study and explore mechanism.

Study design, size, duration: After superovulation and mating of ICR mice, we obtained 8-Cell embryos. In Vitrify Group, the 8-Cell embryos underwent vitrification and thawing and culture in vitro till blastocysts. In InVitro Group, the 8-Cell embryos were directly cultured in vitro till blastocysts. We transplanted these blastocysts into surrogate mice to get offspring of InVitro and Vitrify. Along with offspring of natural conception (NC), we observed their growth and development and glycolipid metabolism until 18 weeks old.

Participants/materials, setting, methods: Single embryo RNA-seq and Whole Genome Bisulfite Sequencing (WGBS) were performed to get transcriptome and DNA methylation atlas. Immunofluorescence of blastocysts was performed to test level of 5mC/5hmC and Tet2. Glucose and insulin tolerance test (GTT/ITT), serum lipid level test were performed on offspring to examine the glycolipid metabolism status. Liver transmission electron microscope photographs were take to observe ultramicrostructure. Liver RNA-seq and WGBS were performed to verify the variation on pathways concerning glycolipid metabolism.

Main results and the role of chance: Pseudo-time analysis from RNA-seq of 16-Cell, morula and blastocyst showed a relative developmental time lag in Vitrify. Differentially expressed genes (DEGs) of vitrify group at the blastocyst stage are mainly enriched in lipid metabolism, insulin signal, TOR signal regulation, gamete production related DNA methylation pathways. GSEA analysis revealed down-regulation of insulin response pathway genes. WGBS showed a trend of genome-wide methylation increase in vitrify group. Immunofluorescence confirmed this observation as the fluorescence intensity ratio of 5mC/5hmC was increased. RNA-seq, qPCR and western-blot showed a lower expression level of Tet2. These results suggested that the demethylation was insufficient in vitrify group. Blastocyst differentially methylated genes (DMRs) from WGBS were enriched in insulin response pathways. In offspring, birth weight of Vitrify offspring is higher than InVitro. GTT and ITT showed an elevated level of AUC in Vitrify 10-week-female, indicating an impaired glucose tolerance. And the fasting serum cholesterol level was elevated in Vitrify 10-week-female, indicating an impaired lipid metabolism. Under transmission electron microscope, we saw swollen mitochondria in liver of Vitrify 10-week-female. We extracted RNA from 10-week-female for RNA-seq, DEGs were enriched in cholesterol biosynthesis pathway. We extracted DNA from 10-week-female for WGBS, DEGs were enriched in cAMP and AMPK signaling pathway.

Limitations, reasons for caution: We have not confirm the precise molecular site affected by vitrification, clarify the mechanism and conduct the rescue test yet, which we are currently working on. This study is just the first step in this direction, and more efforts are needed.

Wider implications of the findings: Although more clinical trials and basic science studies are required, our findings provide valuable information for preventive and clinical decisions, as 'freeze-all' strategy springs up in many centers. Embryo vitrification needs more cautious consideration. Optimizing embryo vitrification protocols are needed according to the mechanism to minimize unwanted effects.

Trial registration number: 2021YFC2700601

Abstract citation ID: dead093.1098
P-790 Are children born in England after subfertility and fertility treatment at increased risk of autism and attention deficit hyperactivity disorder?

Abstract withdrawn by the authors

POSTER VIEWING
STEM CELLS

Abstract citation ID: dead093.1099
P-791 Supplementation of mitochondria from endometrial mesenchymal stem cells (EnMSCs) improves oocyte quality in aged mice

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Study question: To improve the quality of aging oocytes by supplementing the mitochondria from endometrial mesenchymal stem cells (EnMSCs) via microinjection.

Summary answer: Supplementation with EnMSC-derived mitochondria can improve the quality of oocytes and promote embryo development in aging mice.

What is known already: Maternal aging is one of the major causes of reduced ovarian reserve and low oocyte quality in elderly women. Decreased oocyte quality is the main cause of age-related infertility. Mitochondria are multifunctional energy stations that determine the oocyte quality. The mitochondria in aging oocytes display functional impairments with mtDNA damage, which leads to reduced competence and developmental potential of oocytes. To improve oocyte quality, mitochondrial supplementation is carried out as a potential therapeutic approach. However, the selection of suitable cells as the source of mitochondria remains controversial.

Study design, size, duration: Study design: Eight-month-old ICR mice were purchased and then were further raised to ten months of age for use in the experiments. EnMSCs were stably cultivated and oocytes were extracted from ten-month-old ICR mice.

Study size: About 300 GV oocytes and 134 MII oocytes were counted . 80 fertilized oocytes were transplanted into the fallopian tubes of surrogate mothers.

Study duraton: About one half a year.

Participants/materials, setting, methods: Participants:ten-month-old ICR mice.

Setting and methods: We cultivated EnMSCs from aged mice and extracted mitochondria from EnMSCs. And then GV oocytes were supplemented with mitochondria via microinjection. To further assess the effects of mitochondrial supplementation on embryo development, MII oocytes from aging mice were fertilized by ICSI combining EnMSCs mitochondria microinjection.To test whether mitochondrial supplementation could improve *in vivo* embryo development in aging mice, embryo transplantation was performed after mitochondrial microinjection combined with ICSI.

Main results and the role of chance: In this study, we found that the mitochondria derived from EnMSCs could significantly improve the quality of aging oocytes.Supplementation with EnMSC mitochondria significantly increased the blastocyst ratio of MII oocytes from aging mice after intracytoplasmic sperm injection (ICSI). We also found that the birth rate of mitochondria-injected aging oocytes was significantly increased after embryo transplantation.

Limitations, reasons for caution: Microinjection into oocytes was invasive and some oocytes died after microinjection. The ability of this method to improve oocyte quality was limited because the MII rate of oocytes after mitochondria supplementation was 35.65 ± 1.75% while the rate of oocytes from 6–8 weeks of mice in a previous study was nearly 80%.

Wider implications of the findings: For clinical treatment, the EnMSCs can be obtained from the patient's own menstrual blood as the sources of mitochondrial supplementation. A noninvasive procedure for cell extraction ensures safety, and the findings of this work might provide a valuable strategy for patients with declining oocyte quality due to aging.

Trial registration number: Not applicable

Abstract citation ID: dead093.1100
P-792 The effect of subendometrial injection of autologous platelet rich plasma (PRP) guided by hysteroscopy in recurrent implantation failure patients - A pilot study

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Study question: Does the subendometrial injection of autologous platelet-rich plasma (PRP) guided by hysteroscopy achieve successful pregnancy outcome in recurrent implantation failure patients?

Summary answer: In this study we observed that subendometrial injection of autologous PRP is associated with higher implantation rates in recurrent implantation failure patients.

What is known already: The key success factors in IVF are to have a good endometrium lining and vascularity for successful implantation. These two factors directly affect the pregnancy outcomes of IVF.

Although several approaches have been attempted to address these factors including estrogen treatment, aspirin, sildenafil, vitamin E, Granulocyte-Colony Stimulating Factor (G-CSF), there is no efficient method described. Additionally, some cases do not show a response to the medical treatment. In those cases, specifically, in previously failed IVF patients autologous Platelet Rich Plasma (PRP) injection into the sub-endometrial cavity with an ovum pick up needle has been preferred, as confirmed by literature.

Study design, size, duration: An open label, single center study. Total 130 women were selected for hysteroscopic instillation of PRP, all patients have previous unsuccessful IVF attempts in our clinic or were referred to our clinic. Study participants are of age 23 to 49 years. Study began in July 2020 and was completed in December 2022.

Participants/materials, setting, methods: 2 ml of autologous PRP was instilled in the junctional zone using a wallace needle through the hysteroscope's operating channel to inject the PRP beneath the superficial endometrium from day 6-14 of menstrual cycle. FET preparation was started from Day 2/3 of next menstrual cycle using 6 mg/daily estradiol hemihydrate (EV Active Tab CORONA Remedies).

Main results and the role of chance: Women who received hysteroscopic instillation of PRP in the sub-endometrial junction had higher pregnancy rates (82/130 [63.07%] (P-value = <0.05). In the subsequent cycle monitored to day 15, Endometrial thickness (ET) was 7 mm or thicker in 90 of 130 patients [69.23%] (P-value = <0.05) and 6-7 mm in 8 of 130 patients [6.15%]. Endometrial thickness did not improve in 32 of 130 patients [24.61%] and remained below 6 mm. Subendometrial blood flow increased significantly in 92 of 130 patients [70.76%] (P-value = <0.05). The mean increase in ET was 1.5 to 2 mm.

The instillation of PRP caused no side effects and was well tolerated by all patients. In 47 patients (36.15%) we did not achieve clinical pregnancy while 1 had a biochemical pregnancy

Based on paired 't' test, it is concluded that there is statistically significant improvement in ET, vascularity and successful pregnancy outcome after PRP instillation.

Limitations, reasons for caution: Little is known about the best dosage and time for the instillation of PRP in endometrium. The importance of standard yet simple techniques and a highly repeatable protocol for clinical use is deciding factor which yet to finalize. It involves invasive procedure so may cause infections and anesthesia related complications.

Wider implications of the findings: Although PRP has still not gained universal acceptance in previously failed IVF patients for a variety of reasons, emerging evidences suggests that PRP could be an effective option compared to conventional approach especially, in patients with thin endometrium, advanced maternal age and primary ovarian insufficiency.

Trial registration number: Not applicable

Abstract citation ID: dead093.1101
P-795 Identification of a specific paternal haplotype in the germline and perpetuating progenies by male gamete replication

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Study question: Can we identify and replicate a male germ cell genome to generate progenies carrying a specific genotype?

Summary answer: We were able to replicate male genome and identify the androgenote with a specific genotype to generate conceptuses with identical paternal haplotype.

What is known already: For patients with heritable genetic disorders, current treatment relies on preimplantation genetic testing for monogenic disorders (PGT-M) to select unaffected embryos for replacement; however, this can lead to embryo wastage, causing emotional distress. Although genomic editing of gametes is being attempted, CRISPR-based technologies are inaccurate and can lead to loss of heterozygosity in cases where the embryo uses the maternal genome as a template to edit the paternal genome. Therefore, the use of haploid androgenotes may offer a useful tool to select male gametes with genes of interest or a successfully edited genome before the creation of an embryo.

Study design, size, duration: To generate haploid androgenotes, spermatozoa from hemizygous transgenic mice with GFP expression (B6-EGFP x B6D2F1) were used to inseminate enucleated wildtype oocytes. Constructs were cultured to the 8-cell stage. Two pseudo-blastomeres were isolated for genotyping by DNAseq. A blastomere with GFP expression was used as a male gamete. Experiments were repeated using mice with the *Fbn1*^{tm1Hcd} mutation as a model for Marfan syndrome. Biparental embryos were created to preserve the mutation as a disease model.

Participants/materials, setting, methods: To generate haploid androgenotes, metaphase II oocytes from wildtype B6 mice were enucleated and inseminated using spermatozoa from hemizygous GFP transgenic mice. Haploid androgenetic embryos were cultured in a time-lapse incubator up to the 8-cell stage. Biparental embryos were created by fusing a selected androgenote with an activated oocyte from another cohort. Resulting embryos were genotyped to confirm the paternal haplotype. Experiments were reproduced using spermatozoa from heterozygous *Fbn1*^{tm1Hcd}-mutant mice.

Main results and the role of chance: In the first set of experiments using spermatozoa from transgenic mice hemizygous of GFP, 200 wildtype oocytes were enucleated and generated 195 (97.5%) ooplasts. Insemination of those ooplasts yielded 168 (86.2%) monopronucleated androgenetic embryos, and subsequently generated 116 (69.0%) 8-cell haploid androgenetic embryos, of which 59 (50.8%) expressed GFP. Up to 2 sibling blastomeres were genotyped individually by DNAseq and confirmed to have an identical paternal haplotype. The remaining blastomeres were used to create biparental embryos and yielded a 97.2% fertilization rate after grafting into 145 oocytes, comparable with the fertilization rate of ICSI controls at 95.6%. Although control zygotes yielded an 88.0% blastulation rate, the experimental embryos yielded a lower blastulation rate of 58.2% ($P<0.0001$). Albeit with a lower developmental rate, all experimental blastocysts expressed GFP and displayed normal embryo morphokinesis.

For the experiment using spermatozoa from *Fbn1*^{tm1Hcd} mice, 50 8-cell stage haploid androgenetic embryos were generated. Two individual pseudo-blastomeres from 35 haploid embryos were used for genotyping. The remaining 210 haploid blastomeres were used to construct biparental embryos and yielded 119 (56.7%) blastocysts. PCR on embryo biopsies confirmed that the resulting blastocysts inherited the mutant (n = 63) or wildtype (n = 56) paternal haplotype from the original gamete.

Limitations, reasons for caution: This technique allowed the selection of male gametes with the desired genotype to generate conceptuses with a known haplotype in a reproducible manner. A more prospective experiment is ongoing to simulate clinical application by cryopreserving haploid androgenetic embryos and using only androgenotes with a confirmed genotype to create biparental embryos.

Wider implications of the findings: This approach to utilize gamete substitutes before fertilization ensures the maintenance of rare and unique genetic materials to generate conceptuses with the desired genotype in transgenic mice in disease models. Indeed, once this technique is implemented in humans, genotyped androgenotes can be used to characterize germline heterozygosity.

Trial registration number: N/A

Abstract citation ID: dead093.1102

P-796 Single-cell transcriptomics unveils a role for leptin receptor in human endometrial mesenchymal stromal/stem cells

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Study question: What are the characteristics of leptin receptor (LepR) expressing cells within the endometrial mesenchymal stromal/stem cells (eMSCs) population?

Summary answer: LepR$^+$ eMSCs display better stem-cell capacities as well as distinctively quiescent properties compared to eMSCs and LepR$^-$ eMSCs.

What is known already: Single-cell RNA sequencing (scRNA-seq) reveals that cultured eMSCs are heterogeneous. LepR is highly expressed in bone marrow MSCs, and LepR$^+$ cells contribute towards the formation of fibroblast colony-forming units (CFU-F). Mouse LepR$^+$ MSCs are quiescent and activate upon injuries to form osteoblasts and adipocytes in the bone marrow. In this study, we hypothesized that LepR$^+$ cells play a critical role in the stem cell population in human endometrium.

Study design, size, duration: We performed scRNA-seq on magnetic bead selected eMSCs (CD140b$^+$CD146$^+$ cells). Functional stem cell assays such as clonogenicity and serial subcloning were performed on the different stromal subsets (eMSC, LepR$^-$ eMSC, and LepR$^+$ eMSC) isolated by fluorescence-activated cell sorting (FACS) cultured endometrial stromal cells. The cell cycle analysis was evaluated by multicolor flow cytometry on freshly isolated endometrial stromal cells.

Participants/materials, setting, methods: The endometrial samples were collected from women aged 37–53 years with regular menstrual cycles undergoing hysterectomy. The expression and localization of LepR in human endometrium were determined by flow cytometry and immunohistochemistry, respectively. The *in vitro* colony-forming and self-renewal abilities were assessed on FACS sorted endometrial stromal populations. The cell cycle status of the stromal subsets was determined by flow cytometry and the quiescent property was determined by qPCR (*RB1, RBL2, CDKN1A, CDKN1B*, and *E2F4*).

Main results and the role of chance: Clustering analysis of the scRNA-seq data revealed 5 subpopulations (SP0-4) of cultured eMSCs. A high proportion (>90%) of the cells in SP3 and SP4 were at the G1 phase. The majority of the SP3 cells (92%) were located at the root of the cell trajectory and pseudo time inference analysis.

The expression of LepR was highly specific in SP3 suggesting LepR eMSCs are potentially the origin progenitors.The expression of LepR in endometrial stromal cells was 2.61 ± 1.43%, and in eMSCs was 25.59 ± 19.04%. *In vitro* experiments demonstrated that LepR$^+$ eMSCs exhibit better stem-cell properties, including clonogenic and self-renewal ability, than its negative counterparts.

QPCR validation of cell-quiescence associated genes revealed higher expression of *RB1*, *RBL2*, and *CDKN1B* in the LepR$^+$ eMSCs than eMSCs and LepR$^-$ eMSCs. In addition, multicolor flow analysis on freshly isolated endometrial cells revealed significantly higher proportion of LepR$^+$ eMSCs are in the G0 phase of the cell cycle when compared to eMSCs and LepR$^-$ eMSCs. The above results indicate in vivo LepR$^+$ eMSCs are relatively more quiescent.

Limitations, reasons for caution: The mechanism involved in maintaining or activating quiescent LepR$^+$ eMSCs remains unknown. The role of LepR$^+$ is likely to be different in human compared to mouse. We also lack an *in vivo* model for studying LepR$^+$ eMSCs during endometrial repair.

Wider implications of the findings: Our findings extend the understanding of heterogeneous human endometrial mesenchymal stem cells and unveil a new role for LepR in endometrial regeneration.

Trial registration number: not applicatble

Abstract citation ID: dead093.1103

P-797 Single-cell analysis sheds light on investigation of epithelial stem/progenitor cells in human endometrial organoid

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Study question: How to find endometrial epithelial stem cell (eeSC) in the human endometrial gland?

Summary answer: Endometrial organoid culture model combined with omics techniques can be a promising platform for investigating eeSC functions and endometrial gland development.

What is known already: Endometrial stem/progenitor cells are thought to be involved in endometrial regeneration, with endometrial stromal stem cells having already been extensively studied. On the other hand, study of human eeSC is challenging due to the lack of specific markers to isolate and examine their functional properties. In addition, the *in vivo* phenotype of primary endometrial epithelial cells cannot be maintained for a long duration in two-dimensional culture. The development of three-dimensional endometrial organoid model provides suitable alternatives for studying eeSC, as they can be tailored to be biomimetic and accurately recapitulate the native *in vivo* scenario of the glandular epithelial cells.

Study design, size, duration: This is a basic science study of endometrial organoids. Endometrial biopsies were performed on day 2 after the injection of human chorionic gonadotrophin in women undergoing in vitro fertilization treatment (IVF) but having no fresh embryo transfer in Queen Mary Hospital, The University of Hong Kong. Endometrial organoids were derived from human endometrial gland fragments. The established organoids were digested into single cells for droplet-based single-cell sequencing to investigate their cellular population and component.

Participants/materials, setting, methods: The filtered high-quality single-cell analysis data were clustered and assigned to specific cell types according to their expressions of classical markers. Potential stem cells cluster was identified by trajectory analysis, RNA velocity, *CytoTRACE* and literature search. The existence of specific eeSC markers was validated by immunofluorescence staining and flow cytometric analysis of primary endometrial tissues/endometrial organoids. The clonogenicity of the isolated eeSCs was determined by clonogenic assay.

Main results and the role of chance: The endometrial organoid exhibit morphology and marker expression pattern of human endometrial glands. They can also be expanded for long-term culture. The resemblance between *in vitro* generated organoid and the *in vivo* endometrial gland was further supported by single-cell RNA-seq analysis. Seurat clustering identifies 8 cell clusters in endometrial organoids. RNA velocity analysis revealed a potential eeSC cluster at the starting point of the differentiation trajectory from the immature cell cluster toward the most mature one. Besides, the eeSC cluster had relatively high expression of known stem-cell markers including Tumor-Associated Calcium Signal Transducer 2 (*TACSTD2*) and Aldehyde Dehydrogenase 1 Family Member A3 (*ALDH1A3*). Five genes including Intercellular Adhesion Molecule 1 (*ICAM1*), L1 Cell Adhesion Molecule (*L1CAM*), Annexin A2 (*ANXA2*), Mesothelin (*MSLN*), Integrin Subunit Alpha 3 (*IGTA3*) were subsequently identified as potential markers of the eeSC cluster. Further analysis by immunostaining demonstrated the expressions of *CD54* and *ANXA2* in ~1% and ~30% of the whole organoids cell suspensions, respectively. The expressions of *CD54* and *ANXA2* were also identified in the glandular tissue of human endometrium.

Limitations, reasons for caution: With basal out/apical in phenotype, the endometrial organoids are not exactly similar to the endometrium *in vivo*, hampering the exploration of their surface marker in cell interaction experiments. Like other organoid systems, it is inevitable for endometrial organoids to have heterogeneity in cells and inconsistency in expansion and biological performance.

Wider implications of the findings: Gene manipulation technology like CRISPR-Cas9 could be utilized to genetically alter the eeSc to study their biological properties. The construction of eeSC biobank will offer an opportunity for the development of personalized regenerative medicine with diagnosis, preventive intervention, and treatment approaches for gynecological diseases.

Trial registration number: Not applicableNot applicable

Abstract citation ID: dead093.1104

P-798 Comprehensive evaluation of autologous Platelet-Rich Plasma infusion in ovarian rejuvenation

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Study question: This study aimed to the evaluation of PRP injection in women with ovarian dysfunction (with POR, POI, perimenopause and menopause) to promote the ovarian rejuvenation.

Summary answer: Intraovarian infusion of autologous PRP exhibited remarkable evidence and promising results to restore ovarian insufficiency.

What is known already: Ovarian dysfunctions, such as premature ovarian insufficiency (POI) and poor ovarian response (POR) are conditions, characterized by the collapse of ovarian function and are considered currently as a global public health issue. Primary factors that are associated with female infertility include endocrine dysfunction, failure of embryo implantation, endometriosis, and other related pathologies such as polycystic ovary syndrome (PCOS), various environmental factors and inflammatory disease. For this purpose, the rejuvenation of the ovaries using the intraovarian PRP injections may result in increased release of follicles from the available reservoir, and can potentially increase the possibility of a successful pregnancy.

Study design, size, duration: This study was designed as a randomized prospective observational pilot study. A number of 582 participants were interested to participate. The whole study was performed between May 2018 and December 2021. The patients were subdivided into 5 groups, followeed by autologous blood collection, PRP preparation and intraovarian injection. The follow-up of the patients lasted for 2 months.

Participants/materials, setting, methods: The classification of the participants was performed into the following groups: Group A (22-38 years), Group B (39-44 years), Group C (45-57 years), Group D (48-50 years) and Group E (51-56 years). 60 ml of autologous blood was collected from each patient, followed by the preparation of PRP. Intraovarian PRP injection was performed and monitoring of the ovarian function including the quantification of FSH, LH, AMH and E2 for two constitutive menstrual cycles, was performed.

Main results and the role of chance: For group A, decrease of more than 50% was detected for the FSH and LH whereas, the levels of E2 and AMH were elevated by more than 44% and 31%, respectively in all participants Moreover, statistically significant differences regarding the levels of FSH ($p = 0.001$), LH ($p = 0.046$) , E2 ($p = 0.035$) and AMH ($p = 0.012$), were observed after the 1st and 2nd month of follow-up. In group B, a statistically significant decrease in FSH ($p < 0.001$) and LH ($p = 0.001$) levels were observed in all participants. In parallel, the elevation of E2 and AMH levels was observed. Moreover, 28% of the participants with diagnosed POI, after the PRP infusion, achieved successful pregnancies and live births. In group C, after the PRP infusion, a reduction in FSH and LH levels with a parallel increase of E2 and AMH levels was detected. In group D, the levels of FSH and LH slightly declined after the PRP infusion. Also, the levels of E2 and AMH were increased. In group E, the levels of FSH and LH declin

Limitations, reasons for caution: No classifications between responders and no responders after the PRP administration were performed. Additionally, no presence of control groups in each category nor the determination of the growth factor content of the produced PRP was performed.

The relatively limited time for the follow-up can also be considered a further limitation.

Wider implications of the findings: The administration of autologous PRP can be considered a safe and tolerable alternative approach to the classical ones for ovarian rejuvenation. This in turn could result to better conceiving rates, thus may beneficially improve the social impact of women with ovarian dysfunction.

Trial registration number: 10/10/19

Abstract citation ID: dead093.1105
P-799 Morphine causes global hypomethylation through active and passive demethylation mechanisms in mESCs hindering optimal embryonic development

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Study question: Can morphine create changes in the mESC methylation pattern that are potentially related to the mismatches observed during embryonic development?

Summary answer: Morphine creates an even more hypomethylated state of the mESC genome, which results in a delay in mESC differentiation and, consequently, an increase in pluripotency.

What is known already: Epigenetic changes are essential for normal development. However, some environmental factors can cause epigenetic changes that leads to health problems or diseases. Morphine is known to pass through the placental barrier and impact normal embryo development by affecting the neural tube, frontal cortex and spinal cord development, and, as a consequence, delaying nervous system development. In fact, in-utero morphine exposure has shown alterations in anxiety-like behaviours, analgesic tolerance, synaptic plasticity and the neuronal structure of offspring. Nonetheless, how morphine leads to abnormal neurogenesis and other physiological consequences during embryo development is still unknown.

Study design, size, duration: Considering that DNA methylation is a key epigenetic factor crucial for embryo development, our aim is to elucidate the suitability of using this opioid in pregnant women, analyzing the role of methylation in response to morphine. To study morphine effects on embryo development, we used *mouse embryonic stem cells* (mESCs) culture, one of the most widely used models for studying embryo development *in vitro*.

Participants/materials, setting, methods: mESCs was treated with a chronic morphine treatment (24 h, 10 μM). DNA extraction was done using a classic phenol-chloroform/isoamyl methodology, and RNA extraction with commercial Nucleozol reagent according to the manufacturer's instructions. *Liquid chromatography–mass spectrometry* (LC-MS/MS) measured global genome methylation/hydroxymethylation levels, *Whole Genome Bisulfite Sequencing* (WGBSeq) measured cytosines methylation, and *Real Time quantitative Polymerase Chain Reaction* (RT-qPCR) measured gene expression. The statistical analysis (LC-MS/MS + RT-qPCR) was made by *t-student*.

Main results and the role of chance: By MS/MS approaches, we observed a global methylation decrease and a global hydroxymethylation increase in mESCs, changes that occurred after a chronic morphine treatment (24 h, 10 μM). WGBSeq identified 13329 sensitive cytosines to morphine that are involved in embryo development, signalling pathways, metabolism and/or gene expression. This suggests that morphine might impact methylation levels at developmental genes. Integrative analyses between WGBSeq and RNASeq identified *Tet Methylcytosine Dioxygenase 1* (*Tet1*) as sensitive morphine gene. Morphine increased the gene expression of *Tet1*, modifying the methylation levels at the promoter. Otherwise, RNASeq and qRT-PCR analyses revealed that *DNA Methyltransferase 1* (*Dnmt1*) gene expression decreased after morphine treatment. In conclusion, morphine induces a global hypomethylation in mESC through different mechanisms that involves active and passive demethylation, and the first one has a self-regulating mechanism that significantly enhances the demethylation process.

Limitations, reasons for caution: Taking into account all the ethical problems caused by the use of hESCs, this study has been carried out with mESCs. Although the physiology of mice and humans is somewhat different, this is the most recommended model for this type of pilot study.

Wider implications of the findings: This hypomethylation can potentially lead to a significant delay in optimal embryonic development; The global hypomethylated state of the genome is one of the most significant characteristics of stem cells. Consequently, the possible use of this and other opioids in patients who are pregnant should be strongly discouraged.

Trial registration number: Not applicable

Abstract citation ID: dead093.1106
P-800 Autologous stem cell ovarian transplant induced long-lasting effects on the plasma proteome of women with premature ovarian insufficiency

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Study question: Does autologous stem cell ovarian transplant (ASCOT) induce any long-term modifications in the plasma proteomic profile of women with premature ovarian insufficiency (POI)?

Summary answer: Stem cell mobilization and infusion into the ovary elicit long-term proteomic changes in peripheral blood plasma composition in women with POI, reverting age-related proteomic changes.

What is known already: Patients with POI are challenging to treat, with oocyte donation remaining as the only feasible option to achieve pregnancy. Previous attempts to overcome the fertility problems of these patients have mainly been based on ovarian stimulation, and were likely unsuccessful due to the lack of stimulable antral follicles remaining in the ovaries. Our group described that ASCOT in patients with impaired ovarian function improves ovarian reserve biomarkers, follicle and oocyte quantity enabling pregnancy and delivery of healthy babies. Beneficial effects of ASCOT were associated with the presence of different stem-cell secreted factors in apheresis plasma.

Study design, size, duration: Experimental cohort study with plasma samples of POI women undergoing stem cell mobilization with granulocyte-colony stimulating factor (mobilization arm; N = 3) or stem cell mobilization and infusion into the ovarian artery (ASCOT arm; N = 3). Peripheral plasma samples were collected from all patients at recruitment (*Pre*), during bone marrow stem cell mobilization and collection by apheresis (*Apheresis*), and three months after mobilization or ASCOT (*Post*).

Participants/materials, setting, methods: Proteins were isolated from plasma samples and quantified by SWATH™ to compare the plasma proteomic profile of the different time points (*Pre, Apheresis, Post*), using an ElasticNet penalized linear regression method. To determine the biological processes affected by stem cell mobilization and infusion into the ovary, a Gene Ontology Enrichment Analysis for the differentially expressed proteins (DEPs) was performed, considering significantly enriched those GO Biological Processes with a False Discovery Rate <0.05.

Main results and the role of chance: Discriminant analysis highlighted clear distinctions between the plasma proteome at the three evaluated time points. Both the stem cell mobilization and ASCOT technique induced statistically significant modifications in the plasma composition, reversing some age-related protein expression changes. Specifically, differences between *Apheresis* and *Pre* samples were explained by fourteen DEPs, revealing the functional analysis an enrichment in processes related to the complement cascade, immune system, and platelet degranulation. When the link of these proteomic changes with aging was evaluated, we found three proteins that were upregulated in *Apheresis* [C1QC, LYSC, LYAM1] whose plasma levels decrease with aging, and one downregulated protein [CRP] whose expression rises with age. Regarding *Post* samples, twenty-four DEPs with respect to *Pre* condition were

found within the mobilization arm, being two of them linked with aging [MMP2 and ALDOC]. These DEPs were related with immune response and platelet degranulation but also with processes associated with responses to oxygen-containing compounds and growth hormones, and blood vessel maturation. Within the ASCOT arm, *Pre* and *Post* samples were clearly differentiated by eleven DEPs, although any GO Biological Process were found. Nevertheless, among these DEPs, we found DIAC which increases in plasma with aging and was downregulated after ASCOT.

Limitations, reasons for caution: Considering the small sample size used in this study, further experimental studies will be needed to validate the results. Moreover, further research will be necessary to determine the extent of the regenerative effects of the identified DEPs within the ovaries, and their direct implications in ovarian aging.

Wider implications of the findings: Our findings highlight the potential proteins and biological processes that may promote the follicle activation and growth observed after ASCOT. Identifying plasma proteins that regenerate aged or damaged ovaries could lead to more effective, targeted and/or preventive therapies for affected patients.

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Trial registration number: NCT03535480

Abstract citation ID: dead093.1107
P-801 Human umbilical cord perivascular cells (HUCPVC) reduce ovarian fibrosis and improve pregnancy rates in a mouse model of natural ovarian aging

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Study question: Does repeated intravenous administration of human umbilical cord perivascular cells (HUCPVC) during the period of ovarian aging prevent age-related fertility decline in a mouse model?

Summary answer: Repeated administration of first trimester and term HUCPVC improved pregnancy rates and reduced ovarian fibrosis in a mouse model of natural ovarian aging.

What is known already: Mesenchymal stromal cells **(**MSC), such as first trimester (FTM) and term human umbilical cord perivascular cells (HUCPVC), may be good cell candidates to mitigate side-effects of oncotherapy, including infertility, and are also gaining interest in ovarian rejuvenation strategies. The intraovarian or systemic administration of various sources of mesenchymal stromal cells in animal models of advanced reproductive age have been previously reported to improve ovarian reserve, follicle activation and function, via multiple proposed mechanisms. However, mating studies have not previously been performed to demonstrate fertility preservation. In addition, the optimal dose regimen and timing of treatment has not been established.

Study design, size, duration: Pre-clinical randomized controlled study in a mouse model of natural ovarian aging from 6 months to 12 months, including a negative control group (vehicle-treated), a cell control group (fibroblast-treated) and 3 experimental groups (HUCPVC, 2 sources and 2 dose regimens; n = 10-15 per group). All parameters were compared with young control female mice (6-8 weeks, 6 months). Cell injections and all assessments were blinded.

Participants/materials, setting, methods: 6-month-old ICR mice randomized into 4 groups received 6 monthly tail vein injections of $1x10^6$ HUCPVC (FTM or term), fibroblast cells or HBSS (vehicle control). A 5th group received a single injection of FTM HUCPVC at 11M (n = 5). Pregnancy and litter size data were collected after breeding trials. The total number of follicles per ovary and picrosirius red (PSR) staining were quantified. Serum anti-Mullerian hormone (AMH) and C-reactive protein (CRP) were analyzed by ELISA.

Main results and the role of chance: No differences in mortality rates (P = 0.2) and animal weights were observed. The weight gain in FTM and term HUCPVC treated animals was significantly greater than in the other groups, over the duration of the study (P = 0.04). Animals that received 6 injections of FTM and term HUCPVC showed significantly higher pregnancy rates at 12M (100%, 80%) (P = 0.007; P = 0.048, respectively), when compared to animals that received HBSS (40%) and were similar to pregnancy rates of 6-8weeks (100%) and 6M (77%) groups. Fibroblast-treated and 1x FTM HUCPVC-treated groups showed 60% pregnancy rates. Age-associated declines in litter sizes and ovarian reserve indicators were observed as expected, but not rescued by cell treatments, except for levels of AMH which were increased in the group that received 1x FTM HUCPVC injections when compared to HBSS controls (P = 0.04). Age-associated increases in ovarian stroma fibrosis were observed with aging in the control groups (P < 0.0001), and were significantly reduced in 6x FTM HUPCVC, 6x term HUCPVC and 1x FTM HUCPVC groups, when compared to HBSS and fibroblast controls (P < 0.0001; P = 0.01 and P < 0.0001, respectively). An age-associated increase in CRP (P = 0.01) was significantly decreased in 6x FTM HUCPVC-treated groups, when compared to HBSS (P = 0.04).

Limitations, reasons for caution: Pregnancy rate (the main outcome measured in this study) may confound some of the secondary outcomes analyzed (CRP levels). Use of ovarian tissue for histological analysis and limited serum samples precluded further molecular analyses to assess potential mechanisms for the effects of HUCPVC.

Wider implications of the findings: FTM HUCPVC have anti-inflammatory and anti-fibrotic effects, and represent a promising source of MSC to prevent fertility decline in women of advanced reproductive age.

Trial registration number: Not Applicable

Abstract citation ID: dead093.1108
P-802 TFAP2C is required for proper segregation of developmental markers regulating trophectoderm commitment during mouse preimplantation development

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Study question: What is the effect of CRISPR/Cas9-mediated knock-out of trophectoderm markers TFAP2A/C on polarization and lineage commitment in mouse?

Summary answer: CRISPR/Cas9-mediated KO revealed discrepancies in TFAP2A/C function, wherein TFAP2C is dispensable for blastocyst formation but controls the segregation of transcription factors in the mouse embryo.

What is known already: TFAP2C is an important transcription factor that is expressed solely in trophectoderm (TE) in mouse, and both TE and epiblast in human. Recently, genetic ablation of both TFAP2C and TEAD4 (player of the HIPPO-pathway) completely abolished polarization in mouse, which indicates that TFAP2C might already act before the first lineage segregation takes place. In addition, TFAP2C RNA depletion experiments in mouse embryos revealed a compensatory upregulation of TFAP2A, which indicates functional redundancy. Nonetheless, the question remains how exactly TFAP2C regulates polarization on a molecular level in mouse and whether there is also a functional implication of its isoform TFAP2A.

Study design, size, duration: Guide RNAs were designed targeting exon 5 of *Tfap2c* and exon 2 of the *Tfap2a* gene. CRISPR/Cas9 ribonucleoprotein complexes were delivered into mouse zygotes via electroporation. Additionally, appropriate non-targeted and scramble (inactive crRNA) control groups were included. Morphological analysis, immunofluorescence and next-generation sequencing (NGS) were applied to check for gene editing efficiency and the impact of KO on embryonic development and lineage segregation.

Participants/materials, setting, methods: Targeted mouse embryos and controls were cultured for a maximum of 4.5 days *in vitro*. They were stained for different developmental markers, including CDX2 (TE), SOX2 (early ICM), NANOG (epibast, EPI) and SOX17 (hypoblast, PrE) at different stages of development, such as polarization (E2.5), first (E3.5) and second (E4.5) lineage segregation. Immunostaining was used to determine cell number, TE/ICM fraction, marker localization and fluorescence intensity. Embryos were subjected to genetic analysis to determine on-target efficiency.

Main results and the role of chance: CRISPR/Cas9-mediated electroporation of mouse zygotes generated efficiently complete knock-out embryos. Of the 39 mouse zygotes targeted for TFAP2C, 38 (97%) of them were edited. From the 38 embryos edited for TFAP2C, 24 (62%) embryos displayed 100% frameshift mutations, and were considered KO. TFAP2C KO mouse embryos cultured until E4.5 were still able to form blastocysts (13/15, 87%), but were associated with inferior blastocyst quality compared to the control groups. Furthermore, TFAP2c-null blastocysts could hatch but herniated at multiple places, in contrast to wild-type blastocysts, which typically herniate at one place.

In full frameshift TFAP2C KO mouse embryos, we observed delayed CDX2 expression at E2.5 (n = 7). At E3.5 (n = 5), however, CDX2 expression could still be pertained in KO embryos, whereas nuclear localization of SOX2 was disturbed. At E4.5 (n = 6), some primitive endoderm cells were observed in the presumed ICM (SOX17-positive), whereas no NANOG-positive (EPI) cells could be detected. However, some cells of presumed TE of E4.5 TFAP2C-KO embryos displayed erroneous SOX17 or NANOG expression, indicating that they adopted another cell fate irrespective of cell localization.

Secondly, we generated TFAP2A KO mouse embryos (3/5, 60%) which exhibited complete morula arrest (100%; n = 3) at E4.5.

Limitations, reasons for caution: CRISPR/Cas9 is limited by the occurrence of mosaicism (more than one genotype present in an embryo) and potential off-target editing, which we will assess at *in silico* predicted off-target sites via NGS in mouse embryonic stem cells. The observations of the study will be consolidated by increasing the sample size.

Wider implications of the findings: Gene editing studies enable us to unravel the molecular interactions that are required for human preimplantation development. Obtaining novel insights into the molecular networks of the TFAP2-transcription factor family could improve our fundamental understanding on the spatial segregation of key transcription factors, which is crucial for successful implantation.

Trial registration number: Not applicable

Abstract citation ID: dead093.1109
P-804 Endometrial recovery via implantable CD133+ stem cells scaffold in Asherman's syndrome therapy

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Study question: Can we use CD133+ bone marrow derived stem cell (BMDSCs) loaded scaffolds to aid endometrial regeneration locally in Asherman syndrome (AS)?

Summary answer: Gelatin scaffolds supported transdifferentiation *via* decidualization of CD133+ BMDSCs. Implantable CD133+ scaffolds showed a certain degree of endometrial recovery in an AS rat model.

What is known already: We have demonstrated that CD133+ BMDSCs contribute to endometrial proliferation, and functional recovery in murine models of AS (Cervelló et al., 2015) and humans (Santamaria et al., 2016). This advanced cell therapy is already in Phase II Clinical trial regulated by EMA. Here, we use bioengineered loaded-cell scaffolds to obtain a more localized cell therapy.

Study design, size, duration: Gelatin scaffolds were prepared by cryogelation and analyzed by micro-computed tomography. For *in vitro* studies, CD133+ were cultured into the scaffolds and decidualized with 1 mM of 8-Bromoadenosine 35′-cyclic monophosphate sodium salt (cAMP) for 7 days. For studies *in vivo*, the scaffolds used as the control group (gelatin scaffold only) and the CD133+ loaded gelatin scaffolds were implanted into acid-damaged horns of rat AS induced model followed after one and three weeks.

Participants/materials, setting, methods: DNA quantification was carried out for cell viability on the scaffolds, whereas immunofluorescence, single cell RNA sequencing and RT-qPCR were used to assess decidualization. For studies *in vivo*, endometrial recovery was screened by scanning electron microscopy (SEM), hematoxylin and eosin staining (H&E) and RT-qPCR.

Main results and the role of chance: Cell spreading and proliferation was observed in gelatin loaded scaffolds due to its high pore size and similarity to the native extracellular matrix. IGFBP1 and Prolactin expression determined by immunofluorescence and RT-qPCR demonstrated that gelatin scaffold supported decidualization of CD133 into stromal decidual cells. Additionally, single cell data revealed a higher expression of stromal specific canonical markers (ENG, CXCL12, PDGFRB) and decidual associated genes (IRS2, COCH) in the decidualized scaffold. During the *in vivo* studies of the implanted scaffolds in a rat model of AS, we studied a set of inflammatory genes and paracrine secretory molecules. For the CD133+ loaded gelatin scaffold, we obtained a decrease in the expression of NF-kB ($p \leq 0.01$). Additionally, the number of glands as well as endometrial thickness showed an increasing trend for the bioengineered cell loaded scaffolds after 3 weeks of implantation.

Limitations, reasons for caution: Endometrial functionality should be further analysed to needed full endometrial recovery.

Wider implications of the findings: Our bioengineered scaffolds mimic the native extracellular matrix, contributing to the viability, proliferation and decidualization of CD133+ BMDSCs. The herein implanted CD133+ BMDSCs scaffolds open new avenues to localized cell therapy for endometrial regeneration, especially for AS patients.

Trial registration number: Not applicable