54th
Scientific Meeting of BSRM

28-29 November 2025

Palais des congrès de Liège

PROGRAM & ABSTRACTBOOK

Friday, 28 November 2025

10:00 - 10:30	Welcome, coffee & registrations
10:30 - 10:40	Welcome by the President, Dominic Stoop
10:40 - 12:00 *Chairs:*	**Oncofertility** *Margherita Condorelli, Hôpital Erasme & Ingrid Segers, UZ Brussel*
10:40 - 10:55	Pre-preservation *Irene IJgosse, Prinses Máxima Centrum voor kinderoncologie BV, Utrecht, Netherlands*
10:55 - 11:10	Hope for tomorrow: clinical approaches to oncofertility *Michel De Vos, UZ Brussel*
11:10 – 11:25	Up-date of laboratory advances for ovarian tissue cryopreservation and clinical outcomes *Isabelle Demeestere, Hôpital Erasme*
11:25 - 11:40	Parenthood after cancer: clinical evidence and outcomes *Michel De Vos, UZ Brussel*
11:40 – 12:00	Debate
12:00 - 13:30	**Lunch**
13:30 - 14:30 *Chairs:*	**SoHO** *Christine Wyns, Cliniques Universitaires Saint-Luc & Kathleen Hostens, AZ Sint-Jan*
13:30 - 13:50	The new SoHO regulation *Stefaan van der Spiegel, EC Europa*
13:50 - 14:10	Perspective Clinic *Johan Guns, UZ Brussel*
14:10 – 14:30	Perspective Lab *Nathalie Vermeulen, ESHRE*
14:30 - 14:45	**Sponsored symposium BIRR** Understanding MDR: Insights and practical experience from BIRR's regulatory journey *Erik Vollebregt, lawyer and regulatory advisor in the life sciences and medical technology sector, The Netherlands*
14:45 - 15:00	Debate: understanding MDR
15:00 - 15:30	**Coffee break**
15:30 - 16:30 *Chairs:*	**When there are few and far in between…** *Daniel Murillo, CHU Saint-Pierre - Jeroen De Smet, AZ Sint- Lucas*
15:30 - 15:50	Endocrine perspective: Aging and Testosterone *Channa Jayasena, Hammersmith & St. Mary's Hospitals, London, United Kingdom*
15:50 – 16:10	Surgical perspective: "TESE for non-obstructive azoospermia – 30 years after" *Herman Tournaye, UZ Brussel*
16:10 - 16:30	Q&A
16:30 – 17:00	**Update on the upcoming new law on gamete donation** *Dominic Stoop, UZ Gent*
17:00 - 17:15	**BSRM Honorary Member 2025 - Michel Degueldre** *introduced by Candice Autin, CHU Saint-Pierre*
19:30	**Reception & dinner**

Saturday, 29 November 2025

09:00 - 09:20	**BELRAP 2023** **Overview IVF and non-IVF data** *Dominic Stoop, President of the College of Physicians Reproductive Medicine*
09:20 - 10:50 *Chairs:*	**Free communications & poster presentations** *Laurie Henry, CHU Liège & Hanne Devroe, UZ Leuven* ***Lab research***
09:20 - 09:30	Refining in vitro maturation conditions to enhance germinal vesicle transfer outcomes *Guangshun Gong, Ghent-Fertility And Stem cell Team (G-FaST), Department for Reproductive Medicine, Department of Human Structure and Repair, Ghent University Hospital*
09:30 - 09:40	Tracing ICSI pipette impact: longitudinal analysis of key performance indicators across five periods *Matija Krunic, Brussels IVF, Center for Reproductive Medicine, UZ Brussel*
09:40 - 09:50	Performing ICSI outside a laminar airflow environment: post-validation *Griet Eylenbosch, Universitair Ziekenhuis Brussel, Brussels IVF* ***Clinical research***
09:50 - 10:00	Establishing cut-off values for oocyte, zygote, and embryo maturation arrest (OZEMA) based on 16,994 ICSI cycles *Eva Decroos, Department of Reproductive Medicine, Ghent University Hospital*
10:00 - 10:10	Preliminary results of custom design "in-silico" gene panel as a diagnostic tool for human infertility *E Gonzales-Merino, Université libre de Bruxelles (ULB), Hôpital Universitaire de Bruxelles (H.U.B), CUB Erasme Hospital, Department of Obstetrics and Gynaecology, Fertility Clinic*
10:10 - 10:20	Random start natural micronized progestin-primed ovarian stimulation (NPPOS): a flexible alternative in ovarian stimulation – comparative analysis of 1181 elective freeze-all cycles *Florence Vandierendonck, Department of Reproductive Medicine, Ghent University Hospital* ***Basic research***
10:20 - 10:30	Rebooting life: pronuclear transfer rescues embryo developmental arrest in Padi6-Deficient mice *Hongbei Mu, Ghent-Fertility And Stem cell Team (G-FaST), Department for Reproductive Medicine, Ghent University Hospital*
10:30 - 10:40	Is embryo-maternal communication required for embryonic DNA methylation? A bovine in vitro model to study this epigenetic reprogramming process *Patricia Kubo Fontes, Center of Natural and Human Sciences, Federal University of ABC, Brazil*
10:40 - 10:50	Does tirzepatide-induced weight loss affect oocyte lipid content and mitochondrial activity: insights from a diet-induced obese mouse model? *Lien Loier, Gamete Research Centre, University of Antwerp*
10:50 - 11:20	**Coffee break**
11:20 - 12:20 *Chairs:*	**The age, too old, the eggs too few** *Marie Hoslet, CHR Namur & Ingrid Inion, ZAS Middelheim*
11:20 - 11:50	Clinical *Laurie Henry, CHU Liège*
11:50 - 12:20	Psychological *Sarah Colman, CHU Saint-Pierre*
Chair:	**BSRM Keynote Lecture** *Pascale Lybaert, ULB*
12:20 - 12:50	The naked Mole rat *Thomas B. Hildebrandt, Leibniz-Institut, Berlin, Germany*
12:50	**BSRM Scientific Awards sponsored by Ferring** *Best lab award: Prize winner receives: 1500 EUR* *Best clinical award: Prize winner receives: 1500 EUR* *Best basic research award: Prize winner receives: 1500 EUR* *Best poster award: Prize winner receives: 500 EUR*

Abstracts of invited speakers

Pre-preservation

Irene Ijgosse, Prinses Máxima Centrum voor kinderoncologie BV, Utrecht, The Netherlands

Yet to be received.

Hope for tomorrow: clinical approaches to oncofertility

Michel De Vos, UZ Brussel

Recent advances in cancer therapies have significantly improved survival rates among young patients. However, these treatments can have detrimental effects on reproductive function, making fertility preservation an important consideration in this population. Research suggests that counselling on fertility preservation, including the option to store gametes for future use, can enhance quality of life. Although data on reproductive outcomes in cancer survivors who ultimately use their cryopreserved gametes or gonadal tissue remain limited, fertility preservation is increasingly practiced in countries where it is supported by public health funding, such as Belgium. In this presentation, I will review both established and emerging fertility preservation techniques currently available in the oncofertility setting.

Up-date of laboratory advances for ovarian tissue cryopreservation and clinical outcomes

Isabelle Demeestere, Hôpital Erasme

Yet to be received.

Parenthood after cancer: clinical evidenceand outcomes

Michel De Vos, UZ Brussel

The potential to have children after successful treatment for cancer remains a priority for many young cancer survivors.
Nevertheless, population-based studies have demonstrated a significant reduction in the chance of a life birth across all cancer diagnoses compared to age-matched controls. Studies of follow-up of reproductive outcomes after cancer are hampered by the lack of linkable databases of cancer registrations and pregnancy-related outcome records in several countries including Belgium. To have an estimate on 1/ pregnancy chances after cancer; 2/ pregnancy outcomes after cancer and 3/ safety of pregnancy after cancer, structured follow-up of patients referred for fertility preservation can provide valuable information.
In this presentation, I will discuss existing literature regarding reproductive outcomes in female cancer survivors who return for infertility treatment after fertility preservation.

The new SoHO regulation

Stefaan van der Spiegel, EC Europa

Just like for pharmaceuticals and medical devices, the European Union regulates safety and quality of human body materials (substances of human origin - SoHO - blood, tissues, cells, organs). A new framework has been adopted (EU)2024/1938 which will have to be implemented by all actors by August 2027. The framework allows for more up-to-date and uniform, EU-wide, technical guidelines for professionals to protect recipients as well as donors and offspring. It also brings more harmonized oversight practices for national authorities. The framework also will bring more legal clarity and a risk-based authorisation mechanism for novel SoHO preparations.

Perspective Clinic

Johan Guns, UZ Brussel

Clinical Implications of the New SoHO Regulation for MAR Centres:
A Comparison with the 2004/23/EC and 2006/17/EC Directives

On August 6, 2024, the new European SoHO Regulation (2024/1938) entered into force, establishing a harmonised legal framework for the quality and safety of Substances of Human Origin (SoHO), including gametes and embryos, across the EU. From August 7, 2027, this Regulation will replace the existing Tissues and Cells Directive (2004/23/EC) and its implementing acts (e.g., 2006/17/EC), marking a significant shift in regulatory oversight for Medically Assisted Reproduction (MAR) centres.

This presentation explores the clinical impact of the SoHO Regulation on MAR practice, highlighting key changes such as the expanded scope of donor, recipient, and

offspring protection, mandatory traceability systems, and stricter vigilance requirements. MAR centres will need to adapt to new obligations, including the appointment of a responsible physician for clinical oversight, implementation of quality management systems, and formal release procedures for SoHO preparations.

Clinically, the Regulation introduces more rigorous standards for donor screening, including genetic risk assessment and post-donation health monitoring, particularly relevant for oocyte donors. It also mandates outcome monitoring for certain SoHO preparations, linking clinical effectiveness to regulatory approval. The Regulation's emphasis on risk categorisation and benefit-risk assessment may influence treatment protocols and the introduction of innovative techniques.

Unlike the previous directives, which required national transposition, the Regulation is directly applicable, reducing variability across Member States and promoting consistency in clinical standards. However, this also means MAR centres must ensure full compliance without relying on national interpretation.

Overall, the Regulation represents both a challenge and an opportunity: while it increases administrative and clinical responsibilities, it also enhances patient safety, data transparency, and cross-border collaboration. This presentation will provide practical insights into navigating the new framework and preparing MAR centres for its implementation.

Perspective Lab

Nathalie Vermeulen, ESHRE

The SoHO Regulation introduces a new legal framework for ensuring the quality and safety of all Substances of Human Origin (SoHO), including oocytes, sperm, and embryos, across the EU. Its implementation will inevitably impact daily workflows and quality systems within IVF laboratories.
This presentation will examine the implications of the SoHO Regulation from a laboratory perspective, focusing on the integration of professional guidelines.
These include technical guidelines issued by the ECDC and EDQM, national standards adopted by member states, and those developed by professional societies such as ESHRE.
The presentation aims to provide attendees with a clearer understanding of what constitutes a compliant, high-quality IVF laboratory under the SoHO Regulation.

Endocrine perspective: Aging and Testosterone

Channa Jayasena, Hammersmith & St. Mary's Hospitals, London, United Kingdom

Testosterone replacement therapy (TRT) is a long-established treatment to restore sexual function in young men with classical, organic hypogonadism. However, TRT is most often prescribed to men with non-classic (also known as functional) low testosterone associated with low health status, such as older age, obesity, or diabetes,

which also cause sexual dysfunction, low mood, tiredness, and weakness fatigue independently of low testosterone. Therefore, it has been controversial whether TRT improve symptoms in men with functional hypogonadism. In addition, some historical studies have suggested that TRT may increase risk of major adverse cardiovascular events.

We recently conducted an individual patient data (IPD) meta-analysis suggesting that TRT modestly improved symptoms but did not increase MACE compared with placebo in >3000 men with low testosterone aged > 40 years. Subsequently, a recent RCT in >5000 men (mean age 65 years) reported that TRT modestly improves symptoms without increased MACE.

In summary, recent evidence suggests that TRT may have modest benefits to improve symptoms in middle-aged and older men with low testosterone. The prospect of growing TRT usage may have implications for fertility treatment in couples, given the suppressive effects of exogenous testosterone on testicular function.

Surgical perspective: "TESE for non-obstructive azoospermia – 30 years after"

Herman Tournaye, UZ Brussel

The introduction of ICSI at our centre in 1992 has completely changed the clinical approach towards male infertility by offering a novel opportunity for parenthood even to azoospermic men. Eventually in 1995 we published the first pregnancies ever using testicular spermatozoa in men with non-obstructive azoospermia. In the meantime, surgical retrieval of spermatozoa for ICSI has become a routine technique in reproductive andrology. But even after 30 years, controversies do remain. Although depending on your preselection of patients one may manipulate sperm retrieval rates, the live birth rates after TESE-ICSI range from 10- 25% in a non-preselected population on an intention to treat basis. And when no sperm are harvested after TESE, even today, round spermatid injection remains an unefficient strategy. Overall, no statistical difference in fetal karyotype abnormalities, neither in congenital malformations nor in neonatal outcome risks are being observed among the offspring obtained. And the same goes for Klinefelter's offspring. Yet, the scarcity of data available in the literature hampers definitive conclusions on the safety of this treatment strategy towards the offspring.

Clinical

Laurie Henry, CHU Liège

Over recent decades, the age of motherhood has steadily increased across the world, reflecting profound social and demographic changes. Yet reproductive biology remains unforgiving: with advancing age, both the number and the quality of oocytes decline sharply, profoundly impacting fertility potential.

Female reproductive ageing is characterized not only by a reduction in ovarian reserve but also by a progressive deterioration in oocyte competence. After the mid-thirties, the proportion of euploid oocytes decreases rapidly, leading to lower implantation rates, higher miscarriage rates, and reduced cumulative live birth outcomes, even in assisted reproduction. Current markers such as AMH and AFC offer only partial insight into this complex process, as oocyte quality—closely linked to mitochondrial function, spindle integrity, and chromosomal segregation—remains the key determinant of success. Advances in ovarian stimulation protocols, the use of preimplantation genetic testing (PGT-A), and elective oocyte cryopreservation have improved outcomes in selected cases but cannot overcome the fundamental biological limits of reproductive ageing. Clinicians thus face growing challenges in counselling women who delay motherhood, balancing optimism with realistic expectations and promoting timely fertility awareness.
Conclusions:
The expression “too old, too few” encapsulates both a biological truth and a societal dilemma. As reproductive specialists, we must strive to bridge this gap between reproductive autonomy and biological feasibility—offering evidence-based guidance, accessible fertility preservation options, and a clear message: while science can support, it cannot indefinitely postpone the biological clock.

Psychological

Sarah Colman, CHU Saint-Pierre

When the question of delaying motherhood is raised, it is often associated with women who choose to prioritize their professional careers. We wanted to take care to present an alternative perspective on this reality faced by women. Our approach will consist of drawing a parallel between the testimonies of our patients at the Assisted Reproduction Center of the CHU Saint-Pierre (Brussels) and a review of some of the existing body of literature.

The nakes mole rat

Thomas B. Hildebrandt, Leibniz Institut, Berlin, Germany

The naked mole rat (NMR), Heterocephalus glaber, has been studied intensively over the last four decades, due to its remarkable array of properties, such as its very long life span, unusual social life, eco-physiological endurance and apparent resistance to neoplasia.
The NMR is a eusocial animal, naturally found in subterranean burrows in east Africa (Kenya, Ethiopia, and Somalia). It lives in large colonies (20 – 300 individuals) headed by one fertile queen, and with a clear division of class: reproducers and subordinate workers. Workers maintain the colony by foraging for food, extending the burrow system, defending the colony and helping the queen care for her offspring. The queen and reproductive males (1-3) have longer life spans than workers and are the only sexually developed individuals in the colony. Females older than 6 months are capable of becoming reproductively active if the queen dies, and will fight to establish dominance. The workers dig the elaborate burrow system in which the colony resides by using their hind limbs to brace themselves, and their teeth as chisels and digging tools.

In order to be able to thrive in their subterranean niche, NMRs developed special physiological and morphological adaptations. These include small eyes (with an expanded somatosensory cortex) and streamlined body shape which assists in movement within the burrows and unusual respiratory physiology that enables them extreme tolerance of the hypoxic and hypercapnic atmosphere in their habitat. Low body temperature, low metabolic rate, tolerance for vitamin D deficiency and efficient mineral metabolism in the absence of sunlight also enable NMRs to survive under harsh conditions.
The most remarkable observation associated with NMRs is their longevity. The naked mole rat is by far the longest-living rodent known, with maximum life expectancy of at least 38 years in captivity. The presentation will provide you detailed information of this unique mammalian species based on 17 years of research on captive and wild NMR.

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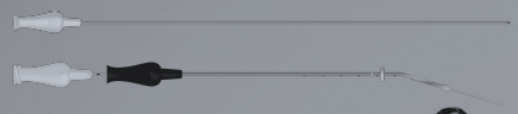

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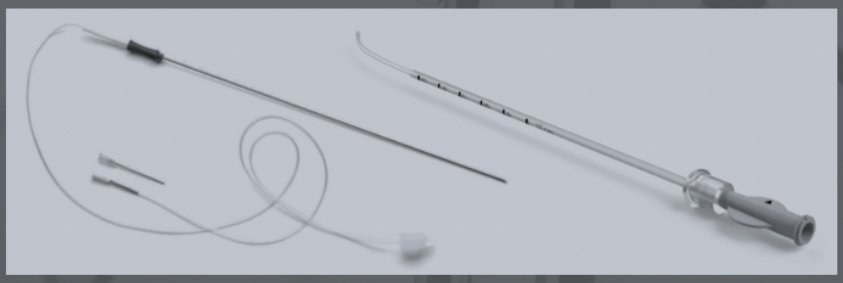

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Free Communications

Lab research

FC-01

Refining In Vitro Maturation Conditions to Enhance Germinal Vesicle Transfer Outcomes

Guangshun Gong, Ghent-Fertility And Stem cell Team (G-FaST)

Objective(s): Germinal vesicle transfer (GVT) represents a promising strategy to rejuvenate aged or compromised oocytes by transferring the nuclear genome into a young healthy donor cytoplasm. Despite its potential, GVT efficiency remains limited due to the low in vitro maturation (IVM) rates of denuded oocytes. Reversible meiosis inhibitors are commonly used to maintain meiotic arrest before IVM. Still, their effects on the competence of denuded oocytes, particularly when combined with GVT, have not been fully optimized. Notably, oocytes reconstructed through GVT have undergone extensive manipulation, which might compromise further maturation or developmental competence. The current study investigated whether replacing the conventional high-dose single inhibitor with a combination of low-dose reversible meiosis inhibitors could improve IVM outcomes of denuded oocytes.
Design and methods: GV oocytes were collected from B6D2F1 mice at 48 h after pregnant mare serum gonadotropin injection. Following cumulus cell removal, denuded oocytes were allocated to two groups: IVM culture (i) with 200 μM 3-isobutyl-1-methylxanthine (IBMX) (control) (n = 115) or (ii) with a dual combination of 50.0 μM dibutyryl cyclic AMP (dbcAMP) and 10.0 μM IBMX for 5 hours (n = 53). GVT was performed by transferring GV nuclei into enucleated recipient cytoplasts, followed by Hemagglutinating Virus of Japan (HVJ-E) - mediated oocytes reconstruction. The oocytes were subsequently subjected to the same IVM conditions, and the maturation rate was evaluated based on the extrusion of the first polar body after 20 h of IVM.
Results: The dual low-dose inhibitors significantly improved the IVM rate of denuded oocytes compared to the group with higher inhibitor (72.20% vs. 44.73%, p = 0.038). In the GVT group, oocytes cultured with the modified inhibitor showed a higher IVM rate than controls (52.20% vs. 47.27%), but this increase did not reach statistical significance. These results suggest that the inhibitor combination enhances IVM efficiency in both denuded and GVT-reconstructed oocytes, with a trend toward improved embryo development in GVT oocytes, as indicated by higher 2-cell rates compared with single-inhibitor treatment (66.60% vs. 55.53%).
Conclusions: Optimizing the combination and dosage of reversible meiosis inhibitors during IVM significantly enhances the maturation rate of denuded oocytes and shows potential to improve the efficiency of GVT procedures. This strategy may contribute to better outcomes in GVT aimed at preserving oocyte quality and developmental competence, particularly in cases of advanced maternal age. By mitigating age-associated defects and potentially reducing aneuploidy risks, this approach holds promise for advancing reproductive medicine.

FC-02

Tracing ICSI Pipette Impact: Longitudinal Analysis of Key Performance Indicators Across Five Periods

Matija Krunic, Brussels IVF

Objective: Evaluate whether changes in ICSI pipette geometry, specifically in injection pipettes, affect key performance indicators (KPIs) and operator usability in a routine clinical IVF setting.

Design and Methods: A prospective validation study was conducted at Brussels IVF in 2025. Each period used pipette sets from a different manufacturer. Injection pipettes in P1, P2, and P4 had a longer tip-to-elbow distance; P3 and P5 used short, tapered designs. Primary KPIs were fertilization (2PN/Injected), degeneration (Deg/Injected), and abnormal fertilization rate (1PN/Injected, 3PN/Injected). Outcomes were summarized as pooled proportions with added binomial logistic regression (successes/trials) adjusted for female age and sperm type (fresh/frozen); adjusted results are shown as odds ratios (OR) vs P1 and model-standardized rates (predicted probabilities; "adjusted percentages"). To control for cycle size (MII/COC), sensitivity models added log(denominator) and, depending on KPI, either Injected/COC (ICSI rate) or log(COC+1). Embryo utilization was defined as (fresh ET + all cryopreserved embryos)/2PN and reported pooled and adjusted. In parallel, operator feedback on robustness, tip visibility, sperm immobilization, oolemma puncture and control was documented.

Results: Cohort sizes were P1 (n=1696 COCs), P2 (n=1894), P3 (n=405), P4 (n=1366), P5 (n=308). Pooled 2PN/Injected was stable across P1-P4 (71.3-73.7%) and lower in P5 (69.6%). In adjusted logistic model vs P1, fertilization rate was lower in P2 (OR 0.84, p=0.04) while the other periods did not show significant differences (p>0.32). Degeneration rate showed no significant period effects (all $p \geq 0.06$). 1PN/Injected showed no differences (p>0.05). 3PN/Injected was higher in P5 (OR 2.64, p=0.02); P2-P4 were not different (p>0.17). Model-standardized rates (predicted probabilities) were close to pooled values; for fertilization rate they were 75.0%, 71.5%, 74.5%, 73.6%, 71.3%. Embryo utilization was similar across P1-P4 (pooled 36.3-39.0%; adjusted 33.9% [30.9-37.1], 36.6% [33.7-39.7], 32.4% [26.4-39.0], 36.1% [32.7-39.6]) and higher in P5 (pooled 51.4%; adjusted 52.7% [42.9-62.2]). This increase may be explained by the smaller sample size, which could have increased variability in the utilization rate. Cycle-size sensitivity shifted adjusted means by ~0.3 percentage points but did not change conclusions. Injection pipettes with shorter, tapered geometries received negative feedback: increased difficulty with sperm immobilization, higher breakage and reduced injection control, especially in cases with high number of oocytes.

Conclusions: Observed trends, including higher degeneration and reduced fertilization in P5, align with the geometric differences in pipette design and operator feedback. These findings confirm that changes in ICSI pipette geometry can affect KPIs, with potential implications for clinical outcomes.

FC-03

Performing ICSI outside a laminar airflow environment: post-validation

Griet Eylenbosch, Universitair Ziekenhuis Brussel

Objectives: The aim of this post-validation analysis was to confirm the safety and efficacy of performing ICSI outside a laminar airflow (LAF).
Design and method: Prospective validation in 2024 showed that (i) ICSI outside the LAF, in background C, was microbiologically safe and that (ii) similar embryological outcomes were achieved in a sibling cohort, despite MiRCA-score of 186/695. As post-validation, this 2-month retrospective analysis was performed after implementing ICSI outside the LAF since April 2025 on 2/6 ICSI workstations. All ICSI procedures, including PGT, with fresh oocytes, day 5 culture and ejaculated sperm (fresh and frozen) performed outside LAF were compared to all procedures inside LAF. Data included female age, number of cumulus-oocyte complexes (COCs), normal fertilization (2PN/inseminated), degeneration (degenerated/inseminated), embryo quality and embryo utilization (transferred+cryopreserved/fertilized); data are presented as mean±SD or adjusted%. Comparisons between groups were conducted using chi-square or t-tests for unadjusted analyses, and multivariable logistic regression adjusted for maternal age, #COCs, and semen type (fresh vs frozen), (Adj) $p<0.05$ was considered significant.
Results: A total of 646 cycles were analyzed including 201 outside and 445 inside the LAF, of which 67 and 141 were PGT-cycles, respectively. Patients were 35.2±4.8 and 35.3±4.8 years old (p=0.672) with 10.5±7.7 and 10.7±8.2 COCs retrieved (p=0.835) and 8.1±6.2 vs. 8.3±6.5 mature oocytes (p=0.774), respectively. Distribution of fresh and frozen ejaculates was similar between groups: 79.6%/20.4% vs. 81.6%/18.4%; p=0.629. Fertilization rates were significantly higher outside compared to inside LAF (77.9% vs. 75.4%; Adj OR:1.15, Adj p=0.045). This effect was mainly attributed to a significantly increased fertilization in PGT cycles outside LAF: 81.4% compared to 76.7% inside LAF (Adj OR:1.28, Adj p=0.028). In non-PGT cycles, fertilization did not differ significantly (76.0% vs. 74.6%, respectively; Adj p=0.236). Overall, degeneration rates were similar outside and inside LAF (5.7% vs. 5.5%; Adj p=0.405), with no subgroup differences with or without PGT. On day 3 of development, ICSI outside LAF generated more top-quality embryos in non-PGT cycles only (62.9% vs. 58.7%, p=0.042); this difference disappeared on day 5 of development. Finally, utilization rates were similar outside and inside LAF: 47.4% vs. 47.0%; Adj p=0.699.
Conclusions: ICSI outside the LAF does not compromise embryological outcomes. If differences are observed, they are in favor of ICSI outside LAF. These findings provide robust evidence supporting the safety and efficacy of performing ICSI outside the LAF as a viable alternative to conventional laboratory conditions, as long as microbiological safety is guaranteed.

Clinical research

FC-04
Establishing cut-off values for oocyte, zygote, and embryo maturation arrest (OZEMA) based on 16,994 ICSI cycles

Eva Decroos, Department of Reproductive Medicine, Ghent University Hospital

Objective(s): Outcomes of ICSI (intracytoplasmic sperm injection) cycles can be adversely affected by oocyte maturation arrest, fertilization failure, or embryo developmental arrest. In 2023, these three phenotypes were collectively categorized under the term Oocyte/Zygote/Embryo Maturation Arrest (OZEMA). The objective of this study was to establish age-specific cut-off values for oocyte maturation rate (OMR), fertilization rate (FR), and blastocyst formation rate (BFR), enabling a diagnostic framework for identifying OZEMA phenotypes.
Design and methods: This retrospective cohort study included 16,994 ICSI cycles from 9,499 patients, aged 18-45 years, treated between July 2015 and September 2024 in a tertiary center. Oocyte donors were excluded from OMR calculations, while cycles involving donor sperm, assisted oocyte activation, or vitrified-warmed oocytes were excluded from FR/BFR analyses, resulting in 14,116 cycles (7,982 patients) for these outcomes. A quantile regression model with a constrained nonlinear term for age (monotone decreasing/convex) was fitted for OMR, FR, and BFR. Cut-off values were defined at the 0.05 and 0.10 quantiles. To ensure reliable interpretation, minimum sample size requirements for oocytes/zygotes were calculated to achieve a 90%/95% confidence interval (CI) halfwidth of at most 0.15, with 50% probability.
Results: Quantile regression enabled the determination of age-specific cut-off values for OMR, FR, and BFR. At the 0.05 quantile, OMR thresholds were 44% at age 25, 39% at age 30, 34% at age 35, and 29% at age 40. Corresponding FR thresholds were 45%, 34%, 24%, and 13%, respectively. For BFR, cut-offs at the 0.05 quantile were consistently 0% across all ages. At the 0.10 quantile, BFR cut-offs were 29% at age 25, 19% at age 30, 8% at age 35, and 0% at age 40. Reliable application of these thresholds required a minimum of 31 oocytes/zygotes to achieve a 90% CI halfwidth of at most 0.15, or 43 for a 95% CI.
Conclusions: This study provides the first age-adjusted diagnostic thresholds for the identification of OZEMA phenotypes by establishing cut-off values for OMR, FR, and BFR. These thresholds are crucial for the clinical diagnosis of these patients, for improving referral for genetic screening and counseling, and for guiding personalized treatment strategies. Furthermore, they offer a standardized framework for future studies, enabling more consistent comparisons across research investigating OZEMA

FC-05

Preliminary results of custom design “in-silico” gene panel as a diagnostic tool for human infertility

E Gonzales-Merino, Université libre de Bruxelles (ULB)

Objective(s): Standard fertility examinations fail to identify an etiology in 15–30% of infertile couples due to the complexity of the reproductive process, and such cases are then classified as “idiopathic.” It is believed that approximately half of the idiopathic cases can be explained by a genetic defect. Although some of them are specific to only males or females, others can affect both sexes. The improvement of high-throughput sequencing (HTS) technologies has considerably modified the discovery of the genetic causes of diseases. It is now possible to analyse several dozen infertility genes simultaneously via HTS, and this new diagnostic tool has the potential to solve a significant proportion of idiopathic infertility cases.

Design and methods: An exome-based “in-silico” reproductive disease gene panel (RD-V1) comprising 341 genes for human infertility and sex development disorders was developed within the genetic diagnostic service of Erasme University Hospital (Université Libre de Bruxelles) and started to be offered national wise in August 2023. Blood samples were collected after obtaining written informed consent from patients with a well-defined infertility phenotype. Libraries were prepared using the kit Twist Human Exome 2.0 Plus Comprehensive Exome (Twist Bioscience, South San Francisco, CA, USA) and sequenced in the Genomics Core Leuven on Novaseq X Plus (Illumina, San Diego, CA, USA) with 2 × 150 bp reads. *In-silico* panel analysis comprising 341 genes related to human reproduction was performed using the Highlander program (https://sites.uclouvain.be/highlander).

Results: By June 2025, 40 patients from Erasme hospital, Fertility Clinic and 142 patients from different fertility clinics in Belgium were analysed via our gene panel RD-V1. Diagnostic yield for patients from our clinic is 12.5% (5/40) while only 2.8% of the samples received from external centers displayed a genetic defect associated with their infertility (4/142). The difference indicates the importance of detailed clinical documentation and patient selection criteria/strategies in such approaches.

Conclusion: The diagnostic practice will allow a better definition of the genotype/phenotype correlations to enable personalized care. Precise diagnosis is the key for seeking and adapting the best treatment and counselling not only to patients but also to their spouses and relatives. To challenge the clinical interest of such a diagnostic tool, a checklist should be applied in order to define the patient group to benefit from the panel test and a larger group of patients needs to be analysed.

FC-06

Random start natural micronized progestin-primed ovarian stimulation (NPPOS): a flexible alternative in ovarian stimulation – comparative analysis of 1181 elective freeze-all cycles

Florence Vandierendonck, Department of Reproductive Medicine

Objective(s): To investigate whether random start natural micronized progestin-primed ovarian stimulation (NPPOS) can provide an equivalent and flexible alternative

strategy for ovarian stimulation in elective freeze-all cycles, compared with conventional day 2/3 start GnRH-antagonist protocol.
Design and methods: This retrospective cohort study was conducted at the Department of Reproductive Medicine, Ghent University Hospital, and included 1181 elective freeze-all cycles performed between January 2022 and December 2024. Indication for freeze-all cycles consisted of egg vitrification, oocyte donation, fertility preservation, PGT and others such as expected high responders.
The final dataset consisted of four groups: GnRH antagonist early follicular start (n=482), NPPOS early follicular start (n=319), NPPOS late follicular start (n=163), and NPPOS luteal start (n=217). The primary endpoint was the number of metaphase II (MII) oocytes retrieved. Secondary endpoints included the number of cumulus–oocyte complexes (COC), follicular output rate (FORT), follicle–oocyte index (FOI), stimulation duration, total gonadotropin dose, and number of monitoring visits. Poisson generalized estimating equations (GEE) models were used to adjust for age, anti-Mullerian-hormone (AMH), body weight, freeze-all indication and gonadotropin start dose. A predefined subgroup analysis was conducted in poor ovarian responders (AMH <1.2 µg/L).
Results: Baseline characteristics were comparable across groups, with 32% of patients classified as poor responders. The number of MII oocytes did not differ significantly (p=0.20) across the four groups: median 12 (antagonist), 12 (NPPOS early follicular), 11 (NPPOS late follicular), and 11 (NPPOS luteal). GEE analysis confirmed equivalence. Pairwise comparisons showed no significant differences in MII oocyte yield between the groups (all p>0.30). Rate ratios ranged from 0.975 to 1.096, none statistically significant, confirming similarity between the NPPOS random start and the antagonist group. FORT (48–52%) and FOI-MII (76–79%) analysis in the NPPOS subgroups remained stable.
The luteal NPPOS group required slightly longer stimulation (11 vs. 10 days, p<0.001), higher gonadotropin consumption, and more monitoring visits. In poor responders, MII oocyte yield was comparable across all four groups (5.2–5.8 per cycle, all p>0.03).
Conclusions: Random start NPPOS using oral micronized progesterone is a safe, effective, and flexible alternative to conventional GnRH antagonist protocols in elective freeze-all cycles.The ability to initiate stimulation irrespective of menstrual phase combined with the use of oral progesterone enhances patient and physician flexibility. This approach may reduce treatment delays and improve convenience without compromising ovarian response.

Basic research

FC-07

Rebooting Life: Pronuclear Transfer Rescues Embryo Developmental Arrest in Padi6-Deficient Mice

Hongbei Mu, Ghent-Fertility And Stem cell Team (G-FaST)

Objective: Oocyte maturation arrest, fertilization failure, and embryonic developmental arrest (EDA), collectively termed OZEMA, often result from defects in oocyte cytoplasmic proteins. Nuclear Transfer (NT) has been proposed as a potential strategy to overcome these defects. Despite reports of live births with NT, the lack of compre-hensive molecular evaluation and long-term follow-up leave its safety insufficiently understood. Using a *Padi6*-knockout mouse model, our research aims to address these gaps by systematically assessing NT-reconstructed embryos via multi-omics, providing critical insights into the developmental competence and molecular characteristics of NT-derived embryos.

Design and methods: Pronuclear transfer (PNT) was performed by transferring pronuclei from maternally-*Padi6*-deficient (*Padi6*$^{mat-}$) zygotes into enucleated wild-type recipients. Reconstructed embryos were cultured for 4 days to evaluate their preimplantation development. Late 2-cell stage embryos were subjected to mass spectrometry-based proteomic analysis. Blastocyst-stage embryos were collected for integrated epigenomic and transcriptomic analyses using bisulfite sequencing (BS-seq) and RNA sequencing (RNA-seq), respectively.

Results: *Padi6*$^{mat-}$ zygotes exhibited a normal 2-cell rate of 93.61% (44/47), similar to the wild-type group (100%, 72/72, P=0.06). However, their developmental competence was severely impaired, with only 2.13% (1/47) forming blastocysts, in stark contrast to the wild-type group (95.83%, 69/72; P<0.001). Following PNT, 44 embryos were reconstructed and all reached the 2-cell stage (100%, 44/44). Notably, 90.91% (40/44) developed into blastocysts, a rate comparable to that of wild-type controls (P=0.71), indicating substantial rescue of preimplantation development.

Proteomic analysis of 2-cell embryos identified a total of 4593 protein groups across all samples. Following data filtering, 3419 protein groups were included for downstream quantitative analysis. Principal component analysis demonstrated a clear separation of *Padi6*$^{mat-}$ embryos from both wild-type and PNT groups, while PNT and wild-type embryos clustered closely together. Differential expression analysis revealed 727 proteins with significantly altered levels between *Padi6*$^{mat-}$ and wild-type embryos, compared to only 13 between PNT and wild-type embryos, supporting the molecular rescue achieved by PNT.

BS-seq analysis showed that PNT blastocysts exhibited a higher global methylation level (16.2%) compared to wild-type embryos (8.5%). However, RNA-seq analysis indicated that this hypermethylation did not consistently downregulate gene expres-sion.

Conclusions: PNT effectively overcomes EDA in *Padi6*-knockout mice. Multi-omics analysis revealed that PNT embryos exhibited normal proteomic signatures at the 2-cell stage and elevated global DNA methylation levels at the blastocyst stage. These findings provide strong evidence supporting PNT as a promising strategy to bypass

cytoplasmic defect-induced EDA, while also highlighting the need for further investigation of its epigenetic implications.

FC-08

Is embryo-maternal communication required for embryonic DNA methylation? A bovine in vitro model to study this epigenetic reprogramming process

Patricia Kubo Fontes, Center of Natural and Human Sciences

Objective(s): Assisted Reproductive Technologies (ART) are associated with epigenetic modifications in human embryos, affecting, for example, DNA methylation (5-methylcytosine, 5mC). This process is particularly critical in *in vitro*–produced embryos (IVPE), possibly due to the absence of embryo–maternal communication. Therefore, we aimed to evaluate the effect of a uterine tube (i.e., oviduct) model on 5mC levels in IVPE.
Design and methods: Cattle were used as an animal model given their similarities to humans in epigenetic reprogramming and early embryo development. *In vitro*–matured cumulus–oocyte complexes (from a slaughterhouse) were fertilized with frozen bull semen. Eight hours post-insemination (hpi), presumptive zygotes (PZ) were cultured in embryo culture media (at 38.5ºC, 5% CO_2, 5% O_2, and high humidity) in two conditions: control ("embryo-only", n=568 PZ, 15 PZ/well) or mimicking embryo–maternal communication ("co-culture", n=572 PZ, 15 PZ + 3 spheroids/well). To simulate the maternal environment, we used spheroids of bovine oviductal cells, a 3D *in vitro* model developed by our group (*Fontes et al. 2025, 10.1002/mrd.70049*). Co-culture lasted four days, corresponding to the *in vivo* tubal phase, followed by standard culture until blastocyst (216-hpi). Yield production (%) and 5mC levels (arbitrary units, au, of immunofluorescence quantification – ImageJ-1.54p) were assessed at 30-hpi (cleavage), 96-hpi (morula), and 216-hpi (blastocyst), and presented in the section below (embryo-only, co-culture% or au). Data from four replicates (random factor) were analyzed in GraphPad (version 8) with a t-test (parametric data, $P<0.05$).
Results: Cleavage (68±14, 61±19%) and morula rates (50±16, 40±13%) did not differ between groups. However, blastocyst rate was lower in co-culture (32±13, 25±14%, $P<0.01$), while hatching was similar (68±27, 82±22%). Strikingly, 5mC levels differed ($P<0.0001$) at 30-hpi (34±17 [n=13], 17±5 au [n=12]), 96-hpi (37±37 [n=16], 16±10 au [n=15]), and 216-hpi (expanded blastocysts only) in both the inner cell mass (ICM: 14±4 [n=10], 26±14 au [n=9]) and trophectoderm (TE: 14±4 [n=10], 30±14 au [n=9]). Importantly, methylation dynamics were opposite: in embryo-only, 5mC progressively decreased (30-hpi > 96-hpi = ICM = TE, $P<0.05$), whereas in co-culture, it increased (30-hpi = 96-hpi < ICM < TE, $P<0.05$).
Conclusions: Embryo–maternal communication seems to modulate embryonic DNA methylation dynamics. Further analysis will elucidate the efficiency and accuracy of this co-culture system. Yet, this *in vitro* oviductal model provides a promising tool to explore how maternal environments shape epigenetic reprogramming and may help optimize conditions to improve human ART outcomes. Supported by FAPESP (21/11747-6, 20/02500-4).

FC-09

Does tirzepatide-induced weight loss affect oocyte lipid content and mitochondrial activity: insights from a diet-induced obese mouse model?

Lien Loier, Gamete Research Centre

Objective(s): Incretin-based therapies, such as the newest Mounjaro® (tirzepatide), are breakthrough anti-obesogenic medications due to their strong metabolic and weight loss effects. Due to reproductive toxicity in animals, tirzepatide is contra-indicated during pregnancy, with discontinuation advised 1 month prior to conception. However, it remains unclear how tirzepatide may affect oocyte quality. This study aims to investigate whether a preconception care intervention (PCCi) using tirzepatide can restore oocyte quality in obese mice.

Design and methods: Outbred Swiss mice received a control or a high-fat high-sugar (HFHS) diet for 7 weeks. HFHS mice then underwent a PCCi, creating 4 groups: (1) C›C, control diet throughout; (2) H›H, HFHS diet throughout; (3) H›C, HFHS diet then switched to a control diet; and (4) H›T, switched to a control diet + Mounjaro® (tirzepatide) injections (0.02-0.05mg/kg, every 48h for 2 weeks) followed by a 2-week wash-out. Weight gain/loss was recorded weekly. At T1 (2 weeks) and T2 (4 weeks) of PCCi, mature oocytes were collected for confocal imaging after JC-1 (n=6), CellROX (n=6), and BODIPY (n=3) staining (±6 oocytes per replicate) to assess mitochondrial membrane potential (MMP), ROS levels and lipid volume, respectively.

Results: Mice on a HFHS diet were 36% heavier than controls (P<0.001) after 7 weeks. Tirzepatide caused faster weight loss than diet normalization, with H›T mice being significantly lighter than H›C (P<0.001) already 48h after the first injection. At T1, H›T mice reached control weight (C›C), while H›C remained 20% heavier than C›C. At that time, lipid content in H›H oocytes was 2 times higher than in C›C (P=0.021) accompanied by increased MMP (P=0.025) and ROS (P=0.043). Diet normalization (H›C) restored MMP and lipid volume to control levels and numerically lowered ROS. Strikingly, H›T oocytes showed elevated lipid levels and MMP similar to the H›H group, which was normalized to control levels again only by T2.

Conclusions: This study shows that tirzepatide treatment may induce acute stress in the oocyte. Elevated lipid levels and MMP in H›T oocytes at T1 likely result from body fat mobilization and excess lipid uptake in the oocyte. By T2, oocytes had mainly matured during the 2 week wash-out period with limited to no body fat mobilization, which resulted in both lipids and MMP in H›T oocytes being normalized to C›C levels. These results provide the first insights in the importance of tirzepatide induced weight loss and subsequent wash-out period in relation to oocyte quality.

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Posters

P-01

Equivalent Efficacy of Zymot and Lenshooke Microfluidic Sorting in Improving Sperm DNA Integrity and High-Resolution Live Morphology Compared with Density Gradient Centrifugation

David Jareño Martinez, ART Center, Centre Hospitalier Interrégional Edith Cavell (CHIREC)

Objective(s): Optimization of sperm selection remains a critical challenge in assisted reproductive technologies (ART). While density gradient centrifugation (DGC) is the most widely adopted approach, it is associated with increased oxidative stress and DNA fragmentation. Microfluidic sperm separation platforms have been proposed as biomimetic alternatives minimizingcentrifugation induced damages. To date, comparative studies between microfluidic and DGC have focused on conventional semen parameters. The objective of this study was to perform a direct comparison of Lenshooke™ and Zymot™ microfluidic devices versus DGC incorporating high-resolution morphological assessment through Motile Sperm Organelle Morphology Examination (MSOME), alongside evaluation of DNA fragmentation.
Design and methods: A prospective experimental study was conducted on 24 normozoospermic semen samples. Each ejaculate was processed in parallel using DGC (SilSelect TM, FertiPro), Zymot™ multi 850□l (Cooper Surgical), and Lenshooke™ CA0 (NordicCell). Standard parameters such as total sperm recovery and progressive motility were recorded according to WHO criteria. Morphological assessment of sperm nuclei was performed by real-time MSOME, classifying spermatozoa into morphological grades based on nuclear shape, chromatin homogeneity, and presence of vacuoles. DNA integrity was assessed by the sperm chromatin dispersion (SCD) assay (Microptic). Normality was evaluated using the Shapiro–Wilk test, and comparisons were carried out intra-individually using ANOVA or Friedman/Wilcoxon tests with Bonferroni correction.
Results: Both microfluidic platforms achieved markedly higher progressive motility compared with DGC (Zymot™: 87.9%, Lenshooke™: 87.2%, DGC: 66%; $p < 0.001$). MSOME analysis demonstrated that Zymot™ and Lenshooke™ enriched the fraction of grade I spermatozoa (normal morphology and absence of vacuoles) to 14.3% and 12.7%, respectively, versus only 9.4% after DGC ($p < 0.05$). DNA fragmentation levels, decreased significantly with microfluidics (Zymot™: 3.6% ± 2.4; Lenshooke™: 4.8% ± 2.6) versus DGC (10.7% ± 3.2; $p < 0.001$). No significant difference in progressive motility, MSOME and DNA fragmentation was observed between Zymot™ and Lenshooke™.
Conclusions: This study represents the first experimental evidence integrating MSOME in the comparison of two microfluidic sperm selection devices with conventional DGC. Both Lenshooke® and Zymot® were equality effective not to only improve motility and reduce DNA fragmentation, but also to select spermatozoa with superior ultra-structural morphology, deselecting large vacuoles, strongly associated with genomic integrity. These encouraging results warrant validation in larger, prospective studies incorporating clinical outcomes to confirm the translational potential of microfluidic sperm selection, including investigations encompassing non-

normozoospermic populations to ensure broader clinical applicability and efficacy across diverse male infertility profiles.

P-02

Relevance of Complementary Gene Analysis on exome data in well-defined infertility phenotype

David Pening, Université libre de Bruxelles (ULB), Hôpital Universitaire de Bruxelles (H.U.B)

Objective(s): Acephalic spermatozoa syndrome (ASS), a rare but severe type of teratozoospermia, is characterized by decapitated flagella in the semen. ASS has been identified to be familial, strongly suggesting that it has a genetic origin. Recently, pathogenic variations in a few genes have been identified in men with acephalic sperm; however, the underlying genetic causes remain largely unknown.
Design and methods: A 20-year-old man who is the third sibling from a consanguineous Romanian family applied to Erasme Hospital Fertility Center for an andrology and infertility workup. Semen analysis indicated a slight oligozoospermia and a motility within normal ranges, however, sperm morphology analysis revealed 100% with pin-shaped. His karyotype was normal and no microdeletions on the Y chromosome were found. A blood sample was collected after obtaining a written informed consent and in-silico gene panel testing (RD-V1) comprising 341 genes related to human reproduction was applied.
Results: Library was prepared using Twist Comprehensive Exome Panel (Twist Bioscience, South San Francisco, CA, USA) and sequenced on the Novaseq 6000 (Illumina, San Diego, CA, USA) with 2 × 150 bp reads. *In-silico* panel analysis was then performed via the Highlander programme (https://sites.uclouvain.be/highlander) by using the reference human genome GRCh38. The mean coverage was 272×, and 99% of target bases were successfully sequenced with a minimum depth coverage of 100×. Initial analysis of RD-V1 gave negative result for causative pathogenic variants. In compliance with the patient's consent, a complementary analysis has been done for nine recently identified genes specific for acephalic sperm phenotype. The latter analysis revealed a novel homozygous nonsense variation in *CCDC188*, a gene recently identified for ASS. It has been demonstrated that disruption of the orthologous gene in mice caused infertility, most of the spermatozoa were acephalic with possibility to find few normal spermatozoa in testes to create embryos through ICSI. A poor ICSI outcome reported on September 2025 after simultaneous injection of a sperm head and a detached tail from fresh semen samples. Based on mice model, we suggested that *CCDC188* mutation-associated infertility in humans is likely overcome by ICSI treatment using testicular sperm.
Conclusion: Clear clinical information is crucial in genome-based analysis. A complementary analysis may reveal the underlying genetic cause, which can considerably help in making better informed decisions as well as in the development of patient-specific therapeutic strategies in medically assisted reproduction applications. The fresh testicular sperm extraction is planned for the patient after detailed genetic counselling.

P-03

HOS test in combination with artificial oocyte activation: Effective strategy for achieving live birth in CFAP43-Related Male Infertility

Elodie Huon, Université libre de Bruxelles (ULB), Hôpital Universitaire de Bruxelles (H.U.B)

Objective(s): Multiple morphological abnormalities of sperm flagella (MMAF) is a severe form of asthenoteratozoospermia, characterized by absent, short, bent, coiled, and irregular flagella. Several genes associated with MMAF have been identified. Although MMAF can lead to male infertility, ICSI can effectively bypass flagellar defects to achieve fertilization and pregnancy. However, in the case of a lack of motility and low vitality, the challenge is to find live spermatozoa with fertilizing capacity.
Design and methods: This case study included a 39 years old man with a history of severe oligoasthenoteratozoospermia (OAT). Semen analysis showed normal volume and pH, albeit with total immotility, necrozoospermia (indicated by 25% vitality), and teratozoospermia. All spermatozoa displayed very short flagella and poor flagellar implantation. The Hypo-Osmotic Swelling (HOS) test was developed to select immotile but viable spermatozoa, as commercial HEPES-buffered medium with low bicarbonate failed to distinguish live sperm in the ejaculate. The patient had a normal karyotype with no Y chromosome microdeletions. After providing informed consent, the patient was enrolled in an ART program and underwent in silico gene panel testing for reproductive disorders.
Results: After two unsuccessful ICSI cycles, an exome-based gene panel revealed biallelic heterozygous variants in the *CFAP43* gene responsible for MMAF with autosomal recessive inheritance. Although artificial oocyte activation (AOA) was performed during the third ICSI cycle, no good-quality embryos were obtained for transfer. During subsequent consultation, it was revealed that the patient had been using high doses of paracetamol and ibuprofen prior to the third ICSI cycle. Both medicines are known to adversely affect different sperm parameters, such as motility, viability, count, and DNA integrity, when used daily at high doses. All medications were discontinued before the fourth ICSI trial. Six mature oocytes were collected from his spouse, and the HOS test in combination with AOA was used, resulting in two fertilized oocytes. Embryo transfer on day 3 resulted in pregnancy, and a healthy baby girl was born in September 2025, with no complications.
Conclusion: The HOS test serves as a useful indicator of sperm vitality, even in cases of very short sperm flagella. ICSI combined with the HOS test and AOA resulted in a live birth in our patient with a *CFAP43* mutation, indicating the importance of a tailored approach in such cases of male infertility.

P-04

Presence of atypical cytoplasmic inclusions with SER aggregates-like appearance in mature oocytes, and their persistence through blastocyst development: case report

Ileana Mateizel, Brussels IVF, Center for Reproductive Medicine

Objective: To document, for the first time, the presence of atypical cytoplasmic inclusions in metaphase II (MII) oocytes and blastocysts. These inclusions exhibited a Smooth Endoplasmic Reticulum aggregates (SERa)-like appearance and were observed throughout the preimplantation development stages, raising questions about their nature, persistence, and potential implications for embryological outcomes.
Design and Methods: The report is based on two consecutive preimplantation genetic testing (PGT) cycles involving the same couple. The atypical cytoplasmic inclusions were documented by images and video recordings using phase contrast microscopy. Embryological and clinical outcomes following the use of these oocytes were also reported. Genetic analysis of the resulting blastocysts was performed via Whole Genome Amplification followed by SNP array using Karyomapping with Bluefuse software (Illumina).
Results: The couple requested PGT for two genetic indications: (1) a heterozygous PTPN11 mutation (c.182A>G; p.Asp61Gly) in the male partner and (2) a duplication of chromosome 22q11.2 in the female partner. A total of 24 MII oocytes were retrieved from the two cycles, all of which exhibited at least one atypical cytoplasmic inclusion. Under static phase contrast observation these inclusions displayed square, round or oval shapes, that shifted into one of the other contours as the oocyte was rotated. Some of these inclusions persisted through blastocyst stage, unlike the previously reported cases where SERa typically disappear after fertilization. Of the 24 MII, 12 were successfully fertilized. Six embryos progressed to the blastocyst stage, of which five underwent biopsy for genetic analysis. One blastocyst was found to have a normal genetic profile and was selected for transfer. This resulted in the birth of a healthy female child, indicating that the presence of these inclusions did not preclude successful implantation or live birth.
Conclusions: A definitive classification of these atypical inclusions as true SERa by electron microscopy was not performed, as the couple chose to discard all supernumerary biological material. By this report we aim to raise awareness of the potential variability in SERa morphology, or of the presence of other inclusions, with SERa-like appearance. Importantly, blastocysts derived from oocytes containing such inclusions may still develop normally and lead to successful healthy live births.

P-05

Extension Analysis in AZFb Deletions: Implications for TESE Outcomes and Reproductive Options

Danijel Jankovic, Université libre de Bruxelles (ULB), Hôpital Universitaire de Bruxelles (H.U.B)

Objective(s): The role of the Y chromosome in spermatogenesis was established in the seventies when large terminal deletions of the long arm of the Y chromosome (Yq) were detected in six azoospermic men upon karyotype analysis. Microdeletions of Y

chromosome occur in about one in 4000 men in the general population, but their frequency is significantly increased in men with azoospermia and severe oligozoospermia. Three AZF regions were identified; AZFa, b, c and the current European clinical guidelines indicate a threshold of less than 5 million spermatozoa/mL for testing. Deletions involving complete AZFb regions are associated with azoo-spermia and chance of sperm retrieval is virtually zero according to European Academy of Andrology (EAA) and the European Molecular Genetics Quality Network (EMQN) best practice guidelines.
Design and methods: A 44-year-old man presented to the Fertility Clinic with primary infertility and azoospermia. He provided a report from 2017, originating from Algeria, which indicated the presence of AZFb deletions, although no extension analysis had been conducted. Two consecutive semen analyses were performed in our clinic, and during the latter analysis one motile spermatozoon was observed after centrifugation. The Y chromosome deletion test with extension analysis was prescribed and microsurgical testicular sperm extraction (micro-TESE) was planned.
Results: Molecular analysis of the Y chromosome microdeletion was performed in two steps, as recommended by the EAA/EMQN guidelines. Basic marker analysis revealed the deletion of two AZFb markers, sY127 and sY134. Extension analysis showed the presence of sY1192, which is not concordant with complete deletion and is defined as an incomplete AZFb deletion characterized by variable semen and testicular phenotypes. Very few immotile and one non-progressive motile spermatozoa were found during the fresh micro-TESE. Intracytoplasmic sperm injection (ICSI) was performed using non-progressive motile spermatozoa, and the remaining sample was frozen. One 8 cell good quality embryo was transferred on day 3, which resulted in a biochemical pregnancy.
Conclusion: Performing an extension analysis is essential, as the results will differentiate between TESE-negative and TESE-positive patients. Only complete test results (including basic and deletion extension markers) can provide the necessary insight into the reproductive options available for men with AZFb deletions. Micro-TESE followed by ICSI is possible for patients with partial AZFb deletion.

P-06

Assessment of oocyte morphological features during insemination can bring additional benefit for predicting prospective usable embryo rate in autologous ICSI cycles

Sandie Janssens, Université libre de Bruxelles (ULB), Hôpital Universitaire de Bruxelles (H.U.B)

Objective(s): Although numerous invasive as well as non-invasive methods are being developed to predict the developmental competence of the resulting embryo**,** oocyte morphology is still one of the most widely used scoring system worldwide. However, the updated Istanbul Consensus Criteria for oocyte scoring concludes that the current data are conflicting and still insufficient to be used routinely in clinical and laboratory prognosis. However, besides the potential of new AI-based tools, inclusion of novel oocyte dysmorphism features and evaluating their contribution to predict embryo

developmental potential can be useful to maximize the medically assisted reproduction (MAR) outcome.
Design and methods: This prospective observational study included 1398 autologous ICSI cycles performed between 17 August 2023 and 1 June 2025 in the Erasme Hospital Fertility Clinic. Only cycles performed with fresh gametes were included in the study. besides distinct morphological features indicated in the literature, the mechanical and cytoplasmic responses of the oocytes during injection were recorded and analysed individually until embryo transfer or cryopreservation. The impact of each morphological feature on fertilization rate and usable embryo rate was then assessed and documented.
Results: A total of 9189 M2 stage oocytes were scored. Among the oocytes with dysmorphisms, 32% (2980/9189) had one distinct morphological abnormality while 45% (4158/9189) displayed two or more abnormal features. Our analysis demonstrated that, although intra-cytoplasmic abnormalities accounted for 35% (3232/9189) of the total abnormalities recorded, their impact was more pronounced compared to abnormalities involving extra-cytoplasmic defects. However, having multiple abnormalities provided only a limited additional negative effect. Notably, abnormal oolemma response such as sudden or difficult breakage, which accounted for 18% (1643/9189) of all scored abnormalities, demonstrated moderate to strong correlations observed for fertilization and embryo development. These findings suggest that the inclusion of additional features, such as operational, environmental or clinical factors in the analysis, could improve the efficiency and enhance the predictive power of the oocyte-morphology assessments.
Conclusion: Oolemma resistance characteristics during ICSI are not currently addressed by the Istanbul Consensus. Relative response of oolemma as and the breakage upon injection during insemination may serve as valuable predictors of embryo development, especially when integrated into AI-based scoring systems. The main limitations of the study are the subjectivity of the operator and confounding biases, which could potentially be contributed by the patient- or clinic-related factors.

P-07

Bacterial contamination in sperm samples for ICSI: a comparison of two preparation techniques and how they affect further contamination during blastocyst culture

Johan Sterckx, Universitair Ziekenhuis Brussel, Brussels IVF

Objective(s): To evaluate the microbial presence in sperm samples processed for ICSI using two different preparation methods.
Design and methods: Prospective pilot study including 25 ICSI-couples between November 2024 and April 2025. Fresh semen samples were split and processed by both: (1) density gradient centrifugation (DGC) with a 90/45 density gradient (SpermGrad™, Vitrolife) followed by two sperm washing steps in HEPES-HSA (Sage™, CooperSurgical), and by (2) sperm separation device (Zymōt™, CooperSurgical) with sperm collection in HEPES-HSA without extra washing step. Following sperm preparation, the sibling MII oocytes were injected either with sperm processed by DGC or by Zymōt, followed by individual culture in cleavage/blastocyst culture medium (Origio®Sequential Series™, CooperSurgical) up to day 7.

Both HEPES-HSA and embryo culture medium contained 10µg/ml gentamicin. All procedures were performed inside a laminar airflow cabinet. For each group, samples were collected from: seminal plasma, processed sperm and embryo culture dish on day 3 and day 7. Sterility testing was performed using the BD Bactec™ system, followed by microbial identification with MALDI-TOF.
Results: Microbial presence was confirmed in all fresh semen samples, with a total of 97 bacterial isolates identified, including both commensal and potentially pathogenic species. After processing, bacterial growth was still observed in 64% (16/25) of DGC-samples and 80% (20/25) of Zymōt-samples (p=0.34).The most frequent potentially pathogenic micro-organisms in fresh semen were *Staphylococcus lugdunensis* (10/25), *Enterococcus spp.* (10/25), and *Streptococcus spp.* (13/25). After processing, *S. lugdunensis* was nearly eliminated (only 1/25 in DGC), *Enterococcus spp.* persisted in few samples (1/25 and 2/25, respectively), while *Streptococcus spp.* remained the most common pathogen (5/25 and 6/25, respectively). One fresh sample contained *Escherichia coli*. No fungal contamination was detected. No further bacterial growth was present in embryo and blastocyst culture media on day 3 or day 7, respectively. The DGC method includes multiple washing steps, whereas a sperm separation device only separates sperm via swim up through a porous membrane. Despite this methodological difference, both approaches showed comparable effectiveness in reducing—but not eliminating—the microbial load. Although bacteria being present in the processed sperm, ICSI effectively prevented carryover contamination into embryo culture.
Conclusions: Sperm preparation by DGC or Zymōt reduced microbial contamination present in raw semen and appeared effective -with combined action of gentamicin- in preventing further contamination in embryo culture media. The persistence of bacteria in processed sperm underlines the need for continued vigilance to minimize microbial risks in ART, particularly for IVF.

P-08
Impact of Isolated Teratozoospermia on Conventional IVF Outcomes: A Retrospective Study

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Objective(s): Sperm morphology is widely used as a semen parameter for assessing male fertility. However, its predictive value for outcomes in assisted reproductive technologies remains debatable. This study aims to compare the outcomes of conventional IVF (cIVF) between patients with isolated teratozoospermia and normal sperm morphology, as defined by the WHO criteria 2021, to promote wider use of cIVF as the first-line approach.
Design and methods: This retrospective study was performed in the embryology lab of Hospital Erasme, spanning a five-year period (2019-2024). A total of 878 cycles resulting in 6135 oocytes inseminated with cIVF were included. In 446 cycles, the sperm morphology was normal (≥4) (control group), while it was abnormal (<4) in 436 cycles (study group). Cycles with abnormal morphology were divided into four groups according to the Kruger criteria for morphology scoring (0,1,2,3) forming the study subgroups. The final insemination volume of the processed sperm was calculated based on the percentage of morphology and progressive motility. Main laboratory

outcomes were compared in a manner that included both normal and abnormal groups, as well as their subgroups 0,1,2,and 3.
Results: No significant differences (p>0.05) were observed between groups in terms of fertilization, day 3 embryo development, and blastulation rates between the control and the study group (Table1). The fertilization rate was identical (62%) in the study group (1837/2964) and in the control group (1965/3171). The Day 3 embryo development rate (>5 cells) was 77.6% (1425/1837) in the study group and 76.2% (1498/1965) in the control group. Finally, the blastocyst rate was 45.1% (829/1837) for the study group and 42% (825/1965) for the control group. The study group was further divided into four subgroups, and each subgroup was analyzed separately, with the results being similar and showing no significant difference.

P-09
Comparison of Automated versus Manual Semen Analysis in Fresh and Density Gradient Processed Semen Samples

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Objective: To evaluate the performance of the automated Low Volume Capillaries (LVC) in combination with the SQA Vision (MES-Medical Electronic Sperm) analyzer compared to manual semen analysis in assessing sperm concentration and motility parameters in both fresh and density gradient centrifugation (DGC) processed human semen samples.
Design and methods: A prospective validation study was performed between February and May 2025 in which a total of 24 fresh semen samples were analyzed. All samples followed the same flow: (i) manual and automated count of the raw semen sample, (ii) DGC and (iii) manual and automated count of the processed sample. Manual counts for concentration and motility were performed by applying 10µl on a Makler counting chamber for raw semen samples and 10µl on a Neubauer for processed samples. Automated measurements were performed using Low Volume capillaries (LVCs) with a sample volume of 5µl, designed for standardized sperm assessment. Capillaries were inserted into the SQA Vision machine, generating concentration of motility (A/B/C)/ml and total progressive motile sperm count (TPMSC) in 60s. Outcome parameters included concentrations of motile sperm subcategories (A, B, A+B, A+B+C) and TPMSC. Paired comparisons were performed using Wilcoxon signed-rank tests for non-normally distributed differences and paired t-tests for normally distributed ones. Agreement between methods was assessed using Pearson correlation coefficients. Three processed samples were excluded from the post-processing analysis due to absent motility after DGC (statistical outliers).
Results: The automated analysis consistently reported significantly higher sperm concentrations across all motility subcategories and TPMSC compared to manual analysis (Table 1)**.**

	Fresh			**DGC**		
	Manual	**LVC**	**p-value**	**Manual**	**LVC**	**p-value**
Conc. A+B+C M/ml	27.6	47.5	<0.001	3.5	11.9	<0.001
Conc. A+B (M/ml)	25.3	38.6	0.003	3.4	10.3	<0.001
Conc. A (M/ml)	14.7	19.8	0.019	3	9.1	<0.001
Conc. B (M/ml)	10.6	18.8	0.001	0.4	1.9	<0.001

TPMSC (M/0.5 ml)	12.7	19.3	0.003	1.7	5.1	<0.001

Table 1: Comparison of motile sperm concentrations measured by manual and MES analysis in fresh and density gradient processed semen samples.

Fresh samples showed high correlation for concentration of A+B+C, A+B and TPMSC between manual and automated counts (r≈0.8), which decreased after DGC processing (r≈0.5). Other parameters showed weaker correlations.
Conclusion: The automated LVC gave systematically higher counts for motile sperm concentrations and TPMSC compared to manual analysis. While the two methods correlate well in fresh samples, agreement weakens after processing, due to lower post-DGC values. Further validation is required before implementation is possible in clinical practice.

P-10
Evaluation of sperm capacitation as a parameter of male infertility diagnosis and impact of the cryopreservation process

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Objective(s): Ejaculated sperm cannot fertilize the oocyte until they undergo a maturation process in the female genital tract known as capacitation. It is characterized by changes in membrane potential (MP) of the spermatozoa (hyperpolarization), changes in sperm pH_i (cytoplasm alkalinization) and acrosome reaction (AR). It is well established that only capacitated sperm will be able to fertilize, yet capacitation is not assessed by the conventional semen analysis. The objectives of the study are to evaluate the effects of sperm freezing on the capacitation related events, and to correlate the capacitation parameters with the success of ART techniques such as IVF and ICSI fertilization rates.
Design and methods: Fresh and frozen normal semen samples (according to the WHO 6th edition) were analysed. We have assessed the impact of the capacitating media (Human Tubal Fluid versus Sperm Medium® -SM) on capacitation parameters (MP, pH_i) and one of its endpoints (AR), as well as the technique of MP measurement (spectrofluorimetry, flow cytometry). Capacitation parameters were also correlated with IVF and ICSI fertilization rates.
Results: More hyperpolarized values of MP were found following swim-up of fresh sperm samples in SM capacitating media (-76 ± 2.4 mV (n=11)) compared with HTF (-65 ± 2.4 mV (n=17)) assessed by spectrofluorimetry (p=0.008). Similar results were obtained using the flow cytometry technique (SM Facs -76 ± 2.2 mV (n=18) vs HTF Facs -71.8 ± 1.9 mV (n=20)) (p=0.004). No difference in mean MP values was found for frozen samples in both capacitating media. Sperm pH_i were more alkaline in fresh samples than frozen ones (p=0.01). The %AR from frozen straws compared with fresh sperm is significantly higher in all capacitating media (HTF and SM) in both DMSO (p<0.01) and progesterone conditions (p<0.05). The origin of sperm (Belgian patient versus foreign bank) does not have an impact, neither does the timing of capacitation assessment (one vs five hours after swim-up). Finally, no significant correlation was found between capacitation parameters (MP, pHi) and success of ART (IVF/ICSI fertilization rates) for frozen straws.

Conclusions: Spectrofluorimetric and flow cytometric assays enable reliable measurement of human sperm membrane potential. An impact of the capacitating media on the mean membrane potential of a sperm population is observed in fresh sample with more hyperpolarized values. Sperm freezing affects the endpoints of capacitation with an increase in the percentage of spontaneous and induced acrosome reaction. We found no statistically significant correlation with the capacitation parameters and IVF/ICSI fertilization rates for frozen straws.

P-11

Outcomes and Efficiency of Embryo Donation: An 8-Year Retrospective Analysis from a Belgian ART Center

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Objectives: The improvements in cryopreservation techniques led to an increasing number frozen-thawed embryo transfers (FET) and a rise in the number of embryos stored in cryobanks. Embryo donation, even if underutilized, remains one option for unused remaining embryos in Belgium. This study aims to evaluate the management, efficiency, and operating costs of an embryo donation program.
Design and Methods: This is a retrospective single-centre study. Data were extracted from the IVF database of CHC MontLégia in Liège from 2014 to 2022 for the donor population analysis and from 2005 to 2023 for the results of donated FET cycles. The administrative costs are based on the gross hourly wage of the secretarial staff and head biologist. The biological costs are based on the prices applied in 2025 for the different laboratory tests.
Results: Out of 3598 IVF treatments initiated from 2014 to 2022, 429 couples indicated their willingness to donate their surplus embryos, 211 confirmed their decision and only 92 had surplus frozen embryos available at the time of the study. Among these 92 couples, only 22 met all eligibility requirements for embryo donation (0.61% of all initiated IVF treatments). From 2005 to 2023, a total of **112 FET cycles** using donated embryos were performed, during which **173 embryos** were transferred. Double embryo transfers (DETs) were performed in **66 cycles (58.9%),** while **single embryo transfers (SETs)** were performed in **46 cycles (41.1%).** The **overall clinical pregnancy rate** was **45.5% (51/112),** and the **live birth rate (LBR)** was **29.6% (33/112**)**. Blastocyst-stage embryos** were transferred in **47 cycles**, with a corresponding live birth rate of **31.9% (15/47)**. The **mean age of recipients** was 40.5 years. At the time of analysis, **31 recipients had completed their treatment and achieved parenthood,** corresponding to a **live birth rate per recipient couple of 52.5%.** It was estimated that the biological screening and validation of a donor couple amounts to approximately €532.9 (administrative time) + €120.2 (serological screening for both partners) + €1343.7 (genetical screening for both partners) = €1996.8
Conclusions: Currently facing an increasing demand in gametes donation and a decrease in availability, embryo donation is an efficient alternative to double gametes donation which is underlined by the observed live birth rate of 31.9% per donated blastocyst transfer cycle, despite the time-consuming process for its management.

P-12

An integrative multi-omics map of adult human granulosa cell regulation

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Objectives: Human ovarian folliculogenesis is a complex and tightly regulated process, relying on coordinated interactions between oocytes and their surrounding somatic cells, in particular granulosa cells (GCs). Cumulus GCs support oocyte maturation through paracrine signaling and metabolic exchange, whereas mural GCs play a key role in steroid hormone production and follicle structure. Disruption of GC function contributes to pathological conditions such as granulosa cell tumors, polycystic ovary syndrome (PCOS), and primary ovarian insufficiency (POI), affecting a total of 10-15% of women of reproductive age. Although the clinical importance of GCs is well established, their molecular regulation in humans remains incompletely understood.
Design and Methods: To address this gap, we collected primary mural and cumulus GCs from women undergoing assisted reproduction at Ghent University Hospital. Following isolation and enrichment for FSH receptor-positive cells, GC identity was confirmed by hormonal assays and GC-specific marker profiling. We then applied an integrative multi-omics approach, combining transcriptomics, proteomics, epigenomics, chromatin accessibility, and three-dimensional (3D) genome profiling, to map the regulatory landscapes of these cells.
Results: Our analyses reveal distinct transcriptional and proteomic profiles between mural and cumulus GCs, consistent with their specialized roles in folliculogenesis. Moreover, we identified candidate cis-regulatory elements involved in oocyte-granulosa communication (GJA1, RYR2, AMIGO2), steroidogenesis (ESR1, STAR, CYP11A1), and ovarian maintenance (VCAN, HAS2). Chromatin interaction data provide insights into the 3D organization of the GC genome, highlighting regulatory regions at key loci implicated in reproductive health, including FOXL2. These datasets represent the first integrative multi-omics resource of the regulatory and 3D genome landscape in human GCs.
Conclusions: By combining multi-omics technologies on primary patient-derived GC material, we provide a comprehensive overview of the molecular regulation of adult human GCs. This work not only advances our fundamental understanding of ovarian biology but also creates a framework to explore how GC dysregulation contributes to reproductive disorders such as POI and PCOS. Ultimately, these insights may inform future strategies to improve diagnosis and treatment of female infertility.

P-13

Refining In Vitro Maturation Conditions to Enhance Germinal Vesicle Transfer Outcomes

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Objective(s): Germinal vesicle transfer (GVT) represents a promising strategy to rejuvenate aged or compromised oocytes by transferring the nuclear genome into a young healthy donor cytoplasm. Despite its potential, GVT efficiency remains limited

due to the low in vitro maturation (IVM) rates of denuded oocytes. Reversible meiosis inhibitors are commonly used to maintain meiotic arrest before IVM. Still, their effects on the competence of denuded oocytes, particularly when combined with GVT, have not been fully optimized. Notably, oocytes reconstructed through GVT have undergone extensive manipulation, which might compromise further maturation or developmental competence. The current study investigated whether replacing the conventional high-dose single inhibitor with a combination of low-dose reversible meiosis inhibitors could improve IVM outcomes of denuded oocytes.
Design and methods: GV oocytes were collected from B6D2F1 mice at 48 h after pregnant mare serum gonadotropin injection. Following cumulus cell removal, denuded oocytes were allocated to two groups: IVM culture (i) with 200 μM 3-isobutyl-1-methylxanthine (IBMX) (control) (n = 115) or (ii) with a dual combination of 50.0 μM dibutyryl cyclic AMP (dbcAMP) and 10.0 μM IBMX for 5 hours (n = 53). GVT was performed by transferring GV nuclei into enucleated recipient cytoplasts, followed by Hemagglutinating Virus of Japan (HVJ-E) - mediated oocytes reconstruction. The oocytes were subsequently subjected to the same IVM conditions, and the maturation rate was evaluated based on the extrusion of the first polar body after 20 h of IVM.
Results: The dual low-dose inhibitors significantly improved the IVM rate of denuded oocytes compared to the group with higher inhibitor (72.20% vs. 44.73%, p = 0.038). In the GVT group, oocytes cultured with the modified inhibitor showed a higher IVM rate than controls (52.20% vs. 47.27%), but this increase did not reach statistical significance. These results suggest that the inhibitor combination enhances IVM efficiency in both denuded and GVT-reconstructed oocytes, with a trend toward improved embryo development in GVT oocytes, as indicated by higher 2-cell rates compared with single-inhibitor treatment (66.60% vs. 55.53%).
Conclusions: Optimizing the combination and dosage of reversible meiosis inhibitors during IVM significantly enhances the maturation rate of denuded oocytes and shows potential to improve the efficiency of GVT procedures. This strategy may contribute to better outcomes in GVT aimed at preserving oocyte quality and developmental competence, particularly in cases of advanced maternal age. By mitigating age-associated defects and potentially reducing aneuploidy risks, this approach holds promise for advancing reproductive medicine.

P-14
Landscape of chemotherapy-induced microenvironmental alterations in the human ovary

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Objective(s): Chemotherapy is life-saving, but its ovarian toxicity threatens fertility and endocrine health in young patients. While ovarian reserve depletion has been documented, the early molecular and microenvironmental changes remain poorly understood. This study aimed to define the molecular signatures and tissue-level alterations underlying chemotherapy-induced ovarian injury.
Design and methods: Cryopreserved ovarian cortex samples from 10 cancer patients (aged 24–30 years) who donated tissue for research were analyzed. Among them, 5 patients had received first-line chemotherapy prior to ovarian tissue cryopreservation and 5 age-matched controls had not. Proteomic profiling by mass spectrometry

identified differentially expressed proteins, validated by western blotting and immunohistochemistry. Stromal and follicular alterations were assessed using markers of apoptosis (TUNEL), DNA damage (γH2Ax), oxidative stress (8-OHdG), proliferation (Ki67), fibrosis (picrosirius red), follicle count and morphology. Markers of inflammation (CCL2, IL-6, TNF-α, IL-1β, IFN-γ, IL-8, IL-10, TGF-β1) were evaluated by qPCR.
Results: A total of 5,209 proteins were detected, of which 237 (4.5%) were differentially expressed after chemotherapy. Pathways related to immune response, hypoxia and apoptosis were upregulated post-chemotherapy, while those involved in cell cycle and DNA repair were downregulated. Extracellular matrix (ECM) remodeling proteins were also dysregulated. Validation confirmed the upregulation of complement C3 (innate immunity; $p = 0.032$), SELENBP1 (hypoxia; $p = 0.030$) and KRT18 (apoptosis; $p = 0.015$) and a slight increase in SERPIN A3 level (ECM; $p = 0.077$) following chemotherapy-exposure, while NCBP2 (DNA repair) tended to decrease ($p = 0.067$). Stromal cell density was significantly higher post-chemotherapy (1.84 ± 0.15 versus $1.62 \pm 0.13 \times 10^6$ cells/mm^3; $p = 0.036$), although stromal fibrosis, apoptosis and oxidative stress did not differ between groups. An imbalance between pro- and anti-inflammatory signals emerged, with elevated CCL2, IL-8, IL-10 expression post-chemotherapy ($p = 0.063$, $p < 0.0001$ and $p < 0.0001$, respectively), while IL-1β, IL-6, TNF-α, IFN-γ, and TGF-β1 remained unchanged. Germ cells were also significantly impacted by first-line chemotherapy: follicles displayed increased apoptosis ($p = 0.013$), DNA damage ($p = 0.033$) and morphological defects ($p = 0.061$), despite preservation of the overall follicular reserve.
Conclusions: First-line chemotherapy significantly alters both the ovarian germ cells and stroma, emphasizing the need to further investigate the underlying molecular mechanisms and their impact on ovarian function and fertility preservation. By identifying candidate proteins and pathways involved in this process, this study offers a foundation for future pharmacoprotection investigations aimed at safeguarding ovarian health during and post-cancer treatment.

P-15

Assessment of environmental knowledge and needs among infertile couples undergoing assisted reproductive technology (ART): insights from a pilot survey

Sarah Marcelle, CHC groupe sante

Objective(s): This study aims to evaluate the interest, knowledge, and exposure of infertile couples undergoing assisted reproductive technology (ART) regarding environmental toxins. Ultimately, we will seek to identify the most appropriate means of information delivery according to the patients in order to optimize environmental health education.
Design and methods: We conducted a cross-sectional, single-center study based on an anonymous and voluntary survey among infertile women and couples undergoing ART at the CHC MontLégia (Liège, Belgium) between December 2023 and December 2024. At registration, all patients systematically received an information leaflet with a QR code providing access to an anonymous online questionnaire. The survey included 38 structured questions assessing demographic characteristics, interest and knowledge regarding environmental toxins, self-reported exposure through frequency of use of common products, and preferred methods for receiving information. Data were analyzed descriptively. Knowledge scores, exposure levels, and preferences

were compared across four predefined age groups (<25, 25–30, 31–40, >40 years) using Chi-square tests, with P<0.05 considered significant.
Results: A total of 1,092 questionnaires were distributed, with a 23.5% response rate (n=257). Most respondents were women (92%), and the majority were aged 31–40 years (61%). Nearly all participants (93%) believed environmental toxins influence fertility, pregnancy, or children's health, and 84% expressed interest in receiving further information. Informational brochures and dedicated web pages were the preferred formats (56% each). The overall knowledge score was modest, with correct responses in 49.7% of cases. Knowledge varied widely by domain: high for textiles (96%) and interior renovation (71%), but low for the impact of parental environment on the fetus before ART (15%), gardening (36%), cooking practices (44%), and air quality (40%). Exposure patterns revealed frequent use of non-stick pans (78%), plastic containers (66%), and home fragrance products (47%). Eco-labeled cleaning products were used by 48% of participants, while pesticides and herbicides were avoided by 70%. Age-group comparisons showed no significant differences across most outcomes.
Conclusions: This study confirms infertile patients' strong interest in environmental health and highlights persistent knowledge gaps, particularly regarding everyday sources of exposure. It also identifies preferred formats for educational tools, such as brochures and online resources, which appear most accessible and acceptable to patients. These findings reinforce the need to integrate structured and targeted environmental health information into ART care pathways, especially in the preconception period, in order to reduce harmful exposures, optimize fertility outcomes, and promote the long-term health of future children.

P-16
Studying CRISPR/Cas9 gene correction of infertility-related mutations in early mouse embryos using next-generation sequencing

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Objective(s): This project aims to study the safety and efficiency of CRISPR/Cas9 gene correction of infertility-related mutations in early mouse embryos. Desired and undesired gene editing outcomes were identified and quantified using next-generation sequencing (NGS). Editing efficiency and fidelity were compared across developmental stages to determine the optimal time point for gene correction.
Design and methods: CRISPR/Cas9 was used to correct pathogenic mutations in mouse *Plcz1,* causing male infertility due to fertilization failure, and in mouse *Padi6,* causing female infertility due to early embryonic arrest. CRISPR/Cas9 was introduced during fertilization, (i) co-injected in wild-type oocytes with mutant sperm during piezo-ICSI, or via electroporation in (ii) zygotes or (iii) 2-cell stage embryos. After four days of in vitro embryo culture, DNA was extracted for NGS. Targeted NGS using the Illumina MiSeq system assessed on-target editing outcomes, while shallow whole-genome sequencing (NovaSeqX) was used to detect chromosomal abnormalities resulting from unresolved double-stranded breaks.
Results: Gene correction via the homology-directed repair (HDR) pathway was most effective when CRISPR/Cas9 was delivered by zygote electroporation (HDR: 23.6%, n=55), compared to oocyte injection (10%, n=40), with no detectable HDR at the 2-cell

stage (0%, n=16). However, later-stage editing led to increased mosaicism (10% at fertilization, 34.5% at zygote, and 75% at the 2-cell stage). Most embryos displayed undesired on-target editing, including small deletions, insertions, mosaicism, and loss of heterozygosity.
Conclusions: Our results suggest that zygote electroporation is the most efficient method for CRISPR/Cas9-mediated gene correction in early mouse embryos, based on HDR rates. However, this approach is also associated with elevated mosaicism, raising concerns about the uniformity of correction. While these findings are based on limited sample sizes, ongoing experiments aim to validate these trends and further refine the gene editing strategy for infertility-associated mutations.

P-17
Primordial Follicle Vulnerability to Maternal Diet-induced Obesity: Implications for Preconception Care Outcomes

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Objectives: High-fat/high-sugar (HF/HS) diets and maternal obesity compromise fema-le fertility through reduced oocyte quality and altered oocyte mitochondrial features. However, the stage of folliculogenesis at which oocytes first become vulnerable re-mains unclear. The aim of this study was to examine if diet-induced obesity (DIO) induces mitochondrial defects in the dormant primordial follicle pool, and to compare that to the effects on ovulated oocytes. We also checked whether preconception diet normalization can alleviate these effects.

Design and methods: Adult female Swiss mice were fed either a control or a HF/HS diet for 7 weeks. Ovaries were then collected for transmission electron microscopy (TEM) to assess mitochondrial ultrastructure in primordial follicle oocytes (10–27 follicles from 6 ovaries per group). In a second experiment, some HF/HS mice were switched to a control diet (Diet Normalization) for 6 weeks, while others (HF/HS and controls) were maintained on their corresponding diets for comparison. Mature cumulus oocyte complexes (COCs) were then collected after hormonal stimulation for TEM analysis (3–5 COCs from 3–5 mice per group).
Results: Primordial follicle oocytes from the HF/HS-fed females showed a significant increase in the proportion of abnormal mitochondria (with vacuolization, elongation, and disrupted inner membrane) compared with controls (22.4% *vs.* 13.4%, $P < 0.05$). In MII oocytes from the HF/HS-fed mice, the proportion of abnormal mitochondria rose dramatically to 46.44% *vs.* 3.02% in controls ($P < 0.05$). Preconception diet normalization reduced abnormalities in mature oocyte mitochondria to 9.5% ($P < 0.05$ *vs.* HF/HS), but complete recovery was not achieved ($P < 0.05$ *vs.* controls).

Conclusions: These findings demonstrate for the first time that primordial follicle oocytes are vulnerable to dietary changes, showing altered mitochondrial ultrastructure. Nevertheless, the biggest impact on mitochondrial morphology appears to occur during follicular growth or the final stage of maturation since more abnormalities were detected in the ovulated oocytes. The persistent mitochondrial defects after diet normalization may reflect irreversible damage originating from affected primordial follicles. These results suggest that early and sustained metabolic health optimization in obese females may therefore be essential to preserve or fully restore oocyte competence and maximize IVF success.

P-18

Stuck at One Cell: CHEK1 Variant Shuts Down Embryogenesis Before It Starts

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Objective(s): This study aimed to elucidate the functional consequences of a rare heterozygous missense variant in the *CHEK1* gene (c.1325G>A, p.Arg442Gln) identified through a whole-exome sequencing (WES)-based gene panel associated with infertility. We investigated the impact of this variant on early embryo development using a mouse model to gain a better understanding of its role in embryo arrest.
Design and methods: A targeted WES gene panel covering 51 genes implicated in infertility and gamete dysfunction was developed collaboratively by the Center for Medical Genetics and the Department for Reproductive Medicine at Ghent University Hospital. DNA was extracted from peripheral blood samples, followed by bioinformatic analysis focusing on infertility-related genes. The identified *CHEK1* variant, located in exon 12, results in an arginine-to-glutamine substitution at a highly conserved residue and is extremely rare in the general population. We generated *in vitro* transcribed cRNAs from wildtype (WT) and mutant *CHEK1* plasmids. Female B6D2 mice were hormonally primed to obtain metaphase II (MII) oocytes, which were microinjected with either WT or mutant *CHEK1* cRNA along with mouse sperm using PIEZO-assisted ICSI, next to a control group without cRNA injection. In a subset of mutant cRNA-injected oocytes, we evaluated whether co-treatment with CCT244747, a CHEK1 inhibitor, could rescue the observed developmental phenotype. Embryo development was monitored by assessing 2-cell cleavage rates at day 1 and blastocyst formation at day 4 post-injection.
Results: Clinically, the variant carrier's prior split IVF/ICSI cycle showed normal fertilization (ICSI 4/4; IVF 5/8 with 2/8 3PN) but no subsequent cleavage, indicating arrest at the 1-cell stage. In the mouse model, 175 MII oocytes were injected across three experiments (WT 57; mutant 59; control 59); post-injection survival was 100% in all groups. The mutant *CHEK1* group exhibited a markedly reduced 2-cell rate (15.3%) versus WT (77.2%) ($p<0.0001$), consistent with very early developmental arrest. Microscopy showed abnormal timing of pronuclear events at the zygote stage. Blastocyst formation was also diminished in the mutant group (33.9%) compared with WT (82.5%) ($p<0.0001$), while controls (79.7%) were comparable to WT. Co-treatment with CCT244747 partially rescued development, increasing blastocyst yield to ~59% in mutant-injected oocytes.

Conclusions: Functional modeling demonstrates that *CHEK1* p.Arg442Gln severely compromises the earliest stages of embryo development, perturbing pronuclear dynamics and preventing timely first cleavage. These findings support the variant's pathogenicity and its contribution to infertility. Partial rescue with CHEK1 inhibition suggests a potential therapeutic avenue for carriers of *CHEK1* hyperactive variants, warranting further translational investigation.