EUROPEAN ACADEMY OF ANDROLOGY

Program Booklet

including Free Paper Abstracts

ECA2026 MODENA

14th European Congress of Andrology

16–18 SEPTEMBER 2026 MODENA, ITALY

CONTENTS

ECA 2026 PROGRAM

The document contains the program of ECA 2026 (held in Modena, Italy, on 16-18 September 2026), including the abstracts of free papers.

16 SEPTEMBER 2026 WEDNESDAY

AUDITORIUM

OPENING LECTURE

Wednesday, 16 September 2026 - 16:00-16:30

A-0163 SPERMATOGONIAL STEM CELL-BASED THERAPIES: FROM INNOVATION TO IMPLEMENTATION

Ellen Goossens

Belgium

ANDROLOGY AWARD

Wednesday, 16 September 2026 - 16:30-17:00

A-0326 LOW HUMAN SPERM MOTILITY COEXISTS WITH SPERM NUCLEAR DNA DAMAGE AND OXIDATIVE STRESS IN SEMEN

Kamil Gill

Poland

A-0325 EFFECTS OF GLUCAGON-LIKE PEPTIDE 1 RECEPTOR AGONISTS ON TESTICULAR DYSFUNCTION: A SYSTEMATIC REVIEW AND META-ANALYSIS

Gianmaria Salvio

Italy

GOLDEN COMMUNICATIONS

Wednesday, 16 September 2026 - 17:00-18:00

A-0024 GENETIC ARCHITECTURE OF PENILE VOLUME IN THE GENERAL POPULATION

Gunnar P Kordes [1], Philipp Beeken [1], Lynn Ogoniak [2], Jan Ernsting [3,4,5] Nils Johannaber [1], Benjamin Risse [3,4], Alexander S Busch [1]

[1] Department of General Pediatrics, University Hospital Münster, Germany; [2] Institute of Medical Informatics, University of Münster, Germany; [3] Institute for Geoinformatics, University of Münster, Germany; [4] Faculty of Mathematics and Computer Science, University of Münster, Germany; [5] Institute for Translational Psychiatry, University of Münster, Germany

Background:
Male genital volume reflects cumulative androgenic signaling across fetal and postnatal developmental windows, yet the genetic determinants underlying interindividual variation in adult penile volume remain poorly characterized, largely due to sample size constraints, potential cohort bias, and measurement bias. Defining the genetic architecture of penile volume through large-scale, population-based genomic analysis is therefore vital for understanding male genital ontogeny.

Methods:
We leveraged magnetic resonance imaging (MRI) data from a population-based cohort of ~30,000 adult men of European ancestry. Total erectile tissue volume (comprising bulb, crura, corpora, and glans) was quantified under resting (flaccid) conditions using automated deep learning–based MRI segmentation. Genome-wide association analysis (GWAS) was performed using Regenie (adjusting for age, age², genotyping batch, and first 10 PCs). SNP-based heritability and genetic correlations were estimated via linkage disequilibrium score regression (LDSC).

Results:
Mean total penile tissue volume was 117.8 ± 27.0 mL. We identified 20 independent genetic loci associated with penile volume at genome-wide significance ($p < 5 \times 10^{-8}$). SNP-based heritability (h^2) was estimated at ~30% (SE ≈ 2%), indicating a substantial polygenic component. Several loci were located near genes involved in androgen signaling and local steroid metabolism, including SRD5A2, CYP21A2, and members of the AKR1 gene family, consistent with the established role of androgen conversion and tissue-level androgen activity in male genital development. Additional loci highlighted pathways involved in somatic growth and developmental size regulation. Genetic correlation analyses revealed strong overlap with lean mass and height (rg ≈ 0.30–0.40), while correlations with circulating adult testosterone were modest (rg ≈ 0.09) and no association was observed with SHBG.

Conclusions:
Penile volume is a substantially heritable, highly polygenic trait whose genetic architecture is shaped by androgen metabolism and somatic growth pathways – reflecting both local endocrine exposure and genetically determined tissue responsiveness. These findings provide a population-level genomic framework for male genital development and refine the biological context for interpreting under-virilization and disorders of sex development in clinical andrology.

A-0101 Diffusion-Generated Synthetic Testicular Ultrasound Images for AI Development in Male Infertility Assessment

Daniele Renda Livraghi [1,2], Giorgia Spaggiari [1,2,3], Antonio R. M. Granata [1,3], Carlotta Pozza [4], Marta Tenuta [4], Andrea M. Isidori [4], Manuela Simoni [1,2], Nicola Morelli [5], Kevin Marchesini [5], Federico Bolelli [5], Costantino Grana [5], Daniele Santi [1,2,3]

[1] Unit of Endocrinology, Department of Medical Specialties, Azienda Ospedaliero-Universitaria of Modena, 41126, Modena, Italy; [2] Department of Biomedical, Metabolic and Neural Sciences, University of Modena and Reggio Emilia, 41126, Modena, Italy; [3] Unit of Andrology and Sexual Medicine of the Unit of Endocrinology, Department of Medical Specialties, Azienda Ospedaliero-Universitaria of Modena, 41126, Modena, Italy; [4] Section of Medical Pathophysiology and Endocrinology, Department of Experimental Medicine, Sapienza University of Rome, 00161 Rome, Italy; [5] Enzo Ferrari Department of Engineering, University of Modena and Reggio Emilia, 41125, Modena, Italy

Background: Testicular ultrasound is a cornerstone of male infertility assessment, particularly considering testicular volume and parenchymal inhomogeneity as key parameters associated with spermatogenic function. However, testicular parenchymal echogenicity assessment suffers from substantial operator dependency, limiting reproducibility and standardization. Artificial intelligence (AI) approaches offer transformative potential to convert subjective ultrasound features into objective, quantitative biomarkers. Nevertheless, the development of robust AI models is severely constrained by limited availability of large, shareable testicular ultrasound datasets due to privacy regulations and data scarcity. Denoising Diffusion Probabilistic Models (DDPMs) have demonstrated remarkable success in generating high-quality synthetic medical images across multiple modalities, yet their application to testicular ultrasound remains unexplored.

Aim of the study: To investigate DDPMs to synthesize realistic B-mode testicular ultrasound images as privacy-preserving surrogates for AI model development and validation.

Methods: Synthetic image quality was rigorously evaluated through quantitative distribution-level metrics and a clinician-driven Visual Turing Test involving endocrinologists with varying expertise levels from two clinical centres (Modena and Rome). A two-stage filtering pipeline ensured feature-space consistency and anatomical plausibility. Images utility was assessed through two downstream tasks: (i) self-supervised pretraining for binary classification of testicular parenchymal homogeneity versus inhomogeneity and (ii)

training of segmentation models for testicular region delineation, with synthetic data fully replacing real images during training.

Results: Twenty-two endocrinologists were involved and all of them, irrespective to their clinical expertise, evaluated 200 testicular images. All subjects were unable to reliably distinguish synthetic from real ultrasound images (accuracy:0.493±0.092, sensitivity:0.473±0.127, specificity:0.511±0.154, d-prime -0.037), confirming visual realism. In downstream classification tasks, self-supervised pretraining on synthetic testicular images significantly improved performance compared to training from scratch and achieved results comparable to pretraining on real ultrasound data. Segmentation models trained exclusively on synthetic images achieved Dice similarity coefficients on real test images nearly equivalent to those obtained when training on real datasets, demonstrating effective substitutability. Application of the two-stage filtering pipeline further enhanced downstream task performance, validating the importance of quality control in synthetic data generation.

Conclusions: This study provides the first evidence that diffusion-generated synthetic testicular ultrasound images are visually realistic and useful for AI model development in male reproductive medicine. Our findings demonstrate that generative AI can provide privacy-preserving, scalable surrogate datasets that enable robust development of AI tools for objective assessment of testicular parenchymal characteristics. This approach has significant implications for advancing AI-assisted reproductive endocrinology while maintaining patient privacy and overcoming data scarcity limitations inherent to specialized medical imaging domains.

A-0110 THE REMODELING OF NUCLEAR TRANSPORT AT THE MITOSIS-TO-MEIOSIS TRANSITION IS A CRUCIAL DETERMINANT FOR SUCCESSFUL SPERMATOGENESIS

Joana Martins Almeida [1], Margot J. Wyrwoll [2], Antoni Riera-Escamilla [3], Inês Ferreira [1], Catarina Lopes [4], Laura Quintas [5], João Guimarães [5], Sabine Kliesch [6], Corinna Friedrich [7], Nuno Santos [4], Donald F. Conrad [3], Joris Veltman [2], Frank Tüttelmann [7], Paulo Navarro-Costa [1]

[1] EvoReproMed Lab, Lisbon School of Medicine, Lisbon, Portugal; [2] Institute of Genetics and Cancer, University of Edinburgh, Edinburgh, UK; [3] Oregon Health & Science University, Oregon, USA; [4] Gulbenkian Institute for Molecular Medicine, Lisbon, Portugal; [5] ERA Chair iSTARS, Lisbon School of Medicine, Lisbon, Portugal; [6] Department of Andrology, Centre of Reproductive Medicine and Andrology, University Hospital Münster, Münster, Germany; [7] Institute of Reproductive Genetics, Centre of Medical Genetics, University of Münster, Münster, Germany

In our previous work, we identified a core genetic program essential for male germ cell development in species as diverse as humans, mice and fruit flies. We observed an enrichment for nuclear transport genes in this core program, prompting us to explore the functional significance of the nuclear transport pathway in male germ cell development and its potential implications for human reproductive disease.

Here, we show that male meiotic cells are remarkably sensitive to the disruption of nuclear transport. Using a pathway-wide in vivo RNAi screen in Drosophila (n= 151 genes), we observed that the silencing of 35.6% of nuclear transport genes specifically at the mitosis-to-meiosis transition led to severe spermatogenic impairment, compared to only 10.7% when silencing was induced at earlier stages of spermatogenesis.
To clarify the functional basis behind this requirement, we measured the biomechanical properties of Drosophila meiotic nuclei. Using atomic force microscopy, we noted that spermatocyte nuclei are significantly more rigid than those from spermatogonia (mean Young's modulus: 2423 vs 588 Pa, respectively). Furthermore, by characterizing the Nuclear Pore Complex (NPC) dynamics at the mitosis-to-meiosis transition, we observed a sharp decrease in N-acetylglucosamine modification of NPC components as cells progressed through prophase I, coupled to localized deposition of the nucleoplasmic interface component TPR around the chromosomes.

Because reduced N acetylglucosamine levels have been linked to less permissive RNA export – a condition that can be further exacerbated by the increased rigidity recorded in meiotic nuclei – we evaluated the specific contribution of the nuclear transport pathway to intranuclear RNA localization using nascent RNA labelling. Of the 50 nuclear transport genes found essential for male fertility in Drosophila, 37 were associated with defects in intranuclear RNA localization and/or levels in spermatocytes.

Importantly, for three of these genes (NUP98, IPO7 and RBM26) we observed an equivalent spermatogenic impairment phenotype in humans and fruit flies. More specifically, we identified 28 men with severe spermatogenic failure who carried potentially causal heterozygous variants (either loss-of-function or missense) in these predicted haploinsufficient genes.

Together, our findings identify a previously unrecognized requirement for efficient nuclear transport in meiotic cells as a crucial determinant for successful spermatogenesis. This requirement manifests as an increased dependency on a broader repertoire of nuclear transport genes upon meiotic entry, a likely consequence of the remodeling of the nuclear envelope into a more selective barrier at this stage. Interfering with this process undermines the correct execution of the meiotic gene expression program, revealing an overlooked link between the fine-tuning of nuclear transport in meiosis and human infertility.

Opening Ceremony

Wednesday, 16 September 2026 - 18:00-18:30

17 September 2026 Thursday

Auditorium

Meet the expert

Thursday, 17 September 2026 - 08:00-08:30

A-0170 Practical interpretation of a WES report in diagnosing male infertility

Frank Tüttelmann

Germany

EAA-ESSM lecture

Thursday, 17 September 2026 - 08:30-09:00

A-0164 The role of the couple in the Andrology field from sexual dysfunction to infertility

Linda Vignozzi

Italy

EAA-ESE lecture

Thursday, 17 September 2026 - 09:00-09:30

A-0165 The Metabolic impact of PDE5 inhibitors: 20 years of trials and Novel findings:

Andrea Isidori

Italy

News on FSH in male infertility

Thursday, 17 September 2026 - 10:00-11:00

A-0171 New insights into FSH receptor signalling

Livio Casarini

Italy

A-0172 Clinical effects of FSH in male patients with couple infertility

Hussein Kandil

UAE

A-0139 Deep Phenotyping of Male Infertility Improves the Response to FSH Treatment in Normogonadotropic Oligo-Asthenozoospermia

Giuseppe Grande [1], Andrea Graziani [1,2], Alberto Scala [2], Nicola Caretta [1], Antonella Di Mambro [1], Andrea Garolla [1,2], Alberto Ferlin [1,2]

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Introduction: The European Association of Andrology and the Italian Society of Andrology and Sexual Medicine guidelines recommend follicle-stimulating hormone (FSH) treatment in men from infertile couples affected by normogonadotropic oligoasthenoteratozoospermia, with the aim of improving quantitative and qualitative sperm parameters and increasing pregnancy rates, either natural or through assisted reproductive techniques. The Italian guidelines propose stricter selection criteria to optimize treatment efficacy, recommending FSH therapy in men with oligozoospermia and/or asthenozoospermia, FSH levels <8 IU/L, and no evidence of obstruction of the seminal tract. This distinction is clinically relevant, since impaired spermatogenesis and partial obstruction of the seminal tract may present with similar alterations in semen parameters unless a detailed diagnostic work-up is performed. Aim: To evaluate the efficacy of FSH treatment in a carefully phenotyped population of infertile men with oligozoospermia and/or asthenozoospermia, FSH plasma levels <8 IU/L, and no evidence of seminal tract obstruction. Materials and Methods: We conducted a prospective study including male partners of infertile couples referred to the Unit of Andrology and Reproductive Medicine, University of Padua (Italy), between June 2024 and December 2025. All patients underwent a comprehensive diagnostic evaluation according to the Italian position statement, including clinical and medical history, physical examination, hormonal assessment, testicular ultrasound, and complete semen analysis. Semen microbiological evaluation was performed in patients with signs or symptoms suggestive of genital tract infection, while transrectal ultrasound (TRUS) was performed when inflammation or obstruction of the seminal tract was suspected. A total of 50 patients with primary spermatogenic impairment (reduced sperm concentration and/or total sperm count), normal FSH levels (1.5–8.0 IU/L), and no evidence of male tract infection or obstruction were included. Patients with inflammatory conditions of the male genital tract were treated with anti-inflammatory therapy before enrollment. All patients received recombinant FSH (150 IU three times per week) for 3 months. Semen analysis was repeated at the end of treatment. A clinically significant response was defined as an increase >100% in sperm concentration or total sperm count. Results: After treatment, 42 patients (82%) showed a significant improvement in sperm concentration and/or total sperm count. Mean sperm concentration increased from 4.5 ± 4.6 to 18.7 ± 14.5 million/mL ($p < 0.05$), while total sperm count increased from 9.3 ± 10.5 to 39.8 ± 32.5 million ($p < 0.05$). Only a small proportion of patients were classified as non-responders. These findings indicate that careful patient selection substantially improves the likelihood of therapeutic success with FSH. Conclusions: This study highlights the clinical value of deep phenotyping in the diagnostic work-up of male infertility. A comprehensive evaluation allowing the exclusion of inflammatory and obstructive conditions of the seminal tract enables the identification of a subgroup of infertile men with true primary spermatogenic impairment who are likely to benefit from FSH therapy. In this selected population, the response rate to treatment appears higher than that reported in unselected cohorts. These findings support a precision-medicine approach to male infertility, in which accurate diagnostic characterization should guide therapeutic decisions and improve the effectiveness of medical treatment before resorting to assisted reproductive techniques.

A-0122 Intracytoplasmic Sperm Injection Outcomes in Men with High Sperm DNA Fragmentation: Testicular Versus Ejaculated Sperm

Ahmad Majzoub [1,2], Ahmad Almalki [1,3], Ahmed Alsaeedi [1], Kareim Khalafalla [1,3], Khalid AlKubaisi [1,3], Sami AlSaid [1,2], Nadir Fadol [1], Mohamed Arafa [1,2,4], Haitham Elbardisi [1,2,3]

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Background and Aim:

Sperm DNA fragmentation (SDF) has been associated with impaired reproductive outcomes, and the use of testicular sperm in men with elevated SDF has been proposed as a strategy to improve intracytoplasmic sperm injection (ICSI) outcomes. We aimed to compare clinical pregnancy and miscarriage rates among ICSI cycles involving men with elevated SDF according to sperm source, using a low-SDF ejaculated sperm group as a control.

Methods:
This retrospective comparative study included 544 ICSI cycles divided into three groups: men with high SDF who underwent ICSI using testicular sperm (n=76), men with high SDF who underwent ICSI with ejaculated sperm (n=136), and men with low SDF who underwent ICSI with ejaculated sperm (n=332). Men with elevated SDF were counseled regarding the option of testicular sperm retrieval; those who consented formed the testicular sperm group, while those who did not proceeded with ejaculated sperm. Clinical pregnancy and miscarriage rates were compared across groups. Baseline clinical, semen, hormonal, and cycle-related variables, including sperm preparation method, were also assessed. Continuous variables were reported as median [IQR] and compared using the Kruskal-Wallis test, while categorical variables were reported as frequency (%) and compared using chi-square analysis. Multinomial logistic regression was performed using the low-SDF ejaculated sperm group as the reference category to adjust for potential confounders.

Results:
Clinical pregnancy rates were comparable across the three groups: 26.3% (20/76) in the high-SDF testicular sperm group, 33.8% (46/136) in the high-SDF ejaculated sperm group, and 35.2% (117/332) in the low-SDF ejaculated sperm group (p=0.331). Miscarriage rates were also not significantly different: 20.0% (4/20), 12.2% (5/41), and 8.2% (9/110), respectively (p=0.263). Fertilization rates were 57.1 [41.7], 73.0 [29.8], and 75.0 [31.7]%, respectively (p<0.001). Several baseline and laboratory variables differed significantly among groups, including male age (39.0 [12.0] vs 36.0 [11.0] vs 35.0 [9.0] years), female age (35.0 [8.0] vs 31.0 [9.8] vs 31.0 [8.0] years), semen concentration (22.5 [24.8] vs 23.0 [28.0] vs 32.0 [37.0] million/mL), total motility (14.5 [25.0] vs 40.0 [23.8] vs 53.0 [22.0]%), progressive motility (0.0 [0.0] vs 0.0 [7.5] vs 10.0 [20.0]%), normal morphology (1.0 [2.0] vs 12.5 [17.2] vs 10.0 [15.0]%), SDF level (58.0 [33.8] vs 40.0 [16.8] vs 18.0 [7.0]%), and AMH <3 (22.7% vs 9.9% vs 9.8%) (all p<0.05). On adjusted multinomial regression using the low-SDF ejaculated sperm group as the reference category, male age (p=0.040), fertilization rate (p<0.001), and sperm preparation method (p<0.001) remained independently associated with study group, whereas female age (p=0.668), AMH category (p=0.851), and clinical pregnancy (p=0.825) did not.

Conclusion:
Clinical pregnancy and miscarriage rates did not differ significantly across the three groups, whereas fertilization was higher in the low-SDF ejaculated sperm group. Adjusted analysis suggested that fertilization and sperm preparation differed between groups, but these findings should be interpreted cautiously given the retrospective non-randomized design, baseline differences, and potential residual confounding.

GIANTS OF ANDROLOGY

Thursday, 17 September 2026 - 11:00-12:00

A-0319 MOST IMPORTANT TOPICS OF ANDROLOGY

Ewa Rajpert-De Meyts

Denmark

A-0320 MOST IMPORTANT TOPICS OF ANDROLOGY

Francesco Lombardo

Italy

A-0321 Most Important Topics of Andrology

Dirk Vanderschueren

Belgium

A-0322 Most Important Topics of Andrology

Mario Maggi

Italy

A-0323 Most Important Topics of Andrology

Manuela Simoni

Italy

Plenary lecture

Thursday, 17 September 2026 - 12:00-12:30

A-0166 Results from the large Nestorone + testosterone combination gel efficacy trial

Peter Liu

USA

IBSA symposium

Hyaluronic acid for the treatment of acute Peyronie's disease: from clinical trials to real-world case discussions

Thursday, 17 September 2026 - 12:30-13:00

Merck symposium

Translating Gonadotropin Biology to Patient Care: the Known and Unknown

Thursday, 17 September 2026 - 13:00-14:00

Biomarkers in Andrology

Thursday, 17 September 2026 - 14:00-15:00

A-0173 Total and free testosterone measurements in European laboratories

Nick Narinx

Belgium

A-0174 Variability in SHBG assays and SHBG gene polymorphisms and the effect on cFT

Bruno Lapauw

Belgium

A-0255 Novel sperm molecular biomarker for selecting spermatozoa with intact DNA

Mikel Albizuri [1,3], Malen Zabala [3], Ainize Odriozola [1,2,3], Irune Calzado [1,2], Manu Araolaza [1,2], Iratxe Ajuria-Morentin [2,4], Lorena Crisol [5], Maria Aranzazu Guembe [5], Roberto Matorras Weining [2,6], Nerea Subirán [1,2,3]

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Limited progress in conventional semen analysis has highlighted the need for improved diagnostic tools in male infertility. Although sperm DNA fragmentation (SDF) has emerged as a relevant marker associated

with impaired fertilization and pregnancy outcomes, its clinical application remains controversial due to methodological heterogeneity and inconsistent cut-off values. Current SDF assays are time-consuming and unsuitable for sperm selection in assisted reproduction, as they imply the sample destruction. CD10 (neprilysin), a membrane-bound protein expressed on human spermatozoa, has been implicated in sperm function, fertility, and DNA integrity. Emerging evidence indicates that CD10 expression is increased in spermatozoa with high levels of DNA fragmentation, positioning CD10 as a useful negative marker for excluding damaged cells and indirectly enriching for spermatozoa with intact DNA.

A prospective study that involves a total of 370 men from couples who started assisted reproductive treatments at University Hospital of Donostia, the Galdakao-Usansolo Hospital and the Cruces Hospital (Osakidetza) between 2020 and 2025. Samples from each hospital were analyzed in parallel in different research labs nearby the hospitals. CD10 expression was inversely associated with sperm quality and genomic integrity. The proportion of CD10-positive spermatozoa correlated negatively with conventional seminal parameters and was significantly higher in samples with seminal pathologies compared with normozoospermic controls. ROC analysis confirmed this capacity, with AUC values of 0.81 for all pathologies combined, 0.82 for asthenozoospermia, 0.82 for teratozoospermia, and 0.97 for oligozoospermia, demonstrating a good diagnostic discrimination. CD10 levels showed significant positive correlations with sperm DNA fragmentation (totalSDF, dsSDF and ssSDF), with the strongest association observed for double-strand damage (dsSDF; $R^2 = 0.57$). Consistently, CD10 accurately identified samples with high DNA fragmentation, yielding AUCs of 0.78 for total SDF, 0.82 for dsSDF, and 0.77 for ssSDF, with the best performance for double-strand damage. Interoperability was assessed by two independent operators, yielding consistent results across multiple hospital sites. Next-generation sequencing of CD10-sorted sperm subpopulations revealed stable ploidy and preserved read-length profiles in CD10-negative cells, whereas CD10-positive sperm exhibited markedly shortened reads, consistent with extensive DNA fragmentation. Together, these findings indicate that CD10 identifies and enriches for a genomically compromised sperm subpopulation, particularly characterized by double-strand DNA damage, supporting its potential clinical utility as a biomarker of sperm genomic instability and functional sperm quality.

Elevated CD10 expression in human spermatozoa is significantly associated with poorer sperm quality and increased DNA fragmentation. The selection of CD10-negative spermatozoa enables the isolation of higher-quality sperm with intact DNA, potentially improving outcomes in assisted reproductive technologies (ART). CD10, therefore, can serve as a reliable biomarker to identify and exclude genomically compromised spermatozoa, enabling the selective molecular isolation of high-quality sperm with intact DNA. This approach could improve diagnostic accuracy in male infertility and enhance assisted reproductive technology outcomes by enriching sperm populations with optimal genomic integrity.

Patent number: WO2019158621A1, EP19703750.0, US20200399593A1

A-0099 Testicular biopsy transcriptomics reveals molecular signatures predictive of negative sperm retrieval in non-obstructive azoospermia

Alexandra Kondic, Sofie Fagerström, Jonatan Axelsson, Angel Elenkov

[1] Lund University, Department of Translational Medicine; [2] Skåne University Hospital, Centre of Reproductive Medicine

Background:
Non-obstructive azoospermia (NOA) is commonly treated using microsurgical testicular sperm extraction (mTESE). However, the procedure is invasive, costly, and sperm retrieval rates remain relatively low at approximately 30–40%. Except for the very rare complete deletions in the AZFa and b sections of the Y chromosome, other relialble biomarkers capable of predicting sperm retrieval outcomes prior to surgery are currently lacking. Less invasive approaches such as testicular sperm aspiration (TESA) can be used as an initial diagnostic procedure. However, even after a negative TESA, salvage mTESE can still result in sperm retrieval in approximately 10% of cases. Consequently, many patients undergo unnecessary mTESE procedures following a negative TESA. Identification of molecular biomarkers within negative TESA samples that can predict the likelihood of sperm retrieval would therefore have significant clinical value. Sertoli cell-only syndrome (SCO), a common histological pattern in NOA, is associated with lowest sperm retrieval rates while producing a strong transcriptomic signature, making it a promising target for biomarker discovery.

Methods:
Bulk RNA sequencing from 18 NOA patients was performed on testicular tissue extracted during mTESE. First question was to analyze whether there is transcriptomic concordance between paired TESA and mTESE samples which were obtained from a single individual. Secondly, differential expression analysis was performed using DESeq2 package in R in order to establish a set of biomarkers. A two-step analytical strategy was applied: (1) identification of transcriptomic signatures distinguishing SCO from non-SCO tissues, and (2) identification of molecular differences associated with sperm retrieval success within SCO samples in order to define biomarker set unique for the cases with lowest prognosis for sperm retrieval in the SCO group.

Results:
Paired TESA and mTESE samples demonstrated strong transcriptomic concordance (Pearson r = 0.94). Principal component–based classification using the top differentially expressed genes achieved strong predictive performance for SCO identification (AUC = 0.94). As expected, the SCO transcriptomic signature was characterized by depletion of germ cell–associated genes including SPATA4, ACRBP, AKAP3, ODAD2, and IZUMO4. Within SCO samples, differential expression between sperm retrieval–positive and –negative cases revealed genes associated with immune signaling and extracellular matrix organization to be indicative for presence of active spermatogenesis.

Conclusions:
In this pilot study, we demonstrate that transcriptomic profiling of testicular tissue may enable molecular stratification of NOA patients. TESA-derived RNA signatures may contain sufficient information to identify a subset of patients unlikely to benefit from salvage mTESE. These findings support the feasibility of a two-step molecular classification strategy for predicting sperm retrieval outcomes and guiding surgical decision-making which need to be tested in a larger patient population.

EAA-ASA LECTURE

Thursday, 17 September 2026 - 15:00-15:30

A-0167 FETAL AND ADULT LEYDIG CELLS: A TALE OF THEIR ORIGIN AND FATE

Jacques J. Tremblay

Canada

DEVELOPMENTAL AND FUNCTIONAL DIMENSIONS OF OBESITY AND MALE REPRODUCTION

Thursday, 17 September 2026 - 16:00-17:00

A-0175 OBESITY AND MALE FERTILITY: LOOKING BEYOND SEMEN ANALYSIS

Andrea Salonia

Italy

A-0309 NOVEL ANTI-OBESITY MEDICATIONS AND MALE FERTILITY: EMERGING EVIDENCE

Konstantinos Michalakis

Greece

A-0106 TESTICULAR FUNCTION IN YOUNG MEN AND RISK OF CARDIOMETABOLIC DISEASE UP TO 20 YEARS LATER – A REGISTER-BASED FOLLOW-UP STUDY OF MORE THAN 5000 MEN

Laura Smidt Hansen [1,2], Astrid Linnea Beck [1,2], Julie Abildgaard [1,2,3], Anders Juul [1,2,4], Jørgen Holm Petersen [1,2,5], Niels Jørgensen [1,2], Lærke Priskorn [1,2]

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Copenhagen, Copenhagen, Denmark; [5] Section of Biostatistics, Department of Public Health, University of Copenhagen, DK-1353 Copenhagen, Denmark

Background: The role of testicular function may extend beyond reproduction, potentially serving as a biomarker of general health. In fact, poor semen quality has been linked with increased risk of cardiovascular morbidity, hospitalization, and mortality.

Objective: To investigate testicular function and its associations with cardiometabolic health from a longitudinal perspective by utilizing baseline data from a well-characterized cohort of Danish young men from the general population, coupled with register-based follow-up.

Material and methods: This register-based follow-up study includes 5,265 men from the Danish Young Men Study (DYMS), a cohort established to investigate reproductive function in young adulthood. At baseline, participants completed a questionnaire, provided a blood sample for the assessment of reproductive hormones and health markers, delivered a semen sample, and underwent physical examinations. After a median follow-up of 12.1 years (5th–95th percentile: 2.1–20.8), the men had a median age of 32.4 years (5th–95th percentile: 22.4–40.9). Baseline data were linked to the nationwide Prescription Register and National Patient Register to obtain information on cardiometabolic prescriptions and diagnosed diseases. Men were divided into quartiles based on semen quality parameters. The associations between semen quality in early adulthood and later cardiometabolic disease were analyzed using Cox regression analyses, with follow-up from baseline until the first cardiometabolic prescription or diagnosis, or censoring. Results are expressed as hazard ratios (HR).

Results: After adjustment for period of ejaculation abstinence, smoking and BMI, we observed that the lower the quartile of baseline total sperm count, the higher the risk of cardiometabolic conditions (Q4: reference, Q3: HR=0.97, Q2: HR=1.06, Q1: HR=1.16; p-trend=0.21). A similar dose-dependent pattern was observed for total progressive motile sperm count (Q4: reference, Q3: HR=1.06, Q2: HR=1.14, Q1: HR=1.21; p-trend=0.14).

BMI modified the association, which was less pronounced in men with normal BMI but more pronounced and statistically significant in men with BMI ≥ 25 kg/m² (N=898) (total sperm count: Q4: reference, Q3: HR=0.93, Q2: HR=1.34, Q1: HR=1.59, p-trend=0.01, and total progressive motile count: Q4: reference, Q3: HR=0.72, Q2: HR=1.20, Q1: HR=1.47, p-trend=0.01, respectively).

Conclusion: Semen parameters in youth were significantly associated with risk of cardiometabolic health in men with BMI ≥ 25 kg/m². Thus, semen quality could serve as an additional biomarker for future cardiometabolic health, enabling earlier intervention and prevention. Men seeking help for infertility may be a relevant target group for such preventive initiatives.

A-0136 MULTIPARAMETRIC PROFILING OF MALE INFERTILITY: GENOMIC AND ULTRASTRUCTURAL SPERM DAMAGE IN RELATION TO OXIDATIVE STRESS, AGE AND BODY MASS INDEX

Girolamo Francesca[1], Governini Laura[1], Ponchia Rosetta[1], Boschi Letizia [1], Saracini Giada[1], Luongo Francesca Paola[2], Alice Luddi[1], Paola Piomboni[1]

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Background
Sperm genome integrity is a key determinant of reproductive success, reflecting the efficiency of spermatogenesis, spermiogenesis, and epididymal maturation. Sperm DNA fragmentation serves as a critical indicator of biological damage and is linked to chromosomal instability and structural sperm defects. Oxidative stress is a major contributor, disrupting redox balance and promoting molecular alterations that impair sperm function. Few studies, however, have simultaneously analysed DNA fragmentation, chromosomal abnormalities, ultrastructural defects, and oxidative status. This study aimed to investigate the relationships among these parameters using a multiparametric approach and to assess the influence of age and body mass index (BMI) on sperm quality.

Methods
Twenty-four semen samples were collected in 2025 from healthy men aged 20–53 years at an assisted reproduction unit. Twelve samples were normozoospermic, and twelve showed abnormal semen

parameters. DNA integrity was evaluated using the TUNEL assay and sperm chromatin dispersion (SCD) test. Chromosomal aneuploidies were assessed by fluorescence in situ hybridisation (FISH) targeting chromosomes 18, X, and Y. Sperm ultrastructure was examined via transmission electron microscopy. Oxidative status of seminal plasma was assessed by measuring lipid peroxidation and total antioxidant capacity. Multiparametric regression analysis explored correlations among variables and their associations with age and BMI.

Results
The two DNA fragmentation methods were highly correlated ($r = 0.93$, $p < 0.0001$), though receiver operating characteristic analysis indicated modest discriminative performance (AUC = 0.6042, $p = 0.3865$). DNA fragmentation correlated positively with chromosomal abnormalities, particularly disomies of chromosomes 18, X, and XY. TUNEL values correlated with chromosome 18 disomy ($r = 0.7671$, $p = 0.0005$), X disomy ($r = 0.8772$, $p = 0.0004$), and XY disomy ($r = 0.7650$, $p = 0.0058$). Similar correlations were observed with the SCD test. Lipid peroxidation correlated with DNA fragmentation (TUNEL $r = 0.6503$, $p = 0.0257$; SCD $r = 0.6084$, $p = 0.0399$) and with disomies of 18, X, and XY. Multiparametric regression revealed a strong negative correlation between age and total antioxidant capacity ($r = -0.84$, $p < 0.001$). Age was also negatively correlated with Y chromosome disomy ($r = -0.77$, $p = 0.003$), which positively correlated with total antioxidant capacity ($r = 0.83$, $p < 0.001$).

Conclusions
These findings demonstrate a close association between sperm DNA fragmentation, chromosomal abnormalities, ultrastructural defects, and oxidative stress, highlighting the link between genomic instability and redox imbalance. A multiparametric approach provides a more comprehensive assessment of sperm quality than single diagnostic tests. Although based on a limited cohort, the data suggest that age may indirectly contribute to sperm damage via reduced antioxidant capacity. Integrating multiple diagnostic parameters can improve clinical evaluation of male infertility and support more personalized diagnostic strategies.

GENETIC INSIGHTS INTO MALE INFERTILITY

Thursday, 17 September 2026 - 17:00-18:00

A-0178 piRNA DYSREGULATION: SMALL RNAS, BIG IMPACT ON MALE FERTILITY

Kristian Almstrup

Denmark

A-0179 COUPLE-BASED GENETIC SCREENING: MATCHING CARRIERS TO UNCOVER RECESSIVE RISKS IN MALE INFERTILITY

Antonio Capalbo

Italy

A-0215 ASSOCIATION OF SPERMATOZOAL RNA EXPRESSION WITH FERTILIZATION OUTCOMES IN ART

Thomas Greither, Lina Albrecht, Diana Handke, Ivan Hoffmann, Hermann M. Behre

Center for Reproductive Medicine and Andrology, University Medicine Halle, Halle (Saale), Germany

Background: Spermatogenesis is governed by a precisely coordinated spatial and temporal expression of phase-specific RNA transcripts throughout the differentiation process from spermatogonia to mature spermatozoa. This study aimed to investigate spermatogenesis-specific RNA transcripts in mature spermatozoa used for assisted reproductive technology (ART) and to examine their association with clinical parameters such as fertilization success.

Materials and Methods: Aliquots of the motile sperm fraction used for ART were collected from 168 patients, and total RNA was extracted. Following reverse transcription, cDNA was pre-amplified according to the manufacturer's protocol (Standard BioTools, San Francisco, CA, USA). Expression of 65 spermatogenesis-related genes previously linked to impaired male fertility (Greither et al., Cytogenet Genome Res. 2020; Greither et al., Andrology 2023) was quantified in patient samples using the BioMark

HD system (Standard BioTools). Six reference genes were measured in parallel; ACTB, RHOQ, and GAPDH showed homogeneous expression and were therefore combined for normalization of gene expression. In comparison to the BioMark quantification, two transcripts (MNS1 and EQTN) were analyzed via qPCR.

Results: Transcript expression of spermatogenesis-related genes in mature spermatozoa was heterogeneous. High and consistent expression levels were observed for, among others, SAG, LDHC, HORMAD, NRIP1, DMRT3, ODF1, and AKAP4, whereas VASA, CYLC1, TEX11, PRSS21, ASZ1, TNP1, and RNF17 exhibited low expression. In the patient group with higher fertilization rates (n = 104), spermatozoal expression of DAZL, DNAJC5B, AKAP4, CFAP251, and ODF1 was significantly lower than in spermatozoa from patients with fertilization rates below 50% (n = 64; Mann–Whitney U test). MNS1 and EQTN RNA level were not significantly associated with the fertilization rate in ART.

Conclusion: Expression patterns of spermatogenesis-related transcripts in mature spermatozoa may reflect the physiological differentiation trajectory and could potentially serve as a predictor of ART success in the future.

A-0228 Results of the national Reproductive health screening program of the population of the Russian Federation: two years and nearly 10 million participants experience

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Since January 2024, a program of Reproductive health screening (RHSP) for men and women aged 18-49 to assess their reproductive health has been launched in Russia. The screening consists of two stages. During the first stage, the man fills a specially designed questionnaire and visit a urologist. If the questionnaire reveals indications or risk factors, the patient is referred to the second stage (semen analysis, STD testing, scrotal ultrasound, follow-up urological consultation). We analyzed data from the Federal Compulsory Medical Insurance Fund and the Automated System for Monitoring Medical Statistics of the Russian Ministry of Health for 2024–2025. Totally 9.8 million men were examined during the first stage of the RHSP over two years. 616 600 men were referred to the second stage of the screening (6.3% from first stage). Thus, in 2025, 88.4%, 9.6%, and 2.0% of men, were assigned to reproductive health groups 1 (“reproductively healthy”), 2 (“risk factors”), and 3 (“reproductive disorders”), respectively. A total of 118,340 semen analysis were performed, of which 14.9% (n=17,682) were found to have abnormalities for the first time. Among 175,500 STD tests, infections were newly detected in 13.6% of men (n=23,809). Among 175,200 scrotal ultrasounds, pathological findings were detected in 21% of patients (n=36,780). Following a medical examination, 66,267 men aged 18-49 were newly diagnosed with a reproductive system disease. This rate was 0.68% (0.68% of those who completed the first stage) or 677 per 100,000 men of the corresponding age. The incidence of male infertility, varicocele, prostatitis, and epididymoorchitis was 245.6, 544.4, 2074.5, and 144.2 per 100,000 men aged 18-49, respectively. The launch of reproductive health screening is only the first step toward establishing the true incidence of reproductive health problems and creating a comprehensive system for the prevention of urogenital diseases in men.

Room 2

Podium session

Thursday, 17 September 2026 - 08:00-09:00

A-0275 Assessing gene expression and chromatin accessibility dynamics in the early prepubertal Sertoli cell population of the primate testis

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Each Sertoli cell (SC) can support a fixed number of germ cells in adulthood, which determines the spermatogenic output of the testis. Understanding the changes SCs undergo during their maturation between birth and puberty, and their dynamics, becomes essential. To address this, we performed single-nuclei (sn)RNA-seq and snATAC-seq of the marmoset testis (Callithrix jacchus, sampling times: 0, 3 and 12 weeks after birth, n=2 each). For each animal, one testis was fixed, and the other one was flash-frozen for later 10x Genomics Multiome processing. Sequenced libraries were pre-processed with CellRanger ARC. The resulting FASTQ files were analysed with Seurat and Signac. Nuclei were quality-filtered. Samples were integrated with Harmony. The FindAllMarkers function was used to determine the differentially expressed genes (DEGs, min.diff.pct = 0.25, test.use = "MAST", only.pos = TRUE, avg_log2FC > 1) and the differentially accessible chromatin regions (DARs, test.use = "LR", latent.vars = “counts", only.pos = TRUE) for each cluster at each time point. The BioVenn package was used to determine overlapping- and time-specific- DEGs and DARs for each cluster. Published testis markers were used to identify the main testicular cell types, i.e.: Sertoli, germinal, Leydig and peritubular myoid cells, and endothelial, epididymal and immune cells.

We generated a snRNA-seq-snATAC-seq curated dataset of 24199 nuclei (0 weeks: 8341, 3 weeks: 9139 and 12 weeks: 6719 nuclei). Over 50% of the recovered nuclei were identified as SCs for each time point, showing FSHR and AMH expression; these were scattered into two clusters. A small subset with enriched expression for TOP2A, MKi-67, ASPM and CENP-E separates itself from the main SC cluster, suggesting it could be a proliferative subpopulation of SCs; which was further supported by Gene Set Enrichment Analysis (GSEA) showing enrichment in biological processes related to cell cycle and cell division (mitosis). Upon DEG analysis of the main SC cluster, we found 19 DEGs at 0 weeks and 92 DEGs at 12 weeks, with 22 DEGs overlapping the three time points and some shared between two only (0-3 weeks: 6, 3-12 weeks: 4, and 0-12 weeks: 15). One of the overlapping genes was HSD17B3 (a relevant enzyme for testosterone production), which has not been described for SCs at this stage of testis development in primates. We found 681 DARs overlapping the three time points, and some shared between two only (0-3 weeks: 667, 3-12 weeks: 157, and 0-12 weeks: 40).

Our preliminary analysis prompts our multiome dataset as a valuable resource to deepen our understanding of SC changes between birth and puberty. We found gene expression of HSD17B3 in early postnatal SCs, which has not been reported at this stage in primates before. We identified a proliferative subcluster of SCs based on gene expression. We identified DEGs and DARs that are time-point-specific as well as shared during early prepuberty for the SC population. Further analysis is forthcoming to include the different testicular cell types and their interactions to gain a better understanding of testicular gene regulation dynamics during early prepuberty.

A-0042 Sexual Dysfunction in Male IBD Patients on Advanced Treatments: Insights into a Neglected Dimension of Quality of Life

Marilina Romeo [1], Cristina Bezzio [2,3], Walter Vena [1,2], Giulia Migliorisi [3], Andrea Dall'Oca [1,2], Roberto Gabbiadini [3], Laura Loy [3], Giuseppe Privitera [2,3], Antonio Carlo Bossi [1], Ferdinando D'Amico [4], Andrea Sansone [5], Alessandro Pizzocaro [2,6], Alessandro Armuzzi [2,3], Andrea Gerardo Lania [2,6]

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Inflammatory bowel diseases (IBDs), comprising ulcerative colitis (UC) and Crohn’s disease (CD), are chronic relapsing disorders of the gastrointestinal tract. These conditions often begin in adolescence or early adulthood, overlapping with reproductive years and most sexually active period. Beyond intestinal symptoms, IBD affects multiple domains of quality of life, including sexual health. Nonetheless, estimates of prevalence of sexual dysfunction vary widely across studies and evidence is scanty concerning the possible impact of new biologic therapies available in the IBD field.

The aim of the study was to evaluate the prevalence of sexual dysfunction in a cohort of male IBD patients using validated tools, to assess possible determinants for the development of such condition posing greater attention to patients on biological treatment.

Subjects were consecutively enrolled at IRCCS Humanitas Research Hospital (Rozzano, Milan, Italy). Demographic and clinical data were collected for all participants. Clinical disease activity was assessed at the time of enrollment using the Harvey– Bradshaw Index (HBI) for CD and the partial Mayo score for UC. Biochemical disease activity was evaluated using fecal calprotectin (FC) and C-reactive protein (CRP). All enrolled patients filled the international Index of Erectile Function questionnaire (IIEF-15).

The overall prevalence of erectile dysfunction (ED) was 35.7 % (n=58/162), among which 41 (70.6%) mild, 4 (6.8%) moderate and 13 (8.0%) with severe. A similar prevalence was observed in the group of patients treated with biological therapies in our population (37.8%; n=39/103). Compared to normal erectile function (EF) subjects, patients with ED had significantly higher age (45.5 vs 37.0 years, p = 0.006), BMI (25.1 vs 23.5 kg/m 2, p= 0.009) and FC levels (265.0 vs 50.3; p<0.001). On multivariable regression model, only FC (OR 4.347, CI 2.039—9.263; p< 0.001) and clinical remission (OR 0.207, CI 0.082-0.523; p< 0.001) maintained significant association with ED.

Our results are in line with previous literature, describing an extremely high prevalence of ED in IBD male subjects with active intestinal inflammation found as the major determinant of sexual dysfunction, even in the population receiving advanced treatments.

In conclusion, active disease and inflammation remain pivotal risk factors associated to ED, whereas clinical remission independently predicts better sexual function. Sexual health is a major determinant of general health and it should be proactively addressed and managed in IBD subjects regardless of age or treatment regimens.

A-0046 Human Sertoli-to-granulosa cell transdifferentiation caused by a somatic DMRT1 mutation evidenced by a testicular granulosa cell tumor case

Sofia Boeg Winge [1,2], Terje Svingen [3], Niels Erik Skakkebaek [1,2], Maria Rossing [4], Lise Barlebo Ahlborn [4], Birgitte Grønkær Toft [5,6], Lise Aksglaede* [1,2], Gedske Daugaard* [7]

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Testicular granulosa cell tumors are very rare sex cord-gonadal stromal tumors with only 83 cases described to date. While their ovarian counterpart typically arises from a mutation in FOXL2 (c.402C>G; p.C134W), only a few reported testicular cases carry this mutation. Consequently, the genetic origin of testicular granulosa cell tumors is poorly understood. Here, we describe a case of such a tumor, which was initially diagnosed as a Sertoli cell tumor. Subsequent immunohistochemical reevaluation revealed absent expression of SOX9 together with ectopic FOXL2 expression, leading to reclassification as a testicular granulosa cell tumor. The patient presented with gynecomastia along with elevated serum concentrations of estradiol, inhibin B, and AMH. Over a five-year period, the patient experienced eight metastatic events, each preceded by increases in inhibin B and AMH, suggesting their potential as tumor biomarkers. Tumor sequencing excluded the canonical FOXL2 mutation; however, the tumor carried a somatic DMRT1 deletion, providing the first human evidence that loss of DMRT1 can drive Sertoli-to-granulosa cell transdifferentiation in the adult testis. These findings suggest that Sertoli and granulosa cells retain a degree of plasticity into adulthood in humans, as previously described in mice, maintained by the opposing actions of DMRT1 and FOXL2. This case expands our understanding of human gonadal differentiation, identifies DMRT1 dysfunction as a novel mechanism underlying somatic sex-reversal phenotypes in testicular tumors, and highlights the potential of AMH and inhibin B as markers for disease monitoring.

A-0093 Luteinizing hormone (LH) and human choriogonadotropin (hCG) induce ligand-specific steroid secretion profile in the mouse Leydig tumor cell line 1 (mLTC1)

Clara Lazzaretti [1], Carmela Perri [1,2], Flaminia Fanelli [3], Valentina Bissi [3], Giada Mercanile [1,4], Samantha Sperduti [1,5], Monica Lispi [6], Daniele Santi [1,7], Manuela Simoni [1,5,7], Livio Casarini [1,5]

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In Leydig cells, dihydrotestosterone (DHT) production occurs via the classical Δ5, Δ4, and the backdoor pathways. The latter involves the production of 5α- and 5α,3α-steroids and is required for the masculinization of the male fetus. Androgen synthesis can be promoted by luteinizing hormone (LH) and human choriogonadotropin (hCG), which act on the same receptor (LHCGR) expressed in the Leydig cell. LH and hCG induce different intracellular signalling pattern, but it is unknown whether they differentially promote specific steroid/androgen pathways.

The study compared LH- and hCG-dependent Leydig cell steroidogenic pathways by liquid chromatography-tandem mass spectrometry (LC-MS/MS) steroid profiling.

The mouse Leydig tumor cell line 1 (mLTC1) was exposed to the 50% effective concentrations (EC50) of LH and hCG (500 pM LH and 100 pM hCG), calculated from the gonadotropin-induced cAMP response curve, for 8 and 24 h. Steroids were extracted from the supernatant, and a panel of 20 Δ5, Δ4, and backdoor steroids was measured by LC-MS/MS. Data from 6 LH- vs hCG-treated independent experimental replicates were statistically analysed by the Kruskal-Wallis and Dunn's post-hoc test ($p < 0.05$).
LH induced higher progesterone, dihydroprogesterone, and 17OH-dihydroprogesterone production than hCG. However, cell treatment with LH resulted in weak testosterone and DHT synthesis. hCG induced a significant increase of 16OH-progesterone, 17OH-progesterone, androstenedione, testosterone, and DHT, compared to untreated cells, indicating preferential activation of the Δ4 pathway. Interestingly, hCG promoted the production of 5α- and 5α,3α-steroids, such as 17OH-dihydroprogesterone, 17-allopregnanolone, androsterone, and androstanediol, while LH induced the inhibition of backdoor steroids.

Our results demonstrated that LH and hCG induced different sex steroid patterns in Leydig cells. LH has overall weak effects and leads mainly to precursors accumulation and inhibition of the backdoor pathway. hCG leads to activation of the full steroidogenic pathway, where precursors are effectively converted to androgens via both the Δ4 and the backdoor pathway steroids.

In conclusion, LH and hCG induce distinct steroid profiles in murine Leydig cells, suggestive of different physiological specialization of the two molecules, i.e. gametogenesis vs pregnancy support. These findings have potential implications for the use of these two gonadotropins in clinical practice.

A-0148 Slower Pubertal Trajectory Based on Testicular Volume, but Not Tanner Genitalia Staging, Predicts Reproductive Outcomes and Relates with Sperm Epigenetic Markers in Young Adulthood

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Background and Objectives.

Pubertal timing is linked to lifelong health outcomes, yet long-term associations between the pace and trajectory of male puberty and detailed adult reproductive function remain largely unexplored in prospective studies. We tracked boys annually through puberty in the unique prospective Russian Children's Study (RCS) to investigate whether pubertal trajectory assessed by two pubertal metrics predicts overall and reproductive health in young adulthood, and to identify associated sperm epigenetic markers.

Methods.
This prospective cohort study followed 516 boys annually from ages 8 to 19 years; 10 subjects with severe chronic conditions and 48 subjects with less than 3 visits were excluded. We employed group-based trajectory models using lcmm R package to identify subgroups following similar pubertal trajectories based on two annual metrics: testicular volume (TV) assessed by Prader orchidometer and Tanner genitalia stages (G 1-5). Each boy was assigned to a trajectory based on his highest posterior probability from the selected model. At ages 18-21 years, semen samples from 222 subjects were evaluated according to WHO 6th edition criteria. Anthropometric measurements, body composition (Tanita BC-418MA), and serum hormone levels (testosterone, LH, FSH, prolactin, TSH) were collected on the day of semen collection. Intra-assay CVs for hormones ranged from 1.6-5%. Wilcoxon rank-sum test and multivariate linear regression were applied. A subset of 51 ejaculate samples underwent assessment of sperm DNA methylation using RRBS and small non-coding RNA using sncRNA-seq.

Results.
Among 458 subjects, we identified three pubertal progression categories ("slower," "moderate," and "faster") using cubic polynomial function of age, with distributions of 27-43-30% for TV, and 41-30-29% for G. For analysis, we assigned 222 subjects with semen data to slower (S) versus combined moderate/faster (F) pubertal groups. Based on TV trajectories, the S group exhibited lower birth weight (BW) compared to F (median 3.30 kg vs 3.50 kg, p=0.004). In multivariate models adjusted for BW, slower pubertal trajectory (TV-based) was significantly associated with higher serum FSH (β=-1.4, $p=1.2\times10^{-7}$), lower TV (β=6.7, $p=9.3\times10^{-35}$), reduced total progressive motile sperm count (β=41.3, p=0.003), lower BMI (β=2.0, $p=7\times10^{-5}$), and lower fat-free mass index (β=1.3, $p=1.5\times10^{-5}$). No associations were found with penile length, serum testosterone, LH, prolactin, or TSH. Tanner genitalia staging-based trajectories revealed no significant associations with adult outcomes after BW adjustment except association of slower trajectory with lower penile length (β=0.39, p=0.03).
Sperm DNA methylation analysis (2,164,582 CpGs with min 10x coverage) identified 402 DMCs (methylKit) between S and F groups (TV-based, $q<0.05$, $|diff|>10\%$), with significantly higher methylation levels among DMCs in the S group. SncRNA sequencing revealed 104 miRNA, 230 piRNA, and 13 tsRNA in sperm samples, and differential expression (DESeq2) of piR-32951 and 5'-half-GluTTC between groups ($p<0.05$).

Conclusions.

Using unique prospective data from the RCS, we demonstrate that a slower male pubertal trajectory assessed by testicular volume, but not Tanner genitalia staging, is associated with elevated serum FSH, decreased testicular volume, reduced BMI and fat-free mass index, altered semen quality, and distinct sperm epigenetic markers in young adulthood.

A-0057 Impairment of Male Reproductive Function After Isotretinoin Exposure

Fie Bendix [1,2], Lærke Priskorn [1,2], Niels Jørgensen [1,2], Hanne Frederiksen [1,2], Anne Jørgensen [1,3], Lina Mørch [4], Hans Christian Ring [5], Anna Rosenmai [6], Terje Svingen [6], Anders Juul [1,2,7], Anders Rehfeld [1,2]

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Isotretinoin (ISO) is a widely prescribed treatment for acne vulgaris. In 2024 alone, 11,345 Danish men redeemed an ISO prescription (incidence of 3.83 per 1,000). ISO is a stereoisomer of retinoic acid (RA),

and both can activate the Retinoid Acid Receptor (RAR). RA is a key regulator of spermatogenesis and investigated as a potential target for male contraception. Still, the effects of ISO on male reproductive health remain conflicting. Using a multi-model approach, we here aim to investigate the potential side-effects of ISO on male reproductive function.

We investigated the effects of ISO by:

1) A cross-sectional analysis of ISO users and non-users from the Danish Young Men Study (DYMS) cohort (n=5,330; median age=19). ISO exposure was identified through linkage to the Danish Prescription Registry, enabling assessment of in vivo associations between ISO use and reproductive parameters based on each participant's exact examination date. We applied adjusted linear regression models to evaluate differences in semen parameters and hormone levels between non-users and ISO users at varying time points their examination date, adjusting for BMI and age, as well as abstinence time (for semen parameters) and time of blood sampling (for hormone levels).

2) 96-hours ex vivo cultured human testis tissue exposed to ISO to examine changes in steroid hormone secretion quantified using isotope-diluted on-line TurboFlow-LC-MS/MS, and ELISA for inhibin B. Germ cell proliferation and apoptosis were assessed by BrdU incorporation and c-PARP antibody detection with immunohistochemistry staining.

3) A receptor reporter gene assay to assess the effect of ISO on RA-induced RAR-signaling.

Our results show young men using ISO (n=322) at any timepoint before their examination date had significantly lower serum FSH (2.38 vs. 2.62 IUI/L; (IQR): 1.66–3.37 vs. 1.77–3.79; p = 0.008) and higher estradiol (85 vs. 81 pmol/L; (IQR): 66–104 vs. 65–100; p = 0.044) levels, as well as a tendency of reduced sperm counts (125 vs. 125 mill; (IQR): 60-250 vs. 66–260; p = 0.586) when compared to non-users (n= 4,065). These associations persisted among both current users and prior users (with >2 years since last prescription) but were not observed in men first initiating ISO treatment after their examination date or in users of other acne medication. ISO exposure (in concentrations of 10-25uM) also altered hormone secretion in the cultured testis tissue when compared to control with significantly elevated testosterone and androstenedione levels, but significantly lower dihydrotestosterone (DHT) and inhibin B. No effects were detected on germ cell proliferation nor apoptosis after ISO exposure. ISO (at concentrations >12nM) inhibited RA-induced RAR signaling in a competitive manner.

In conclusion, our findings indicate that ISO exposure may impair male reproductive function by interfering with the hormone production and inhibition of RAR-signaling, potentially leading to reduced sperm counts, with potential long-term effects after termination of ISO. Confirmation in controlled in vivo human exposure studies, together with more investigations into the underlying molecular mechanisms, is required and will be crucial to fully understand the reproductive safety of ISO use in young men.

A-0069 Stimulation of the Leydig cells androgenic response using LH receptor agonist as a potential therapy for hypogonadism in Klinefelter syndrome

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Klinefelter syndrome (KS) is the most common sex chromosome aneuploidy which is the leading genetic cause of male infertility and is characterized by the 47,XXY karyotype. Hypergonadotropic hypogonadism is a hallmark of KS. In this condition, elevated luteinizing hormone (LH) fails to restore normal circulating testosterone (T) levels, despite maintenance of intratesticular testosterone (ITT) via Leydig cell (LC) hyperplasia. Factors such as testicular fibrosis and impaired vascularization may limit LH delivery and its stimulatory effects on LCs, meaning even elevated LH may fail to drive steroidogenesis adequately.

We investigated whether direct stimulation of the LH receptor (LHR) with the small-molecule LHR agonist Org 43553 could overcome these limitations and enhance T production. Testicular tissue from 41,XXY* mice, a well-established model for KS, and 40,XY wild type littermate controls was dissected into 1–2 mm^3 fragments and treated ex vivo with LHR agonist Org 43553, human chorionic gonadotropin (hCG, positive control), or vehicle for 4 hours. Testosterone levels in the supernatants were measured to assess LC responsiveness. Complementary, in vivo studies were conducted using the KS mouse model and wild-type controls. Mice received systemic administration of the LHR agonist, hCG, or saline for 24 hours to assess

overall steroidogenic responses. Testosterone levels were measured in blood collected directly from the heart to evaluate peripheral levels, and testes were collected to determine intratesticular T (ITT) concentrations.

Ex vivo stimulation with an LHR agonist elevated testosterone levels in both karyotypes. Both treatments increased T secretion in a dose-dependent manner. Additionally, age influenced this response, as younger mice exhibited a strong stimulation of T production, whereas older mice followed the same overall pattern but were less responsive. Complementary preliminary in vivo data reflected these findings. KS mice respond more strongly to the LHR agonist than wild-type controls. By contrast, hCG induced a robust response in wild-type mice but only a weak increase in KS mice. This pattern is consistent with impaired LH delivery in KS testes, likely caused by fibrosis and disrupted vascularization.

Results from these studies could provide a foundation for preclinical and clinical evaluation of a novel therapeutic approach for managing hypogonadism in Klinefelter syndrome.

A-0100 Can Microlithiasis disrupt Testicular Immune Privilege? Analysis on 1,755 Infertile Men

Civilla Matteo, Tienforti Daniele, Spagnolo Luca, Pisanó Carlo, Cocci Elisa, Barbonetti Arcangelo

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Testicular microlithiasis (TM) is an incidental ultrasonographic finding characterized by multiple small, non-shadowing echogenic foci within the testicular parenchyma. It has been hypothesized that TM might impair testicular immune privilege, potentially promoting the development of anti-sperm antibodies (ASA). However, previous studies investigating this relationship have been hindered by small sample sizes, leaving the clinical significance of TM regarding immune tolerance unresolved

This study aims to evaluate the association between TM and ASA positivity in a large, well-characterized clinical cohort of infertile men and to clarify whether incidental TM indicates a breakdown of testicular immune tolerance.

We conducted a retrospective cohort study of 1,755 infertile men evaluated in the Andrology Unit of the San Salvatore Hospital of L'Aquila (Italy) between September 2001 and May 2022. All participants underwent semen analysis, according to the current WHO guidelines, and scrotal ultrasonography. ASA were assessed using the direct IgG mixed antiglobulin reaction (MAR) test, with a clinically relevant positivity threshold of ≥ 50%. Men with severe oligozoospermia or asthenozoospermia that precluded reliable MAR interpretation were excluded

Out of 1,755 men, 167 (9.5%) were diagnosed with TM. A MAR test positivity ≥ 50% was found in 1.2% (2/167) of men with TM and 3.5% (56/1,588) in the non-TM group (p=0.17). The comparison of semen parameters - including volume, concentration, progressive motility, morphology, and leukocytospermia - showed no significant differences between the two groups. A history of prior orchiectomy was significantly more frequent in the TM group (16.8% vs. 2.3%; p<0.001), reflecting the well-known role of TM as risk factor for testicular cancer.

In conclusion, in the largest clinical cohort analysed to date, testicular microlithiasis was not associated with increased ASA positivity. These findings demonstrate that testicular immune privilege is preserved in men with incidental TM.

A-0226 Machine learning models may predict the presence of validated pathogenic/ likely pathogenic gene variants in men with non-obstructive azoospermia and micro-TESE failure

Mohamed Wael Ragab [1,2], Haitham El-Hussieny [3], Hesham Hanafy [1,2], Ali Mahran [4], Mohamed Arafa [1,5,6]

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Background: Non-obstructive azoospermia (NOA) represents a severe form of spermatogenic failure, frequently managed through microdissection testicular sperm extraction (microTESE), which fails in approximately 50% of cases. Whole-exome sequencing (WES) has emerged as a diagnostic approach for elucidating the genetic etiologies of NOA and associating it with surgical sperm retrieval failure, yet it often identifies variants of uncertain significance, with pathogenic or likely pathogenic (P/LP) variants being relatively uncommon. Enhancing the identification of patients at a higher likelihood of clinically significant genetic findings could optimize selection for WES, improve resource allocation, and genetic counseling. This study evaluates the efficacy of machine learning (ML) models in predicting validated P/LP gene variants in men with NOA and previous microTESE failure.

Methods: We analyzed data from 64 men with azoospermia and at least one failed microTESE who underwent WES. Fifteen clinical, hormonal, and laboratory variables were used as input features. Two supervised ML models were developed: a decision tree classifier and an artificial neural network (ANN). The outcome variable was the presence of a validated P/LP gene variant, as defined by ACMG criteria and validated by a multicenter study. Model performance was assessed using accuracy metrics.

Results: Validated P/LP gene variants were identified in 12 of 64 patients (18.75%). The decision tree model demonstrated superior performance, achieving 85% accuracy in predicting the presence of P/LP variants. In contrast, the ANN model showed a lower predictive accuracy of 69%. Moreover, the decision tree offered greater interpretability, allowing visualization of key clinical decision nodes contributing to prediction, whereas ANN functioned as a less transparent “black-box” model.

Conclusion: ML approaches can aid in predicting clinically significant genetic findings in men with azoospermia and prior failed microTESE. In this cohort, the decision tree model outperformed ANN in accuracy and interpretability. Further validation in larger, multicenter cohorts is warranted.

A-0080 Exome and genome sequencing entering routine andrology practice – Estonian experience

Maris Laan [1], Kristjan Pomm [2,3], Margus Punab [1,2,3]

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Over 50% of male infertility remain unexplained, potentially due to unknown genetic causes. We have established ambitious translational research based on exome (ES) or genome (GS) sequencing in the ESTonian ANDrology cohort (ESTAND) to solve over 1000 unexplained cases with severe forms of infertility. Core outcomes can be summarized as follows (1) nearly 20% of previously idiopathic cases have reached a molecular diagnosis due to disease-causing monogenic or structural variants; (2) ES/GS based analysis has been equally informative for diverse forms of severe male infertility – azoospermia, severe oligozoospermia, cryptorchidism or sperm motility defects; (3) our data shows shared genetic aetiology between several human congenital conditions and male infertility; (4) more specifically, almost 5-fold enrichment of disease-causing findings in hereditary cancer genes were found in infertile (7%) compared to fertile men (1.5%); (5) genetic heterogeneity of male infertility in outbred populations is high – recurrent variants or recurrently affect genes are infrequent; (6) some cases have oligogenic findings; (7) the majority of men with genetic infertility have not reached biological fatherhood; (8) our research pipeline has uncovered novel genes implicated in spermatogenic defect. Based on these results, using ES/GS in the workup of infertile men has proved to be well-justified. At the Tartu Centre of EAA, clinical ES was introduced in 2022, including gene panels for both quantitative and qualitative defects. Our long-term aim is to develop a routine pipeline for GS as the first-tier genetic test for idiopathic male infertility, advancing cost-effective personalised medicine. The patients will benefit from comprehensive genetic counselling about their reproductive and general health risks, including potential genetic comorbidities and risks to the future offspring.

References: PMID: 38614076; PMID: 41338233, PMID: 38654924, PMID: 40060932, PMID: 41694248; and unpublished data contributed by the team members K.Lillepea, A-G.Juchnewitsch, A.Panenko, O.Mõttus, G.Sakrits.

Funding: Estonian Research Council grants PRG1021 and PRG3041.

NYRA SESSION

Thursday, 17 September 2026 - 09:30-11:00

A-0304 UNWRAPPING THE SPERM CHROMATIN - NEW INSIGHTS INTO PROTAMINE FUNCTIONS

Gina E. Merges

Germany

A-0035 OPTIMIZATION OF IN VITRO SPERMIOGENESIS IN THE MOUSE MODEL

Eva Chemin, Laura Moutard, Magali Basille-Dugay, Valentin Rousseau, Ludovic Dumont, Nathalie Rives, Aurélie Feraille, Christine Rondanino

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Treating the long-term adverse effects of cancer therapies in children represents a major public health challenge. Because cancer treatments are known to exert toxic effects on the gonads, fertility preservation strategies are being proposed. In prepubertal boys, testicular tissue sampling followed by cryopreservation is recommended. After thawing, the in vitro maturation of prepubertal testicular tissue is one of the approaches being investigated to restore fertility in patients cured of pediatric cancer. Spermiogenesis, the final stage of spermatogenesis, represents a limiting step in organotypic cultures. Indeed, sperm production using the current culture protocol is rarely observed and occurs only in a small number of seminiferous tubules. Furthermore, the organotypic culture medium does not allow proper maturation of Leydig cells. The objectives of this research project were (i) to study, for the first time, the spermiogenesis stage in prepubertal mouse testicular cultures, and (ii) to evaluate the effects of several supplementations on the progression of in vitro spermiogenesis and Leydig cell maturation. Testicular tissues from 22-day-old postnatal mice were cultured in a basic medium for 6, 14, or 16 days (n=5-6 for each condition). In addition, tissues were cultured for 14 days (D14) in the presence of different factors known to stimulate spermatogenesis and/or Leydig cell maturation: IGF1, FSH, hCG, testosterone, retinol, estradiol and a Smo receptor agonist (SAG). Histological analyses were performed to investigate the progression of spermiogenesis. The expression of Leydig cell markers (Srd5a1, Sult1e1 and Insl3) and of steroidogenic genes (Cyp11a1, Cyp17a1, Hsd3b1, Hsd17b3 and Cyp19a1) was quantified by RT-qPCR. After 14 days of culture in the basic medium, a delay in post-meiotic progression was observed, characterized by the absence of elongated spermatids. In contrast, in the presence of FSH+IGF1, hCG, testosterone, estradiol or SAG, spermiogenesis was completed at D14 in 6.35%, 3.86%, 1.43%, 0.69% and 3.28% of seminiferous tubules, respectively, highlighting the importance of supplementing the culture medium at this stage. However, the efficiency of in vitro spermiogenesis remains lower than that observed under physiological conditions. Moreover, RT-qPCR analyses revealed defects in both Leydig cell maturation, and the expression of genes involved in steroidogenesis during organotypic culture. The Desert Hedgehog signaling pathway, which plays a key role in Leydig cell maturation and steroidogenesis, is currently under investigation. In addition, intratesticular testosterone and estradiol levels are being quantified using Homogeneous Time Resolved Fluorescence technology. The ultimate goal is to develop a sequential culture medium that will improve the progression of meiosis and spermiogenesis, thereby enhancing in vitro sperm production, and paving the way for future clinical applications.

A-0047 TRANSGENERATIONAL EFFECT OF ENDOCRINE DISRUPTORS AT SUBTOXIC DOSAGE ON MALE REPRODUCTIVE FUNCTION

Iva Sabovic [1], Andrea Di Nisio [2], Luca De Toni [1], Andjela Kovacevic [3], Stefano Gotti [4], Carlo Foresta [1], Alberto Ferlin [1]

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Endocrine disruptors (EDs) are compounds that have the ability to affect the endocrine system by interfering with the synthesis, bioavailability, and action of hormones. Among the main EDs we can include bisphenol A (BPA), bisphenol S (BPS) and perfluoroalkyl compounds (PFAS).

The aim of this project is to evaluate the effects of the current daily human exposure to single or combined EDs (PFOS, BPA and BPS) during the highly sensitive prenatal developmental stages In this PRIN study (Grant 20203AMKTW_002) we evaluated the possible transgenerational effects of EDs on testicular function in male F1 mice born from mothers treated in pregnancy and during lactation with subtoxic levels within the recognized tolerability levels for these three substances: group 1: BPA (0.2 ng/kg/bw) N=12, group 2: BPS (0.2 ng/kg/bw.) N=14, group 3: PFOS (0.7 ng/kg/bw.) N = 15, group 4: BPA (0.2 ng/kg/bw) and PFOS (4.4 ng/kg/bw) N=15 and group 5: BPA (0.2 ng/kg/bw), BPS (0.2 ng/kg/bw).and PFOS (0.7 ng/kg/bw) N=15.

Mice were sacrificed at sexual maturity (P = 90 days), and subsequently blood was collected, testes were stocked at - 80°C, and spermatozoa were retrieved from their respective epididymis to analyze the effects of different EDs by assessment of sperm count and motility, quantification of plasma and testes hormones by ELISA and Mass Spectrometry (LC/MS) method, and analysis of protein expression by western blotting (WB) technique. Compared with the unexposed controls, the group exposed to EDs showed a reduced sperm concentration ($P < 0.0001$). In parallel, the percentage of sperm with progressive motility dropped dramatically (-90% on average) in all EDs-exposed groups compared with the control group.

Quantification of hormones in plasma showed decreased levels of testosterone and INSL3 in all groups of exposed mice compared with controls (all $p<0.05$), while FSH and LH levels were increased in exposed mice compared with control (all $p<0.05$). Quantification of steroid hormones in testicular tissues showed a reduction in testosterone ($p=0.0279$). This pattern is supported by a disrupted steroidogenic process, which was consistent with the reduced StAR expression, compared to unexposed controls, as observed by WB analysis ($P=0.0212$ vs CTRL).Testis histology and transmission electronic microscopy analysis revealed a marked alteration of seminiferous tubule architecture upon exposure to EDs, together with Leydig cells vacuolization.Ultrastructural analysis of epididymal spermatozoa further revealed membrane thickening with irregular cell surface in exposed males to EDs, consistent with compromised sperm integrity .Flow cytometry imaging by Filipin III staining, a marker of membrane-cholesterol content, showed increased fluorescence intensity in spermatozoa from-exposed males (all $p <0.0001$). Finally, bioaccumulation of BPA, BPS and PFOS was evaluated by LC-MS, showing that all three EDs were concentrated at the testicular and plasma levels ,all $p<0. 05$ compared with control.

This study demonstrates for the first time a transgenerational effect of EDs at subtoxic dosages on testicular function, with direct damage on spermatogenesis and Leydig cell function, with intratesticular accumulation of these molecules

A-0079 PWWP3B GENE VARIANTS IN MALE INFERTILITY: INSIGHTS FROM HUMAN GENETICS, IN VITRO ASSAYS, AND A MOUSE MODEL

Leonie Herrmann [1], Shoham Kaufman [2], Juan Manuel Paturlanne [3], Assiel Rahimy [1], Sophie Adina Koser [1], Daniela Fietz [4], Jens Rosellen [5], Hans-Christian Schuppe [5], Sabine Kliesch [6], Nina Neuhaus [3], Nitzan Gonen [2], Frank Tüttelmann [1]

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Testicular somatic cells are indispensable for spermatogenesis and overall testicular function. Accordingly, genes with elevated expression in these cells are potential candidates for monogenic causes of male infertility when affected by pathogenic variants. One candidate gene is X-linked PWWP3B (PWWP domain-containing protein 3B) which is expressed in Sertoli cells. Here, we examine the relevance of predicted loss-of-function variants (pLoF) in the PWWP3B gene for impaired spermatogenesis.

We queried exome/genome data of >3,300 men from infertile couples for rare hemizygous pLoF variants in the PWWP3B gene (allele frequency < 0.001; stop gain, frameshift, or splice donor/acceptor variants). We investigated the testicular phenotypes of probands, the impact of genetic variants at protein level using in vitro assays, and the fertility of a mouse model carrying a selected human variant in the PWWP3B gene.

Three men with azoo- or cryptozoospermia had variants in the PWWP3B gene that introduced premature stop codons. Two of these men underwent a testicular biopsy and histological examination showed hypospermatogenesis with highly disorganised testicular architecture, thickened layer of peritubular myoid cells, and phenotypically immature Sertoli cells. The PWWP3B protein was localised in Sertoli cell nuclei in control tissue, but was absent from the proband's biopsies as observed by immunohistochemistry. Overexpression of PWWP3B gene-variants in HEK293T cells revealed reduced protein stability and disrupted subcellular localisation of the variants compared to the wild type. Male mice harboring a PWWP3B gene-variant that was identified in a man with azoospermia exhibited a potential age-dependent infertility phenotype, which is currently under further investigation.

In conclusion, the PWWP3B gene is a highly interesting candidate gene for Sertoli cell-induced spermatogenic failure and might be added to the growing number of diagnostically relevant genes crucial for male fertility.

This work was supported by the Deutsche Gesellschaft für Andrologie (DGA, German Society of Andrology) by a research project scholarship.

Keywords: male infertility, azoospermia, Sertoli cells, candidate gene, human genetics, mouse model

A-0095 Sequencing of the entire X chromosome reveals coding and regulatory variants pointing to novel candidate NOA genes

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Non-obstructive azoospermia (NOA) represents the most severe form of male infertility with genetic factors accounting for approximately 20% of patients. After a comprehensive diagnostic evaluation, ~40% of cases remain idiopathic. Whole-exome sequencing (WES) has led to the discovery of several novel genetic contributors to NOA, but still the rate of idiopathic cases remains high. Whole-genome sequencing (WGS) has recently emerged as a powerful tool, improving detection of exonic variants while also identifying regulatory variants potentially affecting gene expression. The X chromosome represents a compelling target for uncovering novel monogenic contributors to NOA. X-linked genes exhibit enriched or exclusive testis expression, highlighting their role in male reproduction. Furthermore, owing to male hemizygosity, a single deleterious variant is sufficient to impair protein function. In this context, we used WGS to comprehensively analyze X-linked genes potentially involved in male infertility in 44 WES-negative idiopathic NOA patients with known testicular histology recruited at the Fundació Puigvert (Barcelona, Spain). Only rare variants (MAF <1%) identified in single individuals and predicted to be deleterious by in silico tools were retained for further analysis. Pathogenicity was assessed according to the American College of Medical Genetics and Genomics guidelines. A total of 426288 variants were filtered. Among these, 159 coding and 4326 regulatory variants mapped to the X chromosome. According to ACMG classification, no (likely) pathogenic (LP/P) X-linked variants were identified; therefore, subsequent analyses focused on variants of uncertain significance (VUS). We identified nine genes harboring 11 exonic VUS in nine patients. These loci were prioritized according to testicular expression, mouse reproductive phenotype and literature evidence supporting a role in male infertility. Two genes emerged as potentially relevant. KIAA1210, which interacts with DNA topoisomerase II, recently proposed as a novel NOA gene. GAGE12H, putatively involved in germ cell development. In addition, we detected 42 VUS in non-exonic elements. 13 VUS mapping to repetitive regions were discarded while 29 VUS were evaluated for predicted feature–gene interactions and genomic characteristics. Genes whose expression may be affected by the variants in their predicted regulatory elements were prioritized according to the criteria used for the coding variants. This approach identified five genes, including two with established associations with NOA: i) BCORL1, a diagnostic gene with a moderate gene–disease relationship, ii) UTP14A, recurrently mutated in our previous study. Both carriers showed a Sertoli Cell-only (SCO) histology consistent with impaired function of these genes. Three additional loci were identified as candidate contributors to NOA. FOXO4, involved in germ cell differentiation, in a carrier with SCO phenotype. Variants in the regulatory elements of USP9X, essential for murine mitosis-meiosis transition, and of the testis-specific antigens GAGE and PAGE, displaying germ-cell-specific expression, were detected in two patients presenting spermatogonial arrest. In conclusion, our WGS study focusing on the X

chromosome identified coding and noncoding variants in 5 genes potentially relevant to NOA, supporting the role of X-linked genetic factors. To our knowledge, this is the first study exploring rare variants in regulatory elements potentially affecting the expression of genes involved in germ cell development.

A-0240 TRANSGENDER-DERIVED TESTICULAR TISSUE AS A MODEL FOR DEVELOPING ANTI-FIBROTIC STRATEGIES IN KLINEFELTER SYNDROME

Emily Delgouffe [1], Kim Aicha Feryn [1], Frédéric Chalmel [2], Veerle Vloeberghs [3], Guy T'Sjoen [4], Herman Tournaye [1,3], Ellen Goossens [1]

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Introduction: Klinefelter syndrome (KS) is the most prevalent sex-chromosome disorder in males, occurring in approximately 1 in 660 births worldwide. Most patients carry the classical 47,XXY karyotype, which is associated with progressive testicular degeneration, extensive fibrosis, and severe germ cell loss, ultimately resulting in azoospermia in about 95% of affected adults. Although focal spermatogenesis may persist, sperm retrieval through testicular sperm extraction remains challenging due to the profound structural disruption of the seminiferous tubules. Progress in understanding and treating fibrosis in KS is limited by the scarcity of testicular tissue available for research. Because long-term gender-affirming hormone therapy in transgender women is linked to fibrosis, arrested spermatogenesis, and partial rejuvenation of somatic cells, transgender orchidectomy tissue may represent a useful model for developing anti-fibrotic therapies for KS.

Materials & methods: Histological and immunohistochemical analyses were performed on testicular tissues from KS patients (n = 32) and transgender women (n = 106). Additional samples are being collected for future transcriptomic analysis. The degree of tubular hyalinization and interstitial fibrosis was scored on hematoxylin - periodic acid-Schiff-stained sections (score 0-4). Lumen morphology was classified as open, half-open, or absent. Germ cell differentiation was assessed using MAGE-A4, BOLL, CREM and acrosin immunostainings. Peritubular myoid cell integrity was assessed with ACTA2 and categorized as intact, interrupted, disconnected or absent. Sertoli cell maturation was examined using AMH, SOX9 and AR. Leydig cell maturity and functionality will be evaluated using INSL3 and CYP11a1. Additionally, laminin and fibronectin immunostainings will be performed to further characterize the tubular wall and its extracellular matrix composition.

Results: KS tissue exhibited extensive degeneration, with 41% of cases showing complete tubular loss (score 4), and the remaining tissue displaying moderate (15% score 2) or severe (44% score 3) fibrosis. Transgender tissue demonstrated a broader range of changes, from preserved structure (score 0) to severe fibrosis (score 3). Given that extensive fibrosis is characteristic of KS, subsequent analyses focused on the severely fibrotic transgender tissues (26%; n = 28). Severe fibrosis in trans women was frequently associated with absent lumina (46%) and a disruption in the peritubular myoid cell layers (39% interrupted), similar to KS patients (48% absent lumina, 59% interrupted ACTA2 patterns). Germ cell loss was present in both groups but was less pronounced in transgender tissue (36%) compared to KS tissue (81%). Sertoli cell maturation analyses are ongoing, but early observations indicate preserved SOX9 expression in Sertoli cells in both groups, with an AMH^+/AR^+ profile in transgender samples versus a mix of AMH^+/AR^+ and AMH^-/AR^+ profiles in KS tissues with preserved tubules.

Discussion & conclusions: Severely fibrotic testicular tissue from transgender women mirrors key structural features of KS testes, including tubular hyalinization, collapsed lumina, peritubular myoid cell disruption, and germ cell loss. Further analysis of Sertoli and Leydig cell maturity, tubular wall composition, and transcriptomic profiles will provide deeper insight into shared characteristics and may support the development of targeted anti-fibrotic strategies for KS patients.

A-0285 ABNORMAL SPERM PROTAMINATION IS LINKED TO REDUCED SEPTIN12 LEVELS AND IMPAIRED MOTILITY

Ondrej Sanovec [1,2], Magdalena Tuslova [1], Ondrej Simonik [1], Katerina Komrskova [1,3]

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Infertility of unknown origin affects up to 45% of infertile men. A key factor in sperm quality is the efficiency of the histone-to-protamine exchange. In humans, the ratio of Protamine 1 to Protamine 2 serves as a vital diagnostic marker; however, the exact regulatory mechanisms of this transition remain elusive. Interestingly, all existing mouse models for Protamine knockouts result in the loss of sperm motility.

Our previous research identified a decreased abundance and disrupted localization of the cytoskeletal protein Septin12 in the Protamine2-/- model. Septin12 is a crucial component of the sperm annulus—the barrier between the midpiece and principal piece—and its deficiency is a known factor in human asthenozoospermia.

In the presented study, we explored the relationship between abnormal protamination and sperm motility. We utilized flow cytometry for robust analysis of multiple sperm quality parameters. Moreover, we enriched the existing Sperm Chromatin Structure Assay (SCSA) with the Hoechst 33342 staining. This extension ensures more precise gating strategy for the final exclusion of non-spermatic events (excluding leucocytes and other somatic cells) We compared asthenozoospermic and teratozoospermic patients against healthy controls, evaluating histone-protamine exchange, chromatin structure, and DNA damage. These parameters were then correlated with standard spermiogram results and Septin12 abundance.

Preliminary data indicate a strong correlation between sperm motility and the quality of histone-protamine exchange, DNA fragmentation, and chromatin structure. Furthermore, asthenozoospermic and teratozoospermic patients exhibited significantly increased DNA fragmentation, decreased chromatin quality, and incomplete histone-protamine exchange. Finally, our findings suggest a correlation between histone-protamine exchange status, normal sperm morphology, and Septin12 levels.

Our research sheds light on the potential new connections between nuclear proteins and the sperm cytoskeleton, with possible overlap into the field of andrology as a new sperm quality marker.

This work was supported by Charles University Grant Agency (GAUK) 152125 and institutional grant RVO: 86652036

EAA GUIDELINE SESSION

Thursday, 17 September 2026 - 14:00-15:00

A-0188 MANAGEMENT OF BONE HEALTH IN THE ANDROLOGICAL OUTPATIENT CLINIC. PATHOPHYSIOLOGY AND DIAGNOSIS

Sara De Vincentis

Italy

A-0189 MANAGEMENT OF BONE HEALTH IN THE ANDROLOGICAL OUTPATIENT CLINIC/THERAPY. PATHOPHYSIOLOGY AND DIAGNOSIS

Karel David

Belgium

A-0190 MALE CONTRACEPTION

Cristina Meriggiola

Italy

A-0191 TESTICULAR BIOPSIES IN ADOLESCENT AND ADULT ANDROLOGICAL PATIENTS

Niels Joergensen

Denmark

GLP1 AND DUAL RECEPTOR AGONISTS AND SEXUAL DYSFUNCTIONS: PRO'S AND CON'S

Thursday, 17 September 2026 - 16:00-17:00

A-0176 PRO

Angelo Cignarelli

Italy

A-0177 CON

Dimitrios Goulis

Greece

A-0297 BEYOND WEIGHT LOSS: TIRZEPATIDE DIRECTLY REPROGRAMS THE TESTICULAR TRANSCRIPTOME IN MALE MICE WITH OBESITY, DIABETES AND HYPOGONADISM THROUGH A CALORIC RESTRICTION-INDEPENDENT MECHANISM

Valentina Annamaria Genchi [1], Celeste Lauriola [1], Nicola Marrano [1], Giuseppina Biondi [1], Annalisa Natalicchio [1], Sebastio Perrini [2], Luigi Laviola [1], Francesco Giorgino [1], Angelo Cignarelli [1]

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Background and Objective: Obesity and type 2 diabetes are strongly associated with male hypogonadism, characterized by low testosterone and impaired spermatogenesis. Tirzepatide (TZP), a novel dual GLP-1/GIP receptor agonist, has demonstrated remarkable efficacy in promoting weight loss and improving metabolic parameters; however, its effects on testicular function remain unexplored. This study investigated the hormonal and transcriptomic landscape of testicular tissue following TZP administration in a murine model of diet-induced obesity, diabetes, and hypogonadism.

Materials and Methods: Forty-nine male C57BL/6J mice were randomized into four groups: standard diet (SD, n=10), high-fat diet plus streptozotocin (STZ) to induce diabetes (dHFD, n=13), dHFD treated with TZP 10 nmol/kg/day i.p. (dHFD-TZP, n=13), dHFD with isocaloric pair-feeding matched to TZP food intake (dHFD-PF, n=13). Diabetes was induced in all HFD mice by a single injection of STZ (100 mg/kg i.p.) at week 9-10; TZP or vehicle was administered daily from weeks 15 to 20. Serum testosterone was measured by ELISA. Bulk RNA-seq was performed on testicular tissue and analyzed by PCA, PLS-DA, and DESeq2 (padj <0.05), followed by Gene Ontology, KEGG, and Gene Set Enrichment Analysis (GSEA) using MSigDB Hallmark and Reactome databases, and cell type deconvolution with CIBERSORTx based on the murine single-cell reference dataset GSE112393.

Results: Compared to baseline, dHFD-TZP mice exhibited a 70% increase in serum testosterone, not observed in dHFD-PF mice, indicating a caloric restriction-independent hormonal effect. PCA confirmed transcriptional independence between groups, with PC2 (17.7% variance) specifically separating dHFD-TZP from all other groups. dHFD induced profound testicular reprogramming (471 DEGs; 50 high-confidence genes), characterized by upregulation of contractile and myofibrillar programs, extracellular matrix (ECM) and collagen deposition (Col4a5, Col4a6), vascular remodeling (Sox7, Apln, Efnb1), and blood-testis barrier modification (Cldn5), alongside downregulation of steroidogenesis (Sc5d, Lss) and immune homeostasis. In dHFD-TZP vs. dHFD (792 DEGs; 37 high-confidence genes), TZP upregulated mitochondrial bioenergetics (Cox5b, mt-Nd1), spermiogenesis (Tnp1, Prm3), and flagellar motility (Cfap126), while downregulating steroidogenic enzymes (Sqle, Cyp51). The largest transcriptional divergence emerged in dHFD-TZP vs. dHFD-PF (2.034 DEGs; 36 high-confidence genes), highlighting a specific TZP pharmacologic signature. GSEA identified upregulation of spermatogenesis and complement activation, alongside Reactome enrichment of GPCR ligand binding and Galphai signaling, consistent with a potential direct receptor-mediated testicular action of TZP. Conversely, TZP markedly suppressed ECM organization, collagen biosynthesis, epithelial-to-mesenchymal transition, and TGF-β signaling, indicating a specific anti-fibrotic effect. Cell type deconvolution revealed enrichment of elongating spermatids in dHFD-TZP (~31% vs. ~28% in others), with reduced Leydig cell (3.1% vs. 4.8% in SD) and peritubular myoid cell fractions (3.8% vs. ~5.0% in others), and stable Sertoli cell proportions.

Conclusions: TZP exerts direct and extensive pharmacological reprogramming of the testicular transcriptome, qualitatively distinct from caloric restriction. The activation of spermatogenesis and GPCR signaling, combined with robust suppression of pro-fibrotic ECM remodeling pathways, support a direct multi-target testicular action. In contrast, the recovery of serum testosterone levels is more likely mediated by central mechanisms. These findings position tirzepatide as a promising strategy for obesity- and diabetes-related male hypogonadism beyond weight management.

A-0241 Online information and testosterone therapy: A global multilingual content analysis

Bonnie Grant [1], Nipun Lakshitha de Silva [1,2], Maha Gumssani [1], Oliver Quinton [1], Faysal Kayali [3], Isuru Lakmith Gamage [4], Waljit S. Dhillo [1], Mathis Grossmann [4,5], Channa N. Jayasena [1]

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Background
Testosterone prescribing for men has increased substantially worldwide over the past two decades. Concurrently, growing numbers of men seek testosterone treatment for symptoms not supported by robust evidence or aligned with international clinical guidelines. Direct-to-consumer “men’s health clinics” increasingly advertise testosterone therapy online, providing publicly accessible information about male hypogonadism. The advertising of medicinal products is regulated and requires that promotional claims align with licensed indications and available evidence. The accuracy of online information provided by healthcare providers may therefore influence patient expectations and demand for testosterone therapy. We evaluated the accuracy of information presented on websites offering testosterone treatment and assessed the alignment with international clinical guidelines.

Methods
We conducted a global content analysis of healthcare provider websites offering testosterone prescriptions to men. Searches were performed between 2023 and 2024 using three major internet search engines with the terms “male hypogonadism”, “male health clinics”, and “testosterone clinic”. Virtual private networks were used to minimise geographical across seven countries: Argentina, Australia, India, South Africa, the United Kingdom, the United Arab Emirates, and the United States. Searches were conducted in English, Hindi, Spanish, and Arabic, with translations by native speakers. The first 50 results for each search term were screened. Website claims were coded using a coding frame covering fourteen categories related to hypogonadism diagnosis, treatment, benefits, and risks, developed from existing literature and five international clinical guidelines.

Results
A total of 253 websites were included, with most based in the United States (139/253; 54.9%). Overall, 86.2% (218/253) contained at least one claim inconsistent with clinical guideline recommendations. Testosterone therapy was offered to men with serum testosterone concentrations exceeding 12 nmol/L on 9.9% (25/253) of websites. The term “andropause” was used as a diagnosis in 37.6% (95/253), while finger-prick self-testing was offered on 4.7% (12/253). Cardiovascular risk reduction was claimed by 20.6% (52/253). Non-guideline therapies, including testosterone secretagogues (e.g., hCG or selective oestrogen receptor modulators), were offered on 17.8% (44/253) of websites, while 6.7% (17/253) promoted non-testosterone androgens. Microdosing regimens were recommended on 11.9% (30/253). Claims of improved sexual function (70%), body composition (64.4%), energy (63.2%), psychological benefits (62.5%), anti-ageing effects (9.9%), and glycaemic control (15.4%) were also reported. Only 28.5% (72/253) of websites acknowledged potential fertility impairment with testosterone therapy. Compared with non-US clinics, US-based clinics were more likely to offer testosterone therapy at higher testosterone levels and to promote benefits relating to sexual function, body composition, and diabetes control ($p<0.05$)

Conclusions
This global multilingual content analysis observed that online information about testosterone therapy provided by healthcare clinics frequently contains claims that are inconsistent with international clinical guidelines. Such information may contribute to inappropriate expectations and demand for testosterone treatment. Improving the accuracy of publicly accessible online information and strengthening regulatory

oversight of direct-to-consumer medical advertising may help reduce inappropriate testosterone prescribing and improve care for symptomatic men worldwide.

PODIUM SESSION

Thursday, 17 September 2026 - 17:00-18:00

A-0222 Is 'Living Apart Together' a real advantage for patients consulting for sexual dysfunction? A cohort study

Clotilde Sparano [1], Giovanni Corona [2], Giulia Rastrelli [3], Linda Vignozzi [3], Daniele Vignoli [4], Mario Maggi [1]

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Background: Quantitative research on families has introduced a new category of relationship called Living Apart Together (LAT), that is, when a couple in a committed relationship live in separate homes. This study aimed to confirm whether living apart together improves sexual relationships in those suffering from sexual dysfunction. Methods: This study comprised cross-sectional (N = 4852) and longitudinal (N = 1402) analyses. The former explored psychobiological, hormonal and relational correlates using the Structured Interview on Erectile Dysfunction scale. The latter assessed the occurrence of major adverse cardiovascular events in a subset of patients followed for 4.3 ± 2.59 years. Results: Compared to cohabiting couples, patients in LAT relationships were younger and characterised by higher education levels, healthier lifestyles and lower comorbidity burdens (all $p < 0.001$). After adjusting for those confounders, the LAT group reported better sexual functioning, more frequent sexual intercourse, and higher total testosterone levels ($p < 0.001$), and the relationship was often a source of conflicts within the familial context and of shorter duration (all $p < 0.05$). When total testosterone was included in a fully adjusted analysis of covariance, the difference in obtaining a full erection between cohabiting and LAT relationships became non-significant ($p = 0.086$), suggesting a hormonal influence on erectile dysfunction. In the longitudinal analysis, Cox models adjusted for the aforementioned confounders showed that LAT relationships are associated with a twofold greater risk of major adverse cardiovascular events than cohabiting relationships, independent of other risk factors, including total testosterone levels, waist circumference and pathological penile blood flow. Discussion: This study illustrates that partnership arrangements can shape sexual interest and complaints, as reported by the participants. While men involved in LAT relationships show an ostensibly healthier phenotype, they experienced more often major adverse cardiovascular events. Therefore, co-residential relationship appears to provide more effective protection against future major adverse cardiovascular events for the male partner than a LAT relationship.

A-0262 Sex steroids measurement by mass spectrometry in hypogonadal men in a real-world setting: serum estradiol (E2) and E2 to Total Testosterone (TT) ratio do not differ between patients with Klinefelter Syndrome (KS) and non-KS hypogonadism

Sara Cerrato [1], Daniele Renda Livraghi [1], Francesco Costantino [1,3], Filippo Vignali [1], Giulia Canu [2], Maria Cristina De Santis [2], Laura Roli [2], Tommaso Fasano [2], Sara De Vincentis [1,3], Vincenzo Rochira [1,3]

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Introduction: Klinefelter Syndrome (KS) is the most common chromosomal disorder in men and is characterized by hypergonadotropic hypogonadism and multiple systemic comorbidities. It has traditionally been considered a hyperestrogenic condition, according to studies using immunoassays (IA). Liquid chromatography–tandem mass spectrometry (LC-MS/MS), currently regarded as the gold standard for steroid hormone quantification, provides greater analytical accuracy; however, data in KS remain limited, and the hypothesis of hyperestrogenism is still debated.

Aim: To compare circulating sex steroid profiles, particularly estrogens, in a cohort of KS and non-KS hypogonadal men, using both IA and LC-MS/MS in a real-world clinical setting. Secondary objectives included evaluating the agreement and diagnostic impact of the two analytical methods.

Materials and Methods: We performed a cross-sectional study, prospectively enrolling 203 adult hypogonadal men (117 with KS and 86 non-KS), treated and untreated, at the Endocrinology unit of AOU-Modena between May 2023 and September 2025. Clinical and anthropometric data were collected, and fasting morning blood samples were obtained. Serum total testosterone (TT) and estradiol (E2) were measured by two different methods on the same serum sample: IA with the CMIA DxI800 system (Beckman Coulter) and LC-MS/MS with the Chromsystems MassChrom® Steroids. Sex hormone-binding globulin (SHBG) was measured by IA for the calculated free testosterone (cFT) using the Vermeulen formula. Results are expressed as median and interquartile ranges (IQR) in parentheses.

Results: KS patients were younger, taller and had lower BMI compared to non-KS subjects ($p<0.001$). Gonadotropin levels were significantly higher in KS patients ($p<0.001$), consistent with primary testicular failure. No significant differences were observed between KS and non-KS groups in serum TT, cFT, SHBG, E2, or E2/T ratio, regardless of the analytical method used ($p>0.05$). These findings were confirmed using LC-MS/MS, indicating comparable estradiol exposure in the two groups. A significant positive correlation was observed between TT concentrations measured by either IA or LC-MS/MS, in both KS ($R2 = 0.647$, $p<0.001$) and non-KS ($R2 = 0.820$, $p<0.001$), even when analyzing the correlation between total or cFT and E2 measured by LC-MS/MS in both groups (TT: $R2 = 0.102$, $p = 0.001$ vs $R2 = 0.219$, $p < 0.001$; cFT: $R2 = 0.162$, $p < 0.001$ vs $R2 = 0.235$, $p < 0.001$). Notably, IA identified a significantly higher prevalence of biochemical hypogonadism compared to LC-MS/MS (56% vs 44%, $p<0.001$), suggesting potential overdiagnosis when using IAs.

Conclusions: Serum E2 and E2/TT did not differ between KS and non-KS along the entire spectrum of TT values (from low to normal/high) within the normal range, suggesting no difference in aromatization of estrogen in KS patients. These findings, confirmed by LC-MS/MS, suggest that clinical features traditionally attributed to estrogen excess are more likely due to androgen deficiency and/or supernumerary X chromosome. Additionally, immunoassays tend to underestimate testosterone, potentially leading to misdiagnosis and overtreatment, highlighting the importance of using LC-MS/MS in clinical practice.

A-0271 Measuring the prevalence of gender identities according to XYGO in a non-clinical sample of Italian people

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Introduction: The landscape of gender identity is increasingly characterized by a shift from binary frameworks toward a spectrum of diverse identifications. Gender identity , biological sex, and sexual orientation contribute to a complex, multifaceted construct known as sexual identity. This can be explored with XYGO, a new psychometric tool designed to integrate these components into a unified framework. XYGO measures sexual identity through a double-continuum Cartesian system, reducing the risk of using stigmatizing labels while providing standardized data for clinical and research purposes.

Objectives: This study presents the first application of the XYGO tool in a non-clinical population to examine the distribution of gender identities and highlight the relevance of multidimensional assessment in clinical practice.

Methods: The XYGO tool was administered as part of a broader survey on sexual health and literacy. Data were collected via social media between September 11 and September 18, 2025, from a convenience sample (N = 1123).

Results: The sample included 802 women and 321 men. While individuals reporting their gender identity to be fully aligned with their biological sex constitute the majority of the sample (72.9%; G +3 women, 610/802, 76.1%; G -3 men, 209/321, 65.1%), there is a significant presence of gender-diverse identities (27.1%). Notably, individuals reporting an identity on the opposite side of the gender spectrum (G -3

women or G+3 men, n = 15) or agender (G0 women or G0 men, n = 25) were significantly outnumbered by non-binary and fluid identities (n = 264, p <0.001). Statistical analysis also revealed a significant association between biological sex and gender identity, with the men being significantly more likely to identify as gender-diverse than women (34.9% vs 23.9%, p <0.001).

Conclusion: The high prevalence of non-gender conforming (NGC) identities captured by the XYGO coordinates, particularly within the male-aligned cohort, underscores the limitations of traditional binary models. By mapping identities onto a numerical continuum (from -3 to +3), XYGO successfully identifies nuanced gender expressions that often remain invisible in categorical surveys. These findings support the implementation of holistic, coordinate-based measures to improve inclusivity and diagnostic accuracy in sexual medicine and psychosexology.

A-0030 Concentration Matters: Prevalence Of Genetic Disorders Is Associated With The Severity of Seminal Abnormalities

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INTRODUCTION AND OBJECTIVES:

Genetic factors, including chromosomal abnormalities and Y-chromosome microdeletions, represent established causes of male infertility. Current International guidelines recommend genetic screening based on sperm concentration thresholds. EAU/ASRM guidelines recommend karyotype analysis for men with concentration below 5 million/mL and Y-chromosome microdeletion testing particularly in patients below 1 million/mL. However, comprehensive data comparing genetic abnormalities prevalence across distinct concentration ranges in systematically screened cohorts remain limited. Understanding real life genetic disorders prevalence, could help clinical decision-making, resource allocation and correct patient counselling.

The objective of the study is to determine the prevalence of genetic abnormalities (karyotype alterations and Y-chromosome microdeletions) in men presenting with azoospermia and severe oligozoospermia

METHODS:
Retrospective cohort study, analyzing 245 infertile men with severe oligozoospermia (sperm concentration ≤ 5 mill/mL) and azoospermia with palpable vas deferens, consulting at a tertiary andrological unit, between 2018 and 2025. Patients were stratified by sperm concentration: Azoospermia, ≤ 2 mill/mL and 2-5 mill/mL. All patients underwent standard G banding karyotyping and multiplex polymerase chain reaction (PCR) based Y-chromosome microdeletion analysis targeting AZFa, AZFb and AZFc regions

RESULTS:
Among 245 patients, 18 presented genetic abnormalities (7.3%). Distribution by concentration revealed that in the azoospermic group (n=136), 15 patients presented genetic alterations (11.2%); of which 9 were Karyotype abnormalities (6.5%) including Klinefelter syndrome variants (47,XXY and mosaics, n=3), 47,XYY mosaicism (n=1), and balanced chromosomal translocations (n=4). In this group, 7 patients presentes Y-chromosome microdeletions (5.1%) including AZFc (n=4), multiple deletions (n=2), and AZFa (n=1)

In the ≤2 million/mL group (n=70): 3 patients (4.3%) presented genetic alterations. Y-chromosome microdeletionswere found in 3 patients, including AZFc (n=2) and AZFb (n=1) regions. No karyotype abnormalities were detected. Lastly, in the 2-5 million/mL group(n=39), no patients presneted genetic abnormalities. Overall, patients presenting Y-chromosome microdeletions (n=10) slightly exceeded the number of patients with karyotype abnormalities (n=9), with one patient presenting both

CONCLUSIONS:
This study, of infertile men with seminal alterations, showed a concentration dependent gradient in genetic abnormalities prevalence. These findings validate current EAU/ASRM guidelines recommendations for genetic screening in azoospermia and severe oligozoospermia, while highlighting the diminishing diagnostic yield as sperm concentration increases. The absence of any genetic abnormalities in the 2-5 million/ml group could influence resource allocation within genetic testing protocols. Nevertheless, the 4.3% detection rate below 2 million/mL warrants extending systematic genetic evaluation in this patient

group, particularly considering its implications for assisted reproductive technology planning and counselling

A-0070 A SHARED LANGUAGE OF LIFE: CONSERVED ESSENTIAL PROTEINS UNDERLYING MAMMALIAN FERTILISATION

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Eight sperm protein have been identified in mice as essential for the adhesion and fusion stages of gametes: IZUMO1, TMEM95, TMEM81, SPACA6, SOF1, DCST1, DCST2 and FIMP, each relocating to the equatorial segment after acrosomal reaction. Deletion of each of these genes leads to male sterility, resulting in the absence of fertilisation and accumulation of sperm in the oocyte perivitelline space. To date, only IZUMO1 and SPACA6 have been characterized and demonstrated to be important for human fertilisation.

The objective of this study was to assess if sperm protein essential for adhesion/fusion during fertilisation in mice play the same role in humans. To test this, Immunofluorescence experiments were performed to determine the localization and relocation after acrosome reaction of each protein within the sperm. Then, the function of each was assessed using blocking antibodies in In Vitro fertilisation (IVF) assays between human sperm and depellucidated hamster oocytes. Human sperm samples were donated by patients attending the hospital andrology department for research in accordance with French bioethics laws, and used the day of collection. Prior to IVF experiments, sperm parameters were assessed by computer-assisted sperm analysis (CASA), and only samples with normal parameters (in accordance with the World Health Organization guideline) were used. Sperm sample were preincubated with specific antibodies, Rabbit IgG or IVF medium alone before fertilisation, and only experiments in which the control condition exhibited a fertilisation index greater than 3 sperm fused by oocyte were considered, in order to reliably detect an effect of antibodies.

By immunofluorescence experiments, different patterns of localization and relocation were observed among the proteins, depending of the acrosomal status of the sperm. But in fine, when the sperm become acrosome-reacted and fertilisation competent, IZUMO1, SPACA6, TMEM95, TMEM81, DCST1 and DCST2 are localized to the equatorial segment, the site where the sperm contacts the oocyte membrane. This is compatible with the possible involvement of these proteins in the gamete adhesion and fusion stages. An exception is SOF1, whose signal is lost after the acrosome reaction. Our hypothesis is that in mammals, as it has been demonstrated in C. elegans, SOF1 is a secreted protein. We also demonstrated through IVF using depellucidated hamster oocytes that sperm TMEM95, TMEM81, SOF1 and DCST1 appear to be essential for human fertilisation since used antibodies induced a drastic decrease in fertilisation, with at least 90 % inhibition observed for each protein, while none of the antibodies affected sperm motility. IVF experiments were conducted using the IgG isotype control, and no inhibition of fertilisation was observed. Thus, proteins essential for fertilisation in mice seem to be also essential in humans, highlighting their evolutionary conservation.

This study seeks to improve our understanding of the human fertilisation and opens up clinical perspectives, including the identification of new targets for non-hormonal contraceptive and a better understanding of certain idiopathic male infertility cases.

A-0278 ARE LARGE LANGUAGE MODELS READY FOR CLINICAL EXPLAINABILITY IN ANDROLOGY? INSIGHTS FROM A CLINICIAN-BASED EVALUATION

Moreno La Quatra [1], Francesco Pallotti [2], Giorgia Spaggiari [3], Daniele Santi [3], Nicole Dalia Cilia [1]

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Background
Artificial intelligence models are increasingly applied in seminology, offering support for diagnostic tasks. However, limited interpretability remains a major barrier to clinical adoption. Many approaches, such as deep learning models, function as “black boxes,” making it difficult for clinical andrologists to understand

prediction rationale. This lack of transparency hampers biological validation and limits integration into evidence-based decision-making. To address this, we leveraged a validated model to investigate a new critical issue: clinical explainability. We integrated two AI models to generate clinically meaningful justifications for individual predictions, which were then evaluated by trained clinical andrologists.

Methods
We trained a deep neural network to predict the presence of impaired spermatogenesis in a cohort of 169 men seeking fertility evaluation, who performed a full andrological examination, including semen analysis, hormone evaluation (FSH, LH, total testosterone, prolactin), and testicular ultrasonography. The predictive model was based on a previously validated machine learning framework, achieving high diagnostic performance in identifying varicocele-associated spermatogenesis impairment (accuracy 0.94, precision 0.97, recall 0.92). Based on this trained model, we used SHAP (SHapley Additive exPlanation) to quantify the contribution of each input feature to individual prediction. Different LLM (Large Language Models) were then used to provide synthetic structured text narratives to explain specific results to medical experts, which were finally blindly evaluated by three expert andrologists through a Likert scale evaluation questionnaire.

Results
Despite high predictive performance of the underlying model, explainability remained limited from a clinical perspective. Based on a Likert scale evaluation, SHAP visualizations were preferred over LLM-generated explanations by expert clinicians. However, neither approach alone was considered sufficient for clinical decision support, with only 41.8% of clinical cases correctly interpreted according to expert evaluation. LLM-generated explanations, in particular, showed limited clinical usefulness (~22%), with frequent misinterpretation of feature importance (~79%) and inaccurate clinical reasoning (~71%). However, one LLM (Gemini-2.5-Pro) showed a partially divergent trend, achieving comparatively better interpretability scores, although overall performance remained insufficient for clinical decision support.
Conclusions

LLM-generated explanations were inconsistent and were evaluated clinically inaccurate in more than 70% of cases, limiting their applicability in real-world settings. Both SHAP-based visualizations and LLM-generated explanations currently fall short of clinical decision-support requirements, still presenting insufficient accuracy and clarity, highlighting the need for more reliable and clinically grounded explainability tools.

A-0007 Spermatic cord block as a treatment modality in chronic scrotal pain: a tertiary referral center study

Massimiliano Raffo, Josvany Rene Sanchez Curbelo, Maurizio De Rocco Ponce, Luis Malca Caballero, Jose Antonio Arrus Soldi, Ricardo Contreras Garcia, Sebastian Dumas, Gabi Adalid, Eduard Ruiz-Castane, Doron Vantman Luft

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Introduction
Chronic scrotal pain (CSP) is a multifactorial and often idiopathic condition that significantly affects quality of life in men. Treatment algorithms remain undefined, and conservative therapies are frequently ineffective. The aim of this study was to characterize the clinical profile of men with CSP and to evaluate therapeutic responses to spermatic cord block with local anesthesia at a tertiary referral center.

Materials and Methods

We retrospectively assessed data from 30 men with CSP who underwent spermatic cord block injections between 2019 and 2024 at a single academic institution. Exclusion criteria included previous vasectomy. Demographic and clinical variables, pain characteristics, treatment response, number of injections, complications, and use of adjunctive therapies were systematically recorded.

Results
Patients had a median age of 50 years (SD: 14.2); 18 (60%) had a history of pelvic or genital surgery, notably 8 men (44.4%) underwent inguinal hernioplasty and 6 (33.3%) varicocelectomy. CSP was unilateral in 26 men (86.7%) and bilateral in 4 patients (13.3%), with an initial mean VAS of 5.6 (SD: 2.2). The average duration of pain before treatment was 32.8 months (SD: 40.2). A total of 76 injections were administered, with a median of 3 injections per patient (SD: 1.4), ranging from 1 to 7, and a mean interval

between injections of 56.4 days (SD: 102.8). After the first injection, 25 (83.3%) of patients reported pain improvement with a mean VAS reduction to 2.4 (SD: 2.5). 17 patients (58.6%) initially experienced pain relief following the injection, but their pain returned to baseline levels after a few hours. At the end of the treatment, 11 men (38%) reported complete pain resolution, 9 (31%) partial improvement, and 9 (31%) no improvement, with a mean final VAS of 2.5 (SD: 2.6). Complications included spermatic cord hematoma in 3 (10%) of cases. 7 patients (24%) who failed to respond to treatment were referred for specialized pain management, and 4 patients (13.4%) underwent microsurgical denervation, with 3 (75%) of these achieving complete pain resolution.

Conclusion
Spermatic cord block is a moderately effective and low-risk intervention for CSP in selected patients, particularly those who do not respond to conservative therapy and lack identifiable organic causes.

A-0086 Impact of chemotherapy regimens on spermatogonia subpopulations and somatic cells in (pre)pubertal boys' testicular tissue prior to cryopreservation

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Advances in cancer diagnosis, treatment standardization, and supportive care have increased 5-year survival rates to approximately 83%. However, these advances are accompanied by long-term adverse effects, including infertility in adulthood. Increase survival rates following cancer treatments highlight the importance of integrating fertility preservation strategies into the overall care of patients, with consideration of their developmental stage. Chemotherapy-induced gonadotoxicity in (pre)pubertal boy's patients could result from (i) direct damage to germ cells or (ii) alteration of somatic cells (Sertoli cells, peritubular myoid cells or Leydig cells) that could no longer perform their role in supporting spermatogenesis. With a view to restoring fertility in cancer survivors, it is essential to study the impact of chemotherapy on cell populations in cryopreserved testicular tissue.

This retrospective study analysed testicular tissue from 51 (pre)pubertal patients (6 months - 33 years) who had received low- or moderate-risk gonadotoxic chemotherapy prior to cryopreservation. All participants had undergone testicular tissue freezing before conditioning for hematopoietic stem cell transplantation. Spermatogonial subpopulations, the density, maturity and functionality of somatic cells and several fertility parameters (apoptosis and DNA damage) were quantified by immunofluorescence.

For germ cells, the number of Adark/Apale spermatogonia and differentiating B spermatogonia decreased significantly in boys treated with alkylating agents compared with patients exposed to non-alkylating agents ($P = 0.026$). An increase in the number of spermatogonia per seminiferous tubules (S/T) with age was observed in the group of patients treated without alkylating agents, whereas patients who received alkylating agents included in the cyclophosphamide equivalent dose (CED) formula showed a decrease in S/T ($r = 0.9539$, $P = 0.0031$; $r = -0.4694$, $P = 0.0051$). Moreover, the tubular fertility index and S/T decrease in the group of patients treated with vincristine in combination with alkylating agents (decrease of 42.2%, $P < 0.01$), but in this group the CED was also significantly increased ($P < 0.001$). After CED adjustment, multivariate analysis showed persistence of the decrease in S/T correlated with the administered vincristine dose ($P < 0.01$).

For somatic cells, exposure to carboplatin is associated with a decrease in the number of SOX9+ Sertoli cells per testicular tissue section ($P = 0.007$). High cumulative exposure to alkylating agents, defined by a CED ≥ 8000 mg/m², leads to decreased expression of COUP-TFII in peritubular myoid cells ($P = 0.038$) and CYP11A1 in Leydig cells ($P = 0.013$). In addition, INSL3 expression in Leydig cells decreases with increasing CED ($r = -0.33$; $P = 0.019$), independently of patient age. Exposure to vincristine does not show an additional deleterious effect. Hormonal analyses did not reveal any impact of induction chemotherapy on the somatic cells activity.

Finally, although somatic cells appear to be preserved, we confirmed the depletion of spermatogonia in seminiferous tubules when alkylating agents included in the CED formula were used, with a decline when the CED was higher than 4000 mg/m². However, this reduction in the number of spermatogonia is not necessarily predictive of the real impact on the future fertility of patients.

A-0112 Molecular Characterization of a Microtubule-Associated Protein in Human Sperm and Its Association with Asthenozoospermia

Adli Othamne [1], Ezzahra Rachid [3], Kbirou Adil [1,2], Aboutaieb Rachid [1,2]

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Introduction: Male infertility represents a major global health issue, and in Morocco approximately one in eight couples consults for infertility. One of the most common conditions observed in semen analysis of infertile men is asthenozoospermia, which is characterized by reduced sperm motility. Sperm motility is a critical factor in fertilization, and understanding the molecular factors that influence flagellar dynamics is essential for elucidating the causes of male infertility. Microtubule-associated proteins play a vital role in cytoskeleton organization and flagellar dynamics, and their functions in human sperm motility are not fully understood.

Aim: This study aimed to determine the expression of microtubule-associated protein involved in cytoskeletal organization and its potential association with sperm motility alteration in patients with asthenozoospermia.

Materials and Methods: We recruited 60 reproductive-age men between December 2024 and December 2025 from the reproductive biology laboratories and the Ibn Rochd University Hospital. Participant semen samples were collected following 2–5 days of sexual abstinence and subjected to analysis according to the laboratory manual of the World Health Organization (6th edition, 2021). We analyzed sperm parameters (sperm concentration, motility, vitality, and morphology) following routine laboratory protocols. We included only patients with normal morphology (>4%), without known causes of infertility, and with normal seminal vesicle activity (zinc, α-glucosidase, and fructose). We also evaluated sperm DNA fragmentation and chromatin condensation. We compared the target protein expression in spermatozoa by immunofluorescence between controls and men with asthenozoospermia.

Results: A highly significant association was observed between low sperm motility and expression of the studied protein ($p < 0.0001$). No significant associations were observed between expression of the studied protein and other seminal biochemical markers, sperm DNA fragmentation, and chromatin condensation or other spermogram and spermocytogram abnormalities. This indicates that the present results are specific for sperm motility and do not reflect general alterations of semen parameters.

Conclusion: The present study reveals an association between the expression of this microtubule-related protein and sperm motility in asthenozoospermia. It supports the hypothesis that dysregulation in cytoskeletal and flagellar protein expression may lead to defective sperm motility. This protein may be a candidate molecular marker for elucidating the mechanisms involved in asthenozoospermia and offer new insights into the development of diagnostic tools for male infertility.

A-0118 Novel compound heterozygous SPAG17 variants cause human male infertility through multiple morphological abnormalities of spermatozoal flagella

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Introduction: Sperm-associated antigen 17 (SPAG17) encodes an evolutionarily conserved protein essential for the assembly and functional integrity of the axonemal central pair (CP) apparatus in sperm flagella. While SPAG17 deficiency is known to cause primary ciliary dyskinesia and male infertility in mice, human cases remain extremely rare, and no compound heterozygous SPAG17 mutations have been reported in human. This study aimed to characterize the genetic etiology and ultrastructural defects in a

Chinese patient presenting with primary infertility and a classic phenotype of multiple morphological abnormalities of the sperm flagella (MMAF).

Materials and Methods: A 27-year-old male patient with severe asthenoteratozoospermia from a non-consanguineous family was recruited. Whole-exome sequencing was performed to identify candidate pathogenic variants, followed by Sanger sequencing for familial segregation analysis. The functional impact of a novel splice-site variant was validated using a minigene splicing assay in HeLa and HEK-293T cells. Transmission electron microscopy (TEM) was further utilized to evaluate the ultrastructural integrity of the sperm axoneme. This study was approved by the ethical committee of the Affiliated Hospital of Xuzhou Medical University (no. XYFY2025-KL658-01).

Results: We identified the first case worldwide of male infertility caused by novel compound heterozygous variants in SPAG17: a frameshift variant (c.4929dup; p.Val1644CysfsTer5) and a complex splice-site indel (c.632_634+1delinsCCAA). The frameshift variant is predicted to trigger nonsense-mediated mRNA decay. Minigene assays confirmed that the splice-site variant disrupts canonical splicing, leading to intron retention and premature termination codons, resulting in truncated proteins lacking essential functional domains (residues 248–346 and 2076–2180). TEM analysis revealed significant axonemal disorganization, characterized by the absence or displacement of the central pair microtubules, consistent with the critical role of SPAG17 in CP projection assembly.

Conclusions: This study reports the first case of compound heterozygous SPAG17 variants causing human MMAF, significantly expanding the mutational spectrum of the gene. Our findings demonstrate that SPAG17 defects can lead to isolated male infertility without overt somatic ciliary symptoms, highlighting its non-redundant role in human spermiogenesis. These results support the inclusion of SPAG17 in clinical genetic screening panels for idiopathic MMAF and provide a basis for precise diagnosis and reproductive counseling.

ROOM 3

PODIUM SESSION

Thursday, 17 September 2026 - 08:00-09:00

A-0206 ERECTILE DYSFUNCTION AND LIFESTYLE: INDEPENDENT ROLE OF SLEEP QUALITY IN A LARGE REAL-WORLD COHORT

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Background
Male sexual dysfunction, particularly erectile dysfunction (ED), has been increasingly associated with lifestyle-related factors, including diet, physical activity and metabolic health. While these factors have been extensively investigated, the potential role of sleep quality as an independent contributor to ED remains insufficiently defined. Sleep disturbances may influence sexual function through hormonal, vascular and inflammatory mechanisms, but clinical evidence in large cohorts is still limited.

Aim
To evaluate the association between sleep quality and erectile dysfunction and to explore the relationships between sleep, dietary patterns, physical activity and metabolic profile in a large cohort of adult men.

Methods
This single-center, observational, cross-sectional study included 519 adult men aged 18 to 70 years. All participants underwent clinical evaluation, anthropometric assessment and biochemical and hormonal measurements. Validated questionnaires were administered to assess erectile function (IIEF-15), sleep quality (Pittsburgh Sleep Quality Index, PSQI), adherence to Mediterranean diet (PREDIMED and Medilite

scores) and physical activity (IPAQ). Descriptive analyses, Spearman correlation analyses and non-parametric group comparisons were performed. Multivariate logistic regression models were used to identify independent predictors of ED and pathological sleep quality.

Results
The prevalence of objective ED was 56.5%, while impaired sleep quality was observed in 42.2% of the cohort. PREDIMED and Medilite scores were positively correlated and both showed a negative correlation with PSQI.

PSQI score was negatively correlated with IIEF-15. Age was negatively associated with IIEF-15 and positively associated with PSQI.

Patients with ED showed higher PSQI compared to those without ED (median 5.0 [IQR 3.0–8.0] vs 4.0 [IQR 2.0–6.0], p<0.001). Conversely, patients with pathological sleep quality showed lower IIEF-15 scores compared to those with normal sleep quality (median 19 vs 26, p<0.001).

In multivariate analysis, age was the strongest independent predictor of ED (OR 1.89, 95% CI 1.55–2.32, p<0.001), while PSQI was independently associated with ED (OR 1.26, 95% CI 1.03–1.54, p=0.023). Regarding pathological sleep quality, age (OR 1.022 per year, p=0.0008) and waist circumference (OR 1.031 per cm, p=0.046) were positively associated, whereas Medilite score was inversely associated (OR 0.948 per point, p=0.013).

Conclusions
Sleep quality is significantly associated with erectile function and represents an independent factor associated with ED. Mediterranean diet adherence is associated with better sleep quality, suggesting a potential link between lifestyle, sleep quality and male sexual health.

A-0236 Maternal serum concentrations of 4-hydroxychlorothalonil during pregnancy and the association with risk of testicular germ cell cancer in male offspring

Cecilie S. Uldbjerg [1,2], Christian Lindh [3], David M. Kristensen [1,2,4], Youn-Hee Lim [5], Jørgen H. Petersen [1,2,6], Karina M. Sørensen [7], Panu Rantakokko [8], Brent A. Coull [9], Elvira V. Bräuner [1,2], Russ Hauser [10], Niels E. Skakkebæk [1,2,11], Anders Juul [1,2,11], Lærke Priskorn [1,2]

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Introduction: Testicular germ cell cancer (TGCC) is hypothesized to originate from disruptions of fetal testicular development, potentially caused by environmental chemicals. One potential risk factor is 4-hydroxychlorothalonil (4-OH-CHT), a primary transformation product of fungicide chlorothalonil, which has recently been detected in human biological samples through non-targeted screening. Due to concerns over environmental and health impact, chlorothalonil has been banned across the European Union and classified as probably carcinogenic by the International Agency for Research on Cancer.

Objective: To investigate maternal serum 4-OH-CHT concentrations during pregnancy and the association with TGCC risk in male offspring.

Methods: We conducted a nested case-control study of 549 mother-male offspring pairs (103 TGCC cases, 446 matched controls) within a Danish Pregnancy Screening Registry, a registry of more than 100,000 women who provided serum samples during their pregnancy in Denmark during 1985–1994, a calendar period when chlorothalonil was extensively used in agriculture. We followed their male offspring (28–38 years) for TGCC diagnoses in the Danish Cancer Registry. In maternal serum of identified cases

and matched controls, we quantified 4-OH-CHT using LC-MS/MS. We used Cox regression models to estimate hazard ratios (HR) per doubling (i.e., log2-unit increase) of 4-OH-CHT concentrations.

Results: 4-OH-CHT was detected above the limit of detection (0.1 ng/mL) in all 549 maternal serum samples, with a median concentration of 1.56 ng/mL. Median concentrations remained relatively stable during the 1980s but approximately doubled in the 1990s. The HR for overall TGCC was 0.89 (95% CI: 0.66-1.21) per doubling of concentrations, although not statistically significant. By histological subtype, a higher risk was observed for nonseminomas (HR 1.10, 95% CI: 0.71; 1.69), but a lower risk for seminomas (HR 0.74, 95% CI: 0.48; 1.15) per doubling of concentrations. When stratifying analyses by sample decade, a higher risk was observed based on samples from the 1990s (HR 1.63, 95% CI: 0.87; 3.06), but a lower risk based on samples from the 1980s (HR 0.73, 95% CI: 0.51; 1.05) per doubling of concentrations.
Conclusion: In our study sample, all investigated Danish pregnant women during had detectable levels of 4-OH-CHT, with increasing median concentrations over time (1985-1994). We found no overall evidence of an association between maternal serum 4-OH-CHT concentrations during pregnancy and risk of TGCC in male offspring. However, in analyses restricted to those with serum samples from the 1990s calendar period, when concentrations were generally higher, we noted a higher risk of TGCC, particularly for nonseminomas. It remains to be seen whether a longer follow-up that includes more males in the typical seminoma ages range (median 35–39 years) would show a similar higher seminoma risk among those exposed to the 1990s-levels of 4-OH-CHT.

A-0242 Determinants of androgen abuse withdrawal symptoms: a cross-sectional study of community-dwelling men

Bonnie Grant [1], Joseph Kean [2], Nipun Lakshitha de Silva [1,3], Rodrigo Aguilera [4], Oliver Quinton [1], Maha Gumssani [1], Paul Bassett [5], Waljit S. Dhillo [1], Anne Lingford-Hughes [6], Kim Wolff [4], Channa N. Jayasena [1]

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Background
Androgen abuse is an increasing global public health concern, particularly among young men, and is associated with cardiovascular, reproductive, and psychiatric morbidity. Although hypothalamic-pituitary-gonadal (HPG) axis recovery occurs in most men within 12 months of cessation, symptoms during this early recovery period are poorly characterised. This period is clinically important, as withdrawal symptoms increase relapse risk. We therefore examined the relationship between symptoms and biochemical recovery during the first year after androgen abuse cessation, using urinary testing to exclude undisclosed androgen exposure.

Methods
Ethics-approved cross-sectional observational study of community-dwelling men aged 18–60 years, categorised as nonusers, current users, or past users of androgens within the previous 12 months. All participants attended a single fasted morning visit for blood testing and completed validated questionnaires assessing depressive symptoms (BDI-II), anxiety (GAD-7), sexual function (IIEF-15), and quality-of-life (SF-36). Urine toxicology, analysed at a World Anti-Doping Agency-accredited laboratory, excluded ongoing androgen exposure.

Results
A total of 247 men were included: 50 nonusers, 125 current users, and 72 past users with negative urine screening. Groups did not differ by age or ethnicity. Illicit substance use was higher in current and past users, and psychiatric diagnoses were more frequent in current users ($p<0.05$). Current users had higher serum testosterone and lower gonadotrophins compared with both past users and nonusers. Estimated recovery time was 12.8 months for LH and 18.9 months for FSH.

Sexual function was impaired in past users with lower median IIEF-15 total scores than nonusers (68.5 [61.5-73.0], nonusers; 62.0 [49.5-70.0], past users; $p=0.02$) and current users (69.0 [62.0-73.0], current

users; 62.0 [49.5-70.0], past users; p<0.001). Past users also had lower erectile function, sexual desire, and overall satisfaction scores (p<0.001).

Depressive symptoms were highest in past users, with higher median BDI-II scores than nonusers (3.0 [0.0-8.0], nonusers; 10.5 [2.0-17.8], past users; p<0.001) and current users (5.0 [1.5-11.0], current users; 10.5 [2.0-17.8], past users; p=0.02). Moderate or severe depression occurred in 19.4% of past users, compared with 7.2% of current users and 0% of nonusers. Past users also had worst GAD-7 and SF-36 scores across multiple domains (p<0.05).

In multivariable analyses, psychiatric diagnosis was the strongest predictor of depressive symptoms (ratio 3.04, 95% CI 1.65-5.61; p=0.001), anxiety (ratio 2.74, 95% CI 1.70-4.42; p<0.001), and quality-of-life (SF-36 MCS β −9.5, 95% CI −15.7 to −3.3; p=0.003). Lower serum testosterone was associated with higher depression scores (ratio per 20nmol/L testosterone increase 0.49, 95% CI 0.26-0.94; p=0.03), while increasing age (β -8.0, 95% CI -14.5 to -1.5; p=0.02) and higher LH (β -1.2, 95% CI -2.1 to -0.2; p=0.01) were associated with worse sexual function.

Conclusions
Men within the first year after androgen abuse cessation report worse mood, anxiety, sexual function, and quality-of-life than both current users and nonusers. Psychiatric comorbidity was the strongest predictor of symptoms, with biochemical markers of HPG axis recovery showing a smaller association. These findings suggest that withdrawal symptoms are not solely due to hypogonadism and highlight the need for developing psychological and behavioural support during androgen cessation recovery.

A-0246 A Combined Collagenase and Density-Gradient Method Boosts MicroTESE Outcomes in Non-Obstructive Azoospermia due to SCOS and Maturation Arrest

Marjan Sabbaghian [1], Elisabetta Piatti [2], Marina Bellavia [2], Fabrizio Castiglioni [2], Franco Nerva [2], Giacomo Gazzano [3], Germar-Michael Pinggera [4], Giovanni Maria Colpi [2]

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Introduction: Sertoli Cell Only Syndrome (SCOS) and maturation arrest (MA) represent among of the most challenging histopathological patterns encountered during microdissection testicular sperm extraction (microTESE) in patients with Non Obstructive Azoospermia (NOA). Even with microTESE, reported sperm retrieval rates (SRR) remain consistently low, particularly in complete SCOS and complete MA. Optimizing laboratory tissue processing may enhance SRR and ultimately improve ICSI outcomes. This study evaluates a combined biological protocol—collagenase digestion followed by density gradient separation—and compares the resulting SRR with published benchmarks.

Materials and Methods: A total of 206 consecutive NOA cases were retrospectively classified on the basis of testicular histology. Of these, 37 MA cases and 99 SCOS cases were included in the analysis and further stratified into four subgroups: complete SCOS, incomplete SCOS, complete MA, and incomplete MA. MicroTESE micro-biopsies specimens were processed using a standardized two step protocol including collagenase-mediated enzymatic digestion to promote seminiferous tubule dissociation, followed by density-gradient centrifugation to reduce the amount of germ cells and thereby enrich the fraction containing mature spermatozoa, facilitating their identification.

Briefly, samples were enzymatically processed with Collagenase IV (1 ml) at 37°C for 2 h. After centrifugation at 400 x g for 10 min, the resulting cell suspensions were layered onto a two step density gradient (80%/45%) to remove cellular debris and immature germ cells. The final pellet was examined under an inverted microscope to identify mature spermatozoa. SRRs were recorded for each subgroup and compared with the ranges reported in the contemporary literature.

Results: SRRs were as follows: complete MA 8/17 (47.1%), incomplete MA 16/20 (80.0%), complete SCOS 34/86 (39.5%), incomplete SCOS 13/13 (100%). Overall SSR were: all MA cases 24/37 (64.9%) and all SCOS cases 47/99 (47.5%).

In contrast, published SRRs were substantially lower: complete MA 0–20%, incomplete MA 40–60%, complete SCOS 5–20%, incomplete SCOS 30–60%; overall: all MA 20–40%, all SCOS 10–30% (Bernie et al., 2015; Guangmin, 2024; Eugeni et al., 2025).

Across all subgroups, SRRs obtained with the combined collagenase/density gradient protocol consistently exceeded the ranges reported in the literature for these poor prognosis histopathological patterns.

Conclusions: The combined application of collagenase digestion and density gradient separation appears to significantly enhance SRRs in NOA patients presenting with both SCOS and MA. Although certain subgroups encompass a limited number of cases—reflecting the inherent rarity of these histopathological patterns—the overall trend remains consistent and superior to published data. These findings suggest that optimized laboratory processing may play a meaningful and clinically relevant role in improving sperm retrieval even in the most challenging NOA scenarios.

A-0261 Global Prevalence of Klinefelter Syndrome in Prenatal Diagnosis: Higher Detection with NIPT Compared to Conventional Methods — A Systematic Review of Temporal Trends and Screening Modalities

Francesco Costantino [1,2], Antonio Percesepe [3,4], Riccardo Cuoghi Costantini [5], Maria Chiara Decaroli [1,2], Sara De Vincentis [1,2], Vincenzo Rochira [1,2]

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BACKGROUND: Klinefelter syndrome (KS) affects approximately 1 in 500–1,000 males but is often diagnosed only in adulthood during fertility evaluations, suggesting significant underdiagnosis.

AIM: To assess the prevalence of KS, including classical karyotypes (47,XXY; 48,XXXY) and mosaicisms (47,XXY/46,XY), detected through invasive prenatal diagnostic (IPD) procedures.

METHODS: A systematic review (PROSPERO: CRD42024621400) was conducted using PubMed and Scopus for studies from 1974 to January 2024. Data were extracted on KS cases identified through IPD procedures—amniocentesis, chorionic villus sampling, and cordocentesis—with denominators including both male and female fetuses. Countries were stratified according to the Socio-Demographic Index (SDI) from 1 to 5. The SDI is a composite measure of socio-economic development that integrates indicators of income, educational attainment, and fertility rates.

RESULTS: A total of 602 studies met inclusion criteria, comprising 1,363,453 IPD procedures of which 66.38% were performed in studies reporting only medical indications. The global prevalence of KS among all fetuses undergoing IPD was 0.20% (95% CI 0.16–0.24%). Studies utilizing non invasive prenatal tests (NIPT) for screening prior to confirmatory IPD reported significantly higher KS prevalence compared to conventional IPD alone (1.57% vs 0.13%; OR 16.13, 95% CI 11.82-22.01, $p<0.001$). Overall temporal analysis showed a modest increase in prevalence (OR 1.08, 95% CI 1.057–1.096; $p<0.001$); however, this increase was not significant when stratified by NIPT use, suggesting the trend reflects adoption of NIPT rather than true prevalence changes. After stratification for SDI, the prevalence of KS in NIPT studies from SDI5 countries (0.51%) was consistently lower than SDI3 (3.11%) (OR 0.20; $p<0.001$). No other significant differences were observed across SDI categories.

CONCLUSION: The prevalence of KS detected through IPD (2.0 per 1,000 fetuses; approximately 4.0 per 1,000 male fetuses) exceeds traditional estimates of 1.0–2.0 per 1,000 males, though direct comparison is limited by methodological differences. NIPT-based screening significantly improves KS detection compared to conventional IPD alone, revealing cases that would otherwise remain undiagnosed prenatally. The absence of temporal trends in non-NIPT studies confirms that increased detection reflects screening technology advancement rather than rising incidence, underscoring the substantial underdiagnosis of KS in populations without advanced prenatal screening.

A-0263 Mosaic loss of Y chromosome as a therapy-induced biomarker of accelerated aging in young oncological patients

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Cancer treatment in young men of reproductive age raises growing concerns about long-term biological consequences beyond fertility. Although advances in oncological therapies have improved survival rates, these treatments may accelerate biological aging, contributing to the earlier onset of comorbidities in cancer survivors. Mosaic loss of the Y chromosome (mLOY) in peripheral blood leukocytes is a physiological age-related phenomenon and may, therefore, serve as a biomarker for therapy-induced accelerated aging in cancer patients.

In this study, we evaluated the occurrence of mLOY in 79 young oncological patients recruited at the Fundació Puigvert (Barcelona): 67 men with testicular germ cell tumors (TGCT) and 12 with Hodgkin (HL) or non-Hodgkin lymphomas (NHL).

Among TGCT patients, 41 were treated with PEB, 25 received carboplatin and one underwent radiotherapy alone. Among lymphoma patients, 7 with NHL were treated with CHOP (one also receiving adjuvant radiotherapy), whereas 5 with HL received ABVD, two of whom also received radiotherapy.

Samples were collected before treatment (T0) and at least one year after the end of treatment (Tx).

mLOY was assessed in paired pre- and post-therapy samples using a SNP-array (Global Screening Array, Illumina) and data were processed using MADloy software.

Across the entire cohort, only one TGCT patient exhibited mLOY at baseline (T0). After therapy, this subject remained mLOY-positive, while six additional patients developed mLOY (4 TGCT and 2 lymphoma cases) and this overall increase was statistically significant (McNemar test; $p = 0.03$). Consistently, analysis of the Y-chromosome signal as a continuous variable showed a significant decrease between T0 and T1 (Wilcoxon test; $p = 0.03$), whereas no significant differences were observed at T2 compared with baseline.
Stratified analyses by malignancy type, chemotherapy regimen and number of cycles found no significant differences.
Nevertheless, a dose-response pattern was suggested across regimens: among TGCT patients treated with the PEB regimen, mLOY occurred only in those receiving ≥3 cycles, with no cases among those treated with two cycles. Similarly, for carboplatin-based therapy, mLOY developed only in a patient receiving two cycles with no cases after a single cycle.

Overall, 7.6% of patients developed mLOY following treatment, supporting the hypothesis that chemotherapy may accelerate biological aging in young men, particularly when exposed to more intensive treatment.
However, the decrease in Y-chromosome signal observed at T1 was no longer significant at T2, indicating that this effect may be transient. This aligns with previous reports describing a gradual reduction in mLOY after smoking cessation or after the end of radiotherapy in prostate cancer patients, suggesting that clonal dynamics may partially reverse mLOY once the external stressor is removed.

To date, no data in the literature is available on mLOY in young cancer patients who underwent chemotherapy. Our study highlights the need for further investigation in larger cohorts to identify the factors influencing mLOY reversibility, specifically whether it is age-dependent and whether treatment intensity acts as an independent predictor.

A-0298 Problematic online pornography use and erectile dysfunction

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Background: Erectile dysfunction (ED) is a multidimensional clinical condition that requires a comprehensive assessment within a biopsychosocial framework to allow appropriate etiological stratification. While risk factors for organic ED are well established and the association between vasculogenic ED and systemic cardiovascular disease has been clearly demonstrated, the risk factors and pathophysiological mechanisms underlying non-organic (idiopathic) ED remain incompletely understood. In this context, problematic online pornography use (POPU) has been proposed as a potential contributor to idiopathic ED through several neurobiological and psychosocial mechanisms; however, evidence supporting this association and a causal relationship remains limited.

Objective: To investigate the association between idiopathic ED and POPU.

Methods: We conducted a cross-sectional observational study including 130 heterosexual men aged over 18 years, all in stable relationships, who were evaluated for ED. ED severity was assessed using the International Index of Erectile Function (IIEF). ED etiology was classified as organic or idiopathic based on a comprehensive andrological evaluation including medical history, physical examination, hormonal assessment, and nocturnal penile tumescence and rigidity testing (NPTR). Pornography use was assessed according to frequency of use and with the Pornography Consumption Inventory (PCI) and the Problematic Pornography Consumption Scale (PPCS-6). Descriptive analyses and non-parametric between-group comparisons were performed.

Results: A total of 130 men with ED were included, with a mean age of 48.9 years. Organic ED was identified in 35.4% of patients, whereas 64.6% were classified as having idiopathic ED. Mean PCI and PPCS-6 scores did not differ significantly between patients with idiopathic and organic ED. ED severity, measured by total IIEF score, was not significantly correlated with either PCI or PPCS-6 scores and did not differ according to frequency of pornography use. In contrast, greater pornography use frequency was significantly associated with higher PCI and PPCS-6 scores (both $p<0.001$). In addition, patients with organic ED had higher BMI, a higher prevalence of diabetes and hypertension, and lower calculated free testosterone levels than patients with idiopathic ED (all $p<0.05$).

Conclusions: In this cohort of men with ED, problematic online pornography use, assessed using the PCI and PPCS-6, was not associated with the presence of idiopathic versus organic ED or with ED severity as measured by IIEF-15 and NPTR. Classical cardiovascular and metabolic risk factors remain key determinants of the organic etiology of ED, whereas POPU appears to be primarily related to greater pornography use frequency and does not seem to play an independent etiological role in idiopathic ED.

A-0300 Cognitive Behavioral Sex Therapy for Patients with Peyronie's Disease: A Pilot Randomized Controlled Trial

Armin Soave [1], Carla Oltrogge [1], Martin Sangmeister [2], Johannes Moltrecht [1], Anika Buchholz [3], Amra Pepic [3], Andrea Salzbrunn [4], Margit Fisch [1], Timo O. Nieder [2]

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Funding statement: This research project was funded by the European Society for Sexual Medicine Research Grant (RG 22-03).

Background Patients with Peyronie's disease (PD) experience symptom bother and psychological distress. The aim of the present study was to evaluate the feasibility and efficacy of cognitive behavioral sex therapy (CBST) as an adjunct short-term intervention in PD.

Methods Mono-centric, pilot, randomized trial, which enrolled 51 patients, who received conservative or surgical treatment with adjunct short-term CBST (CBST+) or without CBST (CBST-). The CBST+ group received CBST according to a standardized protocol, consisting of 6 sessions of 50 minutes each over a time period of 6 weeks. Symptom bother, psychological and physical symptoms, penile pain, erectile

function and sexual satisfaction was assessed with Peyronie's Disease Questionnaire (PDQ), International Index of Erectile Function Erectile Function Domain (IIEF-EF) and the New Sexual Satisfaction Scale Short-Form (NSSS-SF) prior to, 5 weeks after and 20 weeks after randomization.

Outcomes The co-primary endpoints were:

1. feasibility of CBST, defined as the percentage of patients who complete the CBST protocol in the CBST+ group

2. absolute change from baseline of the PDQ bother score 20 weeks after randomization

3. clinical improvement on the PDQ bother score 20 weeks after randomization compared to baseline

Secondary endpoints included the change of PDQ psychological and physical symptoms, PDQ penile pain, IIEF-EF and NSSS-SF score 20 weeks after randomization compared to baseline.

Results In total, 25 and 26 patients were randomized in the CBST+ and CBST- group. In the CBST+ group, 22 (88%) patients fully completed the CBST protocol. There were no differences in the co-primary and secondary endpoints between the CBST+ and CBST- group. In additional analyses, the CBST+ group had more reduction of PDQ psychological and physical symptoms score 5 weeks after randomization compared to the CBST- group (-3.16; 95% CI: -5.55, -0.77; p=0.011).

Conclusion For the first time, this pilot randomized trial provides evidence regarding the feasibility and efficacy of CBST in PD. CBST is feasible in PD. Although the efficacy seems to be limited, some patients may still benefit from its use as an adjunct short term intervention.

A-0015 Role of Steroid Hormone-Binding Proteins on the Bioactivity of 11-Oxygenated Androgens

Jennifer Afrakoma Nyamaah [1], Astrid Claessens [1,2], Nick Narinx [1,3], Karl Storbeck [4], Pieter Vermeersch [3], Dirk Vanderschueren [1,2], Frank Claessens [5], Leen Antonio [1,2]

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Background: 11-oxygenated androgens (11OA), particularly 11-ketotestosterone (11KT), have emerged as contributors to the bioactive androgen pool. Unlike classic androgens testosterone (T) and dihydrotestosterone (DHT), which bind to steroid hormone-binding proteins (sex hormone-binding globulin (SHBG), albumin), little is known about how these proteins interact with 11KT. Observational differences between saliva (free hormone) and serum (total hormone) 11KT levels suggest that steroid hormone-binding proteins may influence 11KT bioavailability. We aimed to examine 11KT interaction with SHBG and SHBG's effect on 11KT-mediated androgenic activity.

Methods: We used in vitro competitive radioligand binding assays to assess 11KT's ability to compete with tritium-labelled DHT (3H-DHT) for SHBG. Increasing concentrations of unlabelled 11KT were incubated with a saline buffer containing constant concentrations of 3H-DHT (EC50 = 0.32nM) and SHBG (EC50-like concentration = 0.32nM). Aldosterone served as a negative control. Computational docking (AutoDock Vina) was used to model 11KT's fit in the SHBG DHT-binding pocket, based on known SHBG residue coordinates. To assess functional impact, HEK293T cells expressing androgen receptor and androgen response element-driven luciferase reporter were treated with 100 nM 11KT (EC50 determined by dose-response curve) and increasing SHBG concentrations (6–56 nM).

Results: Increasing concentrations of 11KT displaced 3H-DHT from SHBG in a dose-dependent manner. Half-maximal radioligand displacement by 11KT required concentrations one to two orders of magnitude higher than those needed for unlabelled DHT and T. Aldosterone did not compete with the radioligand. Exploratory in silico modelling supports a relatively high 11KT-SHBG interaction. Interestingly, SHBG and 11KT co-treatment did not reduce 11KT-induced luciferase activity, as luminescence remained stable even at higher SHBG concentrations, in contrast to dose-dependent decreases in luminescence observed with T and DHT.

Conclusions: Our in vitro findings suggest 11KT acts as an SHBG ligand, although 11KT required higher concentrations to achieve radioligand displacement compared to DHT and T. This is consistent with in silico predictions suggesting that 11KT has a relatively high affinity for SHBG. Interestingly, 11KT maintains its androgenic activity in cells even in the presence of SHBG.

A-0049 The bio-psycho-social correlates of male infertility

Anna Juhász [1,2,3], Balázs Matuszka [4], Zsolt Kopa [2]

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Introduction: Infertility affects every 6th couple in Europe, and in approximately 50-60% percent of these cases, male factor is also present. Infertility is often accompanied by chronic stress, several emotional-affective symptoms and decreased fertility-related and sexual quality of life. In the literature, the psychological consequences of male infertility are less discussed. However, initial findings confirmed that the prevalence of depressive and anxious symptoms among infertile men is more than five times higher compared to the general population. Chronic stress is known to be a contributor to maladaptive health behaviours and thus potentially to impaired sperm quality, which is associated with a higher risk of all-cause mortality.

Aims: In 2023, our multidisciplinary team launched a gap-filling extended clinical research project at our Andrology Centre, with the objectives of investigating the psychosocial consequences of impaired male fertility -particularly non-obstructive azoospermia (NOA)- to promote evidence-based psychological care in andrological units.

Methods: In the quantitative study, the psychological characteristics of patients with NOA are measured at two time points by a tailored screening tool containing psychological questionnaires about depressive, anxious and stress symptoms, self-esteem, hopelessness, relationship satisfaction, fertility-related quality of life and also emotion regulatory skills. Semi-structured in-depth interviews are conducted with NOA patients following the microdissection TESE procedure. The control groups consist of men suffering from impaired fertility and sexual dysfunctions.

Results: Our preliminary quantitative data show that NOA patients are not significantly affected by the diagnosis of reproductive disorders in both symptomatic or relational dimensions of their quality of life. However, the qualitative findings provide nuances to this picture as the major themes of the in-depth interviews are 'Facing the diagnosis of non-obstructive azoospermia', 'Coping with the challenges of difficult childbearing', and 'Resources from relationship, which reflect the changes of psychological reactions at more timepoints of the journey of infertility. Furthermore, the prevalent difficulties in emotion regulation might also explain the underlying mechanisms of the contradictory preliminary results.

Conclusions: From the psychological perspective, the most critical point of the NOA patient pathway is facing the diagnosis and the negative result of the microdissection TESE procedure. However, based on our available data, there is limited knowledge on the psychological reactions of infertile men, so further investigations of emotion regulatory skills are needed to understand the underlying mechanisms of this phenomenon. Deficits in emotional processing skills of patients could contribute to two significant risk factors of male fertility and health: persistent chronic stress and maladaptive health behaviours. Our preliminary results point to the conclusion that implementing biopsychosocial care in the work of andrological units seems to be recommended.

ESAU-EAA Symposium: Penile Prosthesis - How to deal with difficult cases

Thursday, 17 September 2026 - 09:30-10:20

A-0193 Priapism

Wai Gin (Don) Lee

UK

A-0194 Infection
Fulvio Colombo

Italy

A-0195 Corporal Fibrosis
Ates Kadioglu

Turkey

A-0196 Peyronie's disease
Agustín Fraile

Spain

ESAU-EAA Symposium: Do we need robotic surgery in andrology?

Thursday, 17 September 2026 - 10:20-11:00

A-0200 Debate - Pro
Giorgio Franco

Italy

A-0201 Debate - Contra
Philippa Sangster

UK

ISA/EAA/IFFS Joint Session - Reducing female burden by improving male factor fertility

Thursday, 17 September 2026 - 14:00-15:00

A-0185 Update on ART outcomes
Edgar Mocanu

Ireland

A-0186 Impact of male factor on female fertility
Dimitrios Goulis

Greece

A-0187 Is there a still room for Art in ART?
Luca Gianaroli

Italy

ESAU-EAA Symposium: Non-surgical management of Induratio penis plastica

Thursday, 17 September 2026 - 16:00-17:00

A-0197 News in medical therapy
Paolo Capogrosso

Italy

A-0198 Low-intensity extracorporeal shock wave therapy
Fotios Dimitriadis

Greece

A-0311 Stem Cells
Marco Cosentino

Italy

A-0199 PRP
Eduard Ruiz Casane

Spain

ESAU-EAA Symposium: Transgender and gender diverse persons
Thursday, 17 September 2026 - 17:00-18:00

A-0202 Hormonal treatment of transgender and non-binary persons
Justine Defreyne

Belgium

A-0318 Attitudes of transgender and gender diverse adolescents and their parents on parenthood and fertility preservation before starting hormone therapy
Daniele Tienforti

Italy

A-0204 Surgical management of transgender and non-binary persons
Andrea Cocci

Italy

A-0205 Law, policy and ethics of transgender and non-binary persons
Angelo Schillaci

UK

18 September 2026 Friday

Auditorium

Plenary lecture
Friday, 18 September 2026 - 09:00-09:30

A-0192 Seminal fluid effects on uterine receptivity to embryo implantation: transcriptomic strategies to define molecular mechanisms
Sarah Robertson

Australia

PLENARY LECTURE

Friday, 18 September 2026 - 09:30-10:00

A-0169 ARE WE PROPAGATING INFERTILITY THROUGH IVF? GENETIC INSIGHTS INTO HERITABILITY

Csilla Krausz

Italy

ANDROGEN DEPRIVATION THERAPY IN PROSTATE CANCER

Friday, 18 September 2026 - 10:30-11:30

A-0180 ANDROGEN DEPRIVATION AND BODY COMPOSITION CHANGES

Andrea Berruti

Italy

A-0303 BEYOND ANDROGEN SUPPRESSION: ESTROGEN DEPRIVATION EFFECTS IN PROSTATE CANCER

Channa Jayasena

UK

A-0045 LONGITUDINAL CHANGES IN REPRODUCTIVE HORMONES PRIOR TO CLINICAL DIAGNOSIS OF A TESTICULAR GERM CELL TUMOUR

Nina Mørup [1,2], Trine Holm Johannsen [1,2,3], Gedske Daugaard [4], Hanne Frederiksen [1,2], Jørgen Holm Petersen [1,2,5], Michael Schwinn [6], Mette Skou Bentsen [6], Jakob T Bay [7], Henrik Ullum [8], Bertram Dalskov Kjerulff [9,10], Kathrine Agergård Kaspersen [10], Christian Erikstrup [9,10], Erik Sørensen [6], Sisse Rye Ostrowski [6,11], Ole Birger Pedersen [7,11], Kristian Almstrup [1,2,12], and Anders Juul [1,2,11]

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Testicular germ cell tumours (TGCTs) are the most common malignancy found in young men. Lower testosterone concentrations and reduced semen quality are observed in men just before their diagnosis of TGCT and infertility is a well-known risk factor for developing a TGCT. We aimed to investigate potential changes in reproductive hormone levels in the years preceding a diagnosis of TGCT.

We used a unique cohort of blood donors from the Danish Blood Donor Study (DBDS), to conduct this recall-by-phenotype biobank study. We measured the concentrations of reproductive hormones (FSH, LH, inhibin B, AMH and testosterone) in plasma samples from 72 Danish blood donors who later developed a TGCT (1-20 samples from each case, donated up to 11 years before the TGCT diagnosis) along with 72 sex- and age-matched controls from DBDS (one sample from each control). Age-matched standard deviation (SD) scores were calculated.

We observed a gradual increase in FSH and a decrease in AMH and inhibin B SD scores in the years preceding a TGCT diagnosis and these hormones differed significantly from controls up to three years prior to diagnosis. Cases developing seminomas had significantly higher FSH and inhibin B SD scores within one year prior to diagnosis than controls did. The inhibin B/FSH ratio was lower in cases developing seminomas compared with controls, but not in cases developing non-seminomas. An inhibin B/FSH ratio <23 gave an increased risk of developing a seminoma within one year (odds ratio: 16.9, p<0.0001;

sensitivity=0.87; specificity=0.72). The lower inhibin B/FSH ratio was evident up to six years prior to diagnosis.

In conclusion, changes in AMH, FSH and inhibin B SD scores are evident in patients several years prior to a primary diagnosis of a TGCT. Hormone alterations are histology-specific and may aid in the early detection of TGCTs and classification of the histological subtypes.

A-0142 A Stepwise Imaging Framework for Testicular Lesions: when to observe, when to preserve, when to operate. Insights from a 20-Year Single-Center Experience

Marta Tenuta [1], Francesco Carlomagno [1], Franz Sesti [1], Francesco Angelini [1], Giulia Pellegrini [1], Daniele Apostoli [1], Lucia Manganaro [2], Giorgio Franco [3], Gabriele Savarese [3], Fabio Massimo Magliocca [2], Daniele Gianfrilli [1], Andrea Isidori [1]*, Carlotta Pozza [1]*

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Introduction

Incidental intratesticular lesions are increasingly detected due to the widespread use of high-resolution scrotal ultrasound, creating relevant diagnostic and management challenges. While ultrasound has excellent sensitivity for lesion detection, differentiation between benign and malignant lesions remains difficult because of overlapping imaging features. Consequently, clinical decisions are often driven by clinician experience rather than structured risk assessment. Although second-level imaging techniques such as contrast-enhanced ultrasound (CEUS) and magnetic resonance imaging (MRI) have shown promising results, standardized diagnostic pathways integrating these modalities are lacking. The aim of this study was to develop and internally validate a pragmatic sequential imaging framework for risk-adapted management of intratesticular lesions.

Materials and Methods

We conducted a single-center cohort study including consecutive patients with intratesticular lesions evaluated between 2005 and 2025. Clinical, ultrasound, CEUS and MRI data were prospectively collected. Histology after surgery served as the reference standard when available, while stability during at least 18 months of imaging follow-up defined benignity in conservatively managed lesions.

A sequential diagnostic framework was developed from the observed diagnostic workflow. Lesion size was evaluated as the primary triage variable to define rule-out and rule-in thresholds for malignancy. Lesions within the intermediate size range underwent structured ultrasound risk stratification based on predefined morphological features to derive a point-based score (T-Les score). Second-level imaging (CEUS and MRI) was analyzed within the intermediate-risk group. Diagnostic performance was assessed using logistic regression and ROC analysis, with internal validation through repeated cross-validation.

Results

A total of 750 lesions were included, of which 415 (55.3%) were malignant. TO was performed in 57.6% of cases, TSS in 12.4%, and AS in 30%. Malignancy was confirmed in 94.7% of TO specimens. All malignant lesions under AS showed interval growth, whereas benign lesions remained stable or regressed.

Lesion size demonstrated excellent discrimination for malignancy (AUC 0.92), with <0.5 cm identifying a low-risk group (malignancy 5.2%) and ≥1.8 cm a high-risk group (PPV 94.6%). Intermediate-size lesions (0.5–1.8 cm, malignancy 45.3%) were further stratified using a five-feature ultrasound-based score (T-Les-SCORE), incorporating vascularization, calcifications, margins, echogenicity, and multifocality. The combined score (0–9) achieved good discrimination (AUC 0.902) and defined three clinically interpretable risk strata were identified: low-risk (0–3 points; malignancy ≤8%), intermediate-risk (4–5 points; malignancy ~30–47%), and high-risk (6–9 points; malignancy ≥87%).Clinical modifiers such as prior germ cell tumor and testicular microlithiasis improved model performance (AUC 0.929), while positive tumor markers acted as an absolute indication for surgery.

CEUS analysis within the intermediate-risk group demonstrated good diagnostic performance for qualitative enhancement patterns (AUC 0.78), with suspicious enhancement associated with malignancy. Integration of CEUS findings with the T-Les score further improved discrimination (AUC ≈0.93).

MRI demonstrated similar performance (AUC 0.793) and provided additional reassurance in selected cases with non-suspicious CEUS, although findings were exploratory. Sequential imaging reduced malignancy prevalence in lesions with both negative CEUS and MRI.

Conclusion
This study proposes a pragmatic, stepwise imaging framework integrating lesion size, structured ultrasound assessment, and selective second-level imaging. The model enables risk-adapted management, potentially reducing unnecessary orchiectomy while maintaining oncological safety.

ARE LIFESTYLE INTERVENTIONS ENOUGH IN FUNCTIONAL HYPOGONADISM?

Friday, 18 September 2026 - 11:30-12:30

A-0182 PRO

Giulia Rastrelli

Italy

A-0183 CON

Michael Zitzmann

Germany

A-0032 HYPERCORTISOLISM AND MALE HYPOGONADISM: WHAT IS THE ROLE OF MILD AUTONOMOUS CORTISOL SECRETION

Alessandro Ciarloni, Silvia Rossi, Michele Perrone, Nicola Ambo, Valentina Di Giacomi, Carlo Mazza, Marianna Martino, Gilberta Giacchetti, Gianmaria Salvio, Giancarlo Balercia

Clinica di Endocrinologia e Malattie del Metabolismo, Dipartimento di Scienze Cliniche e Molecolari, Ancona, Italy

Introduction
In approximately half of adrenal incidentalomas (AI), cortisol hypersecretion is observed without typical hypercortisolism signs, a condition defined as Mild Autonomous Cortisol Secretion (MACS). Recent evidence highlighted that many Cushing's syndrome comorbidities, such as hypertension, dyslipidaemia, diabetes mellitus and osteoporosis, may be observed in MACS. However, it is unclear whether hypogonadotropic hypogonadism resulting from hypercortisolism, typical of Cushing's syndrome, is present in men with MACS.

Methods
We conducted a cross-sectional study on male inpatients admitted to the Endocrinology and Metabolic Diseases Clinic of Marche University Hospital (Ancona, Italy) from September 2017 to July 2025 for assessment of recently diagnosed AI. Only patients diagnosed at discharge with MACS (defined by inadequate suppression of cortisol levels after 1 mg DST) or non-functioning adrenal adenoma (NFAA) were included in the study and considered as cases and controls, respectively. Clinical, instrumental, and biochemical data were collected for 75 patients.

Results
Thirty-two (42.7%) patients had a diagnosis of MACS and 43 (57.3%) had NFAA. Subjects with MACS had lower total testosterone (3.56 vs. 4.08 ng/mL) and calculated free testosterone levels (6.87 vs. 6.97 ng/dL); however, this data did not reach statistical significance. ACTH (12 vs. 22 pg/ml, $p< 0.0001$) and DHEA-S (0.520 vs. 0.970 µg/mL, $p=0.02$) were significantly lower in subjects with MACS. The median BMI was within the overweight range for both groups (26.80 kg/m² in cases and 28.35 kg/m² in controls). In multivariate analysis, BMI appeared to exert a negative effect on gonadal function independently from post-1-mg DST cortisol levels.

Discussion
MACS appears to negatively affect adrenal androgen secretion, while the effect seems less evident at testicular level. This finding is not in line with the currently available limited evidence, in which the mild hypercortisolism typical of MACS seems to exert a suppressive effect on gonadal axis. However, it should be noted that previous studies evaluated populations with higher BMIs. It is therefore possible that a

greater component of functional hypogonadism secondary to excess weight was present in these studies and that this may facilitate the suppressive effect of hypercortisolism on gonadal function.

Conclusions
Further studies, taking adequate account of the role of excess weight, are needed to establish a clear correlation between MACS and male hypogonadism.

A-0229 Comparative study between the effects of weight loss by Sleeve gastrectomy surgery versus liraglutide on male fertility

Mohamed Hassan Ali Fahmy [3], Mohamed Abdul Moneim Amin El Masry [3], Mina Saad [1,2], Ehab Fathy Ahmed [3], Mohamed Mahmoud Saleh [3], Mohamed Wael Ragab [1,2]

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Background: Recent epidemiological studies documented a progressive decline in semen parameters, causing about 30% of infertility worldwide. This decline is linked to interactions among genetic and environmental factors, lifestyle changes, and metabolic disorders. Obesity is a significant modifiable risk factor that adversely affects semen parameters through hormonal imbalance, oxidative stress, inflammation, and altered adipokine secretion, particularly leptin. This study assesses the effects of weight loss on semen parameters and male reproductive hormones.

Methods: This prospective comparative study included 60 infertile morbidly obese male patients (BMI > 30 kg/m²) presenting to Kasr El-Aini outpatient clinics. Participants were divided into two groups: a bariatric surgery group (sleeve gastrectomy) and a liraglutide therapy group, with a follow-up period of six months. Semen analysis, reproductive hormonal profiles, BMI, and serum leptin levels were measured at baseline and at the end of the follow-up period.

Results: Both groups exhibited significant reductions in BMI and serum leptin levels ($p < 0.001$), with greater weight loss observed in the sleeve gastrectomy group after six months. The mean increase in sperm concentration was significantly greater in the liraglutide group ($p = 0.046$). Within-group analysis revealed a significant improvement in motility in the liraglutide group ($p < 0.001$), whereas the sleeve gastrectomy group showed a non-significant change ($p = 0.074$). Testosterone levels improved significantly in both groups ($p < 0.001$ for sleeve gastrectomy; $p = 0.021$ for liraglutide). Additionally, a significant reduction in serum FSH was observed in the liraglutide group after six months ($p = 0.049$).

Conclusion: Liraglutide therapy demonstrated significantly superior short-term effects on semen parameters compared to sleeve gastrectomy, supporting its potential role as a conducive male fertility therapeutic option. However, longer follow-up periods are recommended to increase sample size and to evaluate different bariatric techniques.

Plenary lecture

Friday, 18 September 2026 - 12:30-13:00

A-0168 Reinke's crystals in testicular biopsies of azoospermic men

Davor Jezek

Croatia

Room 2

Podium session

Friday, 18 September 2026 - 08:00-09:00

A-0277 The effect of treatment with aromatase inhibitors on sexual desire in men: a meta-analysis

Giovanni Corona [1], Giulia Rastrelli [2], Clotilde Sparano [3], Linda Vignozzi [2], Alessandra Sforza [4], Mario Maggi [3]

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Introduction: The first-line treatment for male hypogonadism is testosterone (T) replacement therapy. However, this treatment may have adverse effects limiting its use. Therefore, alternative treatments should be considered. Among these, aromatase inhibitors (AROi) may represent an option although their use has been suggested to be associated with decreased sexual desire.

Aim: To evaluate the effect of AROi on the incidence of low sexual desire.

Methods: A meta-analysis considering all the available data from clinical trials evaluating men treated with AROi and reporting data on the incidence of low sexual desire. An extensive MEDLINE, Embase, and Cochrane search was conducted. The identification of relevant studies was performed independently by 2 of the authors (G.R. and G.C.), and conflicts resolved by the third investigator (M.M.).

Results: Overall, nine studies were collected including 457 subjects with a mean age and mean BMI of 42.9 years and 32.1 kg/ m2. Overall, the use of AIs significantly increased the risk of low libido with a event rate of 14 (3; 44)%. Interestingly, a meta-regression analysis showed that the risk of low sexual desire was inversely related to estradiol (E2) levels (Slope= -0.027 [-0.034; -0.020]; p<0.0001) and increased as a function of the T/E2 ratio (Slope= 0.009 [0.007; 0.010]; p<0.0001). These findings were confirmed after the adjustment for age and BMI ($r = -0.370$ and 2.981 for E2 and T/E2 respectively; both $p < 0.0001$).

Conclusions: Although AROi are well known to increase gonadotropin levels and, in turn, to induce a rise in T levels. This is linked to improvement in testicular outcomes such as semen parameters; however, their use is associated with a non-negligible incidence of low sexual desire. This effect is more evident in studies reporting a deeper decline in E2 levels. Since low sexual desire is a common and specific symptom of low T, caution should be paid when AROi treatment is chosen to treat men with hypogonadism.

A-0235 Early Life Stress Reprograms Hippo Signaling in the Testis Despite Increased Body Growth: Converging Evidence from Developmental and Mechanistic Models

İrem İnanç [1], Onur Bender [2], Ekin Baysal [3], Arzu Atalay [2], Esra Erdemli [1]

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Background:
The Hippo signaling pathway is a key regulator of testicular homeostasis, controlling cell proliferation, differentiation, and structural organization. Emerging evidence, including our previous findings, indicates that Hippo pathway components are closely associated with cytoskeletal dynamics and sperm release. However, whether early life stress can disrupt this pathway and contribute to long-term testicular dysfunction remains unclear.

Objective:
This study aimed to investigate the effects of early life stress on testicular development and to evaluate its association with alterations in the Hippo signaling pathway.

Methods:
Early life stress was induced in male rat pups using a maternal separation model between postnatal days 1 and 14. Stress response was confirmed by measuring serum corticosterone levels on postnatal day 14. Testicular tissues were collected at postnatal days 14, 22, 32, 52, and 90(n=5 for each group).Histological analyses were performed to assess seminiferous tubule morphology. Immunohistochemical analyses were conducted to evaluate YAP and phosphorylated YAP protein expression. Digital PCR was used to quantify the expression of Hippo pathway components, including YAP, TAZ, LATS1, MST1, and SAV. Body weight was recorded at all time points, and testicular weight was measured at postnatal day 90.

Results:
Early life stress resulted in a consistent and statistically significant increase in body weight across all time

points compared to controls. Despite this systemic growth, histological evaluation revealed a significant reduction in seminiferous tubule diameter in the stress group at postnatal days 14, 22, 32, and 90. No significant difference in testicular weight was observed at postnatal day 90. Immunohistochemical analysis demonstrated decreased YAP protein expression at postnatal days 52 and 90, along with reduced phosphorylated YAP levels at day 90. Digital PCR results showed significant downregulation of YAP gene expression, particularly at day 90. LATS1, MST1, and SAV exhibited dynamic, time-dependent changes, with early upregulation followed by suppression in adulthood, whereas TAZ expression remained relatively stable. These findings indicate a biphasic reprogramming of the Hippo signaling pathway in response to early life stress.

Conclusion:
Early life stress increases overall body growth while simultaneously impairing testicular development and reprogramming Hippo signaling, revealing a dissociation between systemic growth and reproductive function. When considered together with prior mechanistic evidence linking Hippo signaling to cytoskeletal organization and sperm release, these findings support a central role for Hippo pathway dysregulation in stress-related male reproductive dysfunction.

Funding:
This study was supported by the Ankara University Scientific Research Projects Coordination Unit (Project No: TSA-2024-3689).

A-0076 Differential proteomic maturity in sperm cells subjected to selection procedures in couples with unexplained infertility

Marina Leiva [1], Meritxell Jodar [1,2], Maria Corredor [1], Mar Domingo [1], Marta Guimerà [3], Aina Borràs [3,4], Dolors Manau [3,4], Juan Manuel Corral [5], Judit Castillo [1], Rafael Oliva [1,2]

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An important step during Assisted Reproduction Technologies (ART) is the application of sperm selection procedures. While discontinuous density gradient centrifugation (DGC) is the most commonly used method, passive selection seems to provide advantages. In this study we compared the sperm quality and molecular maturity of sperm selected by 40–80% DGC and the ZyMot (TM) Multi passive device in 29 normozoospermic infertile patients, as evaluated through differential proteomics, protamine determinations, seminal parameters and sperm DNA fragmentation. Passive selection significantly improved motility, vitality, and morphology ($p < 0.0001$) compared to both DGC and neat semen, and reduced the proportion of sperm with DNA fragmentation ($p < 0.001$). Proteomic analysis identified 1,882 sperm proteins showing differential abundance in the passively-selected fraction, as compared to neat semen (p-value< 0.05, FC ≥ 1.5). Further analysis of the differential proteins indicated that passively-selected sperm were enriched in proteins linked to essential functions, such as acrosome reaction and the maintenance of the redox balance, which is crucial for a proper chromatin integrity. Of relevance, a significant reduction of protamine 2 precursor was also observed, revealing a more mature state of the chromatin. In contrast, DGC-selected sperm exhibited a proteomic profile equivalent to neat semen, with no significant differences detected. These findings have implications towards favouring the use of passive sperm selection to enhance sperm quality by favouring molecular traits leading to improved fertilization. Supported by a grant from the Instituto de Salud Carlos III (ISCIII; PI20/00936) to RO. JC is Serra Húnter fellow. The authors belong to COST Action Andronet CA20119, supported by COST (European Cooperation in Science and Technology, www.cost.eu).

A-0126 Anti-Müllerian hormone and surgical sperm retrieval in nonobstructive azoospermia: an aetiology-specific systematic review and meta-analysis

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Surgical sperm retrieval in men with nonobstructive azoospermia (NOA) remains difficult to predict, complicating preoperative counselling. While anti-Müllerian hormone (AMH) has been proposed as a biomarker of residual spermatogenic potential, published evidence is conflicting. This systematic review and meta-analysis aimed to evaluate the association between seminal and serum AMH levels and surgical testicular sperm extraction (TESE), with a focus on aetiology-specific associations.

Following PRISMA and MOOSE guidelines, we searched PubMed/MEDLINE, Embase, and Scopus for observational studies reporting preoperative AMH levels in men with NOA undergoing TESE or microdissection TESE (mTESE). Random-effects meta-analyses using standardized mean differences (SMD) were performed and methodological quality of the included studies was assessed using the EPHPP tool. Subgroup analyses were pre-specified according to NOA aetiology: idiopathic, Klinefelter syndrome (KS), and mixed/undefined.

Fifteen studies comprising 1,777 men were included. Seminal AMH showed no significant association with sperm retrieval success (SMD: 0.01, p=0.95). In the overall analysis of unselected NOA populations, serum AMH levels did not differ between retrieval-positive and retrieval-negative patients (SMD: -0.05, p=0.79). However, aetiology-specific analyses revealed directionally opposite and biologically coherent associations. In idiopathic NOA, serum AMH levels were significantly lower in retrieval-positive men (SMD: -0.65, p=0.0002). Conversely, in KS, serum AMH levels were significantly higher in retrieval-positive patients (SMD: 0.62, p=0.02): such association gained even more statistical strength when the analysis was restricted to adult cohorts (SMD: 0.76, p<0.00001). Significant heterogeneity was observed across studies (I^2=89%), largely resulted from aetiologic differences (p=0.0003 for subgroup differences). No evidence of publication bias was detected.

In conclusions, AMH is not a reliable standalone predictor of sperm retrieval success when applied indiscriminately to heterogeneous NOA populations. Its clinical utility is strictly aetiology-dependent, reflecting the distinct testicular pathologies of idiopathic NOA and Klinefelter syndrome. These findings support an aetiology-aware interpretation of AMH in clinical practice, suggesting it should be integrated with aetiologic classification and standard clinical parameters rather than used in isolation for pre-operative counselling.

A-0130 HUMAN TESTICULAR GERM CELL TUMOURS: EVOLVING LINKS BETWEEN IMMUNE LANDSCAPE AND SPECIALIZED VASCULARISATION

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Introduction: Testicular germ cell tumours (TGCT) are one of the most common cancers in young men (14-45 years). They originate from germ cell neoplasia in situ (GCNIS) and can be classified as seminoma (SE) and non-seminomas (NSE), such as embryonal carcinoma (EC). While tumor development is still poorly understood, infiltration of immune cells has been identified as a hallmark of TGCT and pre-invasive GCNIS. The recruitment of immune cells and respective alterations in cytokine/chemokine profiles are likely to contribute to a unique tumour microenvironment (TME). In other cancer entities with tumour-infiltrating immune cells, the presence of specialized blood vessels (high endothelial venules, HEV) involved in lymphocyte recruitment, has been shown to be associated with tumour progression. This study aims to investigate the role of HEVs and their interplay with immune cells in TGCT.

Patients, Material and Methods: Selected testis tissue samples originate from an ongoing cohort study recruiting patients undergoing surgery for suspicion of TGCT (n=165; age 18-79 years; SE ≥80%: n = 78; EC≥50%: n = 28; other NSE/mixed TGCT n=31). For comparison, testicular biopsies obtained from azoospermic men showing GCNIS (n = 6), non-neoplastic inflammatory hypospermatogenesis (HYP+LY; n = 5), or normal spermatogenesis (NSP; n = 5) were included. FFPE tissue specimens were stained with haematoxylin/eosin for histological evaluation (HE), as well as for CD45+ (pan-leukocytes), CD68+

(macrophages), CD11c+ (dendritic cells), CD20cy+ (B cells), CD3+ (pan-T cells), CD25+, FOXP3+ (regulatory T cells, Treg), MECA-79, PECAM-1, and smooth muscle actin (vessels) by immunohistochemistry (IHC). Cryopreserved tissue from respective subgroups was used for gene expression analyses by qRT-PCR.

Results: Histopathology revealed increased numbers of immune cells and vascularisation in HYP+LY, and GCNIS compared to NSP. Highest density and spatial extension of immune cell infiltrates was found in TGCT, with a strong focus on T lymphocytes incl. Treg in SE, while macrophages were more abundant in EC than T cells, as reflected by IHC and semi-quantitative scoring. Other immune cells, such as B cells and dendritic cells, were also significantly more abundant in TGCT. In a proportion of SE and NSE samples, blood vessels and immune cells formed follicle-like structures (FLS) resembling tertiary lymphoid organs, with striking accumulation of B cells. Overall, IHC showed a higher density of blood and lymphatic vessels in SE, NSE, and GCNIS, when compared to HYP+LY and NSP. HEV+ staining was largely restricted to tumour centres in SE in close association with FLS while rarely observed in other TGCT, and absent in GCNIS, HYP+LY, and NSP. IHC results were underpinned by qPCR analyses.

Conclusion and future perspectives: Our results provide suggestive evidence that infiltrating immune cells, predominantly T cells, in conjunction with specialized vascular components, shape the TME in TGCT. Orchestration of immune cell recruitment by HEV most likely allows for the formation of FLS, especially in SE. Understanding the immune landscape in TGCT, its interaction with HEV, as well as its inter-individual heterogeneity in context with clinical data is a prerequisite to decipher TGCT biology and to identify putative targets for future personalized immunotherapy.

A-0256 Proteomic profiling reveals energy metabolism disruptions in unexplained male infertility

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Unexplained male infertility (UMI) affects men with normal semen parameters but reduced reproductive success, and its molecular basis remains poorly understood. In this study, we integrated clinical data with proteomic analysis of pooled capacitated spermatozoa from strictly selected UMI patients and fertile donors to characterize the biological processes underlying UMI. Proteomic analyses indicate that energy metabolism is altered in UMI patients. Using TMT labeling and high-resolution LC-MS/MS, we identified 1,722 sperm proteins and found 268 differentially expressed proteins between groups, with most being downregulated in UMI. Functional enrichment analyses showed that the altered proteins were mainly associated with energy metabolism, intracellular transport, spermatogenesis, and mitochondrial pathways. In particular, our analysis revealed that UMI sperm exhibited marked changes in key metabolic pathways, especially those related to mitochondrial energy production. Several glycolytic enzymes were downregulated, while enzymes involved in pyruvate metabolism and the TCA cycle were upregulated, suggesting an imbalance in metabolic flux. In parallel, multiple components of the electron transport chain and ATP synthase were altered, supporting the idea of impaired oxidative phosphorylation. Together, these findings indicate that UMI is associated with a specific disruption of sperm bioenergetics rather than a generalized defect in sperm quality.

The proteomic profile of UMI sperm also pointed to a disturbance in NADH-producing reactions and redox homeostasis. Enzymes with dehydrogenase activity were overrepresented among the altered proteins, suggesting enhanced NADH generation that is not efficiently matched by mitochondrial oxidation. Consistent with this, enzymatic assays showed significantly increased NADH dehydrogenase and succinate dehydrogenase activities in UMI samples, whereas peroxidase activity did not differ between groups. In addition, NADH dehydrogenase activity showed excellent discrimination between UMI patients and fertile donors, while SDH also demonstrated relevant diagnostic value. These results support the presence of altered NADH bioenergetics and a potential increase in oxidative stress, which may contribute to impaired fertilizing capacity despite apparently normal semen parameters. In fact, although capacitation parameters, fertilization rates, and early embryo quality were similar between groups, clinical outcomes after ART were clearly worse in UMI patients. Pregnancy and ongoing pregnancy rates were significantly lower in the UMI group, despite apparently favorable early embryological results. Embryo euploidy rates were also reduced in UMI cycles, suggesting a possible paternal contribution to embryo aneuploidy and ART failure.

Overall, this study shows that UMI is associated with mitochondrial dysfunction, altered NADH metabolism, and impaired redox balance in human sperm. These molecular changes may explain the reduced reproductive outcomes observed in UMI patients and support the need for new diagnostic markers and more personalized ART strategies.

A-0109 Genotypic Distribution of High-Risk Human Papillomavirus and Its Association with Sexually Transmitted Infection Coinfections in Men Attending a Sexual Health Center in Lima, Peru

Gustavo A. Grandez-Castillo [1], Olenka Farfan-Zapata [1], Guido Bendezu-Quispe [2], J. Antonio Grandez-Urbina [1]

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Background: Human papillomavirus (HPV) is the most prevalent sexually transmitted infection (STI) worldwide, and high-risk (HR) genotypes are associated with cervical and anogenital cancers. In Peru, information on the genotypic distribution of HR-HPV and its relationship with STI coinfections among men remains limited, particularly in sexual health care settings. This study aimed to analyze the genotypic distribution of HR-HPV and its association with STI coinfections in male patients treated at a sexual health center in Lima, Peru. Methods: A cross-sectional study was conducted using clinical records from male patients evaluated at a sexual health center in Lima between August 2024 and November 2025. Patients attended the center because of genital warts and/or signs or symptoms suggestive of sexually transmitted infections. Records with complete information on age, HPV genotyping (high-risk and low-risk), and an 11-pathogen STI panel were included. The panel comprised Mycoplasma genitalium, Mycoplasma hominis, Ureaplasma urealyticum/parvum, Haemophilus ducreyi, Treponema pallidum, Neisseria gonorrhoeae, Trichomonas vaginalis, Chlamydia trachomatis serovars A-K and L1-L3, herpes simplex virus 1 and 2. HPV genotypes were classified as high-risk or low-risk. STI coinfection was defined as positivity for at least one non-HPV pathogen. Patients were stratified by age group and clinical stage (symptomatic with warts versus asymptomatic). Crude associations were assessed using chi-square tests, Fisher exact tests, and odds ratios (OR). Multivariable logistic regression adjusting for age, clinical stage, and geographic origin was performed to estimate adjusted odds ratios (aOR). Results: Of 1,558 records reviewed during the 15-month study period, 801 met inclusion criteria (mean age 35.2 years, SD 9.8). Overall HPV positivity was 66.7% (534/801), with 31.7% (254/801) positive for HR-HPV and 58.7% (470/801) for low-risk HPV. The most frequent HR genotypes were HPV-52 (20.9%), HPV-16 (20.5%), HPV-51 (17.7%), HPV-59 (15.7%), and HPV-73 (12.2%). HR-HPV prevalence was higher among patients aged 51 or older (40.6%) and symptomatic patients (34.1% versus 27.0%; p = 0.050). Overall STI positivity was 66.8% (535/801), predominantly Ureaplasma urealyticum/parvum (48.3%), Mycoplasma hominis (33.5%), and herpes simplex virus 2 (12.4%). STI positivity was higher among patients aged 30 or younger (73.9%; p = 0.008). No significant association was found between HR-HPV and STI coinfection in crude analysis (OR 1.04; 95% CI 0.75-1.42; p = 0.828) or after multivariable adjustment (aOR 1.04; 95% CI 0.76-1.43; p = 0.800). Pathogen-specific analyses also showed no significant associations for Ureaplasma (OR 1.10; p = 0.515), Mycoplasma hominis (OR 1.14; p = 0.420), herpes simplex virus 2 (OR 1.21; p = 0.405), or Chlamydia trachomatis serovars A-K (OR 1.45; p = 0.366). Conclusions: A high prevalence of both HR-HPV and non-HPV STIs was identified. However, no significant association was observed between them, either overall or by individual pathogens, even after multivariable adjustment. These results suggest that HR-HPV and STI screening should be considered as complementary rather than sequential strategies, as the presence of one infection does not appear to predict the other in this clinical setting. This study provides local evidence from an underrepresented male sexual health care setting in Peru.

A-0119 Does Sperm Source Matter in Cryptozoospermia? A Comparison of Testicular and Ejaculated Sperm for ICSI

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Background:
Cryptozoospermia is a severe form of male infertility in which spermatozoa are detectable only after extensive examination of the centrifuged semen pellet. Intracytoplasmic sperm injection (ICSI) represents the main treatment option for these patients. However, the optimal sperm source remains controversial, as some studies suggest superiority of testicular sperm while others report no significant difference between testicular and ejaculated sperm in terms of ICSI outcomes. This study aimed to compare ICSI outcomes in men with cryptozoospermia using testicular versus ejaculated sperm.

Methodology:
This was a retrospective chart review of 2073 cases that underwent ICSI for male factor infertility at our center between January 2014 and January 2025. Men diagnosed with cryptozoospermia were included and divided into two groups according to the sperm source (testicular vs ejaculate). Clinical and laboratory data were collected, including the ages of both partners, medical history, physical examination findings, semen analysis results, hormonal profiles, sperm retrieval procedures, and ICSI outcomes. Categorical variables were compared between the study groups using the chi-square test, whereas continuous variables were compared using Student's t-test. A two-sided p-value < 0.05 was considered statistically significant.

Results:
A total of 115 cases of cryptozoospermia were identified. The sperm source was ejaculated sperm in 68 cases and testicular sperm in 47 cases (10 microsurgical testicular sperm aspiration and 37 testicular sperm aspiration). Baseline female characteristics and ovarian response parameters did not differ significantly between the two groups, including female age, BMI, AMH level, estradiol level, and ovarian response. The overall clinical pregnancy rate was 26.5% (26/98 cycles). Clinical pregnancy was significantly higher when ejaculated sperm was used compared with testicular sperm (42.6% vs 12.8%, p < 0.001). Similarly, fertilization rates were significantly higher in the ejaculated sperm group compared with the testicular sperm group (62.16 ± 27.16 vs 40.48 ± 34.43; p < 0.001). In contrast, cleavage rates (95.85 ± 17.79 vs 92.93 ± 24.31; p = 0.502) and blastulation rates (45.44 ± 29.14 vs 49.97 ± 33.48; p = 0.503) were comparable between the two groups.

Conclusion:
In men with cryptozoospermia undergoing ICSI, the use of ejaculated sperm was associated with significantly higher fertilization and clinical pregnancy rates compared with testicular sperm. These findings support prioritizing the use of ejaculated sperm on the day of ICSI whenever feasible, including consideration of collecting multiple semen samples.

A-0210 TRANSCEND-XR: Improving knowledge of late treatment effects among adolescent and young adult testicular cancer survivors

Davor Ježek [1], Maria O'Malley [2], Josephine Hegarty [2], Ismail Toygar [2], Maria Inês Clara [2], Jack P. Gleeson [3,4], David Murphy [5], Sheena McHugh [6], Frances Shiely [6,7], Aileen Murphy [8], Ann Kirby [8], Ida J. Korfage [9], Sophie Terhaard [9], Agnes van der Heide [9], Aisha Hussain [10], Matias Katajavaara Seidler [10], Lynda Corrigan [11], Patrice Kearney Sheehan [11], Daniele Raggi [12], Alison Reid [12], Michal Chovanec [13,14], Patrizia Gianntempo [15], Jourik A. Gietema [16], Justine Fleur van der Feen [17], Francisco Lupiáñez-Villanueva [17], Ana Roca-Umbert [17], Joanne Reid [18], Paulo Alves [19,20], Joana Soares [21], Karen Galvin [21], Michal Kubo [22], Adeline Salasc [23], Anna Patrikidou [24], Mohamad M. Saab [2]

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Testicular cancer (TC) is the most common cancer in adolescents and young adults (AYAs) assigned male at birth, with incidence increasing across Europe. While cure rates exceed 90%, many survivors face serious late effects such as cardiovascular disease, secondary cancers, lung and kidney toxicity, infertility, hypogonadism, and psychosocial issues. Awareness of these risks is limited, and follow-up care is often inconsistent, hindering early detection and risk reduction. Tailored educational and supportive interventions for AYA TC survivors are critically needed. The TRANSCEND-XR project (TesticulaR cANcer late effects and unmet Supportive Care Needs of AYA survivors using eXtended Reality) seeks to develop, assess, and expand an innovative XR-based educational programme. Its goal is to improve knowledge about late effects and address unmet supportive care needs among AYA TC survivors. TRANSCEND-XR is a 66-month project funded by Horizon Europe, involving 15 partners across 12 European countries. Following the Medical Research Council framework for complex interventions and the Preconscious Awareness to Action Framework, it comprises three phases. Phase 1 aims to understand the incidence, severity, and impact of late effects and unmet needs through a survey of 500 survivors across Europe. Simultaneously, the XR intervention is developed collaboratively using the World Café participatory approach, engaging survivors, care partners, and clinicians in repeated cycles of ideation, prototyping, and refinement. Technical validation will evaluate usability, immersion, presence, and user acceptance. Phase 2 comprises implementation and evaluation through two trials: a pilot feasibility trial and a pragmatic randomised controlled trial across eight European clinical sites. Following a pilot study (n=15, 3 clinical sites) to refine and finalise the TRANSCEND-XR intervention, 230 AYA TC survivors will be randomised to receive TRANSCEND-XR plus standard care or standard care alone. The primary endpoint is knowledge of late effects at 12 months, measured using the Survivor Knowledge Questionnaire (Lee et al., 2019). Secondary outcomes include unmet supportive care needs, help-seeking behaviours, testicular self-examination practices, health-related quality of life, and healthcare utilisation. A concurrent health economic evaluation will estimate cost-effectiveness using decision-analytic modelling and Markov approaches, incorporating quality-adjusted life years and incremental cost-effectiveness ratios. A mixed-methods process evaluation will examine implementation fidelity, contextual influences, and mechanisms of impact, while an embedded empirical bioethics study will address digital equity, autonomy, privacy, and acceptability. Phase 3 focuses on impact, sustainability, and translating policy into practice. The findings will guide ethics-by-design recommendations, inform clinical guidelines, and contribute to EU digital health policy frameworks. Dissemination will follow FAIR data principles and encourage cross-sector collaboration to ensure long-term scalability and adoption. TRANSCEND-XR aims to significantly improve survivorship education for AYA TC survivors by integrating co-creation, thorough evaluation, health economics, implementation science, and empirical bioethics. The project will produce high-quality evidence on the feasibility, effectiveness, and cost-efficiency of an immersive XR intervention integrated into routine follow-up care. Ultimately, TRANSCEND-XR aspires to increase knowledge, encourage appropriate help-seeking and self-management behaviours, endeavouring to reduce unmet supportive care needs, and enhance long-term health outcomes and quality of life for AYA TC survivors.

PRIMARY CILLIARY DISKINESIA: PHENO-GENOTYPING, SPERM HANDLING AND MAR

Friday, 18 September 2026 - 10:30-11:30

A-0181 GENOTYPING, SPERM HANDLING, LAISS, EMBRYO QUALITY AND ICSI OUTCOME

Kaylee Holleman

The Netherlands

A-0310 MRNA AND GENE THERAPY IN HUMAN STEM CELLS

Johanna Raidt

Germany

A-0096 Investigating the interaction between Perinuclear theca and GARIN proteins in sperm development and male fertility in mice

Andjela Kovacevic, Kathrin Burckhardt, Joana Gerstenberg, Simon Schneider, Gina E. Merges, Hubert Schorle

University of Bonn, University Hospital Bonn, Institute of Pathology, Department of Developmental Pathology

During spermiogenesis haploid round spermatids differentiate into spermatozoa containing flagellum, sperm head with compacted chromatin, and a sperm-specific secretory organelle - acrosome which is required for fertilization. Essential for maintaining the sperm morphology and for connecting the acrosome to the nuclear envelope is a unique cytoskeletal scaffold called perinuclear theca (PT). Testis-specific structural proteins Cylicin 1 (CYLC1), Cylicin 2 (CYLC2) and Calicin (CCIN) are major components of the sperm PT. Deficiency of Cylc1/CYLC1, Cylc2/CYLC2 or Ccin/CCIN have been reported to cause severe morphological anomalies of the sperm head including acrosome malformations and detachment from the nuclear envelope leading to male sub-/infertility in mice and humans.

Mass Spectrometry analysis of epididymal sperm protein lysates from Cylc1-, Cylc2-deficient as well as double Cylc1/Cylc2-deficient male mice revealed that the loss of Cylicins leads to significantly different abundances of PT-specific proteins. Furthermore, among 20 most significantly depleted proteins in Cylc2- and Cylc1/Cylc2-double deficient sperm we found four members of the Golgi associated RAB2 interactor (GARIN) protein family: GARIN2,3,4 and 5b. GARINs are a family of proteins with a binding affinity to Rab2a/b - small GTPases involved in vesicle transport and membrane trafficking which are essential for the acrosome development during spermiogenesis. Of note, RAB2a/b are significantly less abundant in Cylc1-, Cylc2- and Cylc1/Cylc2-double deficient sperm as well. Garin3-knockout mice have been shown to display morphological defects of the sperm head and male infertility, while the loss of GARIN5b has been reported to cause male subfertility in mice. However, the interaction between GARINs and structural proteins of the PT has not yet been investigated.

Western blot analysis and immunofluorescence staining on testicular tissue from wild type (WT), Cylc1-, Cylc2- and Cylc1/Cylc2-double deficient male mice revealed no alterations in protein levels or localization of GARINs. Of note, while GARINs localize in the perinuclear region of the WT murine epididymal sperm, in the epididymal sperm from Cylc2- and Cylc1/Cylc2-double deficient mice GARIN3, GARIN5b as well as RAB2b are absent, indicating their loss during spermiogenesis or sperm maturation.

In this study, we investigate the interaction between PT-specific proteins CYLC1, CYLC2 and CCIN with GARIN family members using in-vitro co-immunoprecipitation approach. This interaction represents a missing link between structural proteins of the sperm head and vesicle trafficking regulators, both essential for acrosome development and its attachment to the nuclear envelope, and thus male fertility in mammals.

A-0071 Human stem cell-derived Sertoli-like cells recapitulate early Sertoli cell transcriptomic signatures

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Sertoli cells (SCs) are central regulators of testicular development and spermatogenesis, providing structural and biochemical support to developing germ cells. However, limited access to human foetal testicular tissue restricts our understanding of SC development, and their role in germ cell maturation. Advancing stem cell technology have enabled the generation of Sertoli-like cells (SLCs) using human pluripotent stem cells. The similarity of these SLCs to their in vivo counterparts remains elusive, impeding further development of stem cell based testicular models.

In this study, we generate and developmentally characterize SLCs from human-induced pluripotent stem cells (hiPSCs) to establish a fully-functional human testicular soma for future in vitro Spermatogenesis (IVS). Using directed differentiation via an intermediate mesoderm stage, followed by exposure to FGF9, PGD2, and Activin A, we induced Sertoli cell fate, resulting in SLCs that upregulated key SC markers

(SOX9, WT1, VIM, AMH) and downregulated pluripotency genes (NANOG, POU5F1), consistent with lineage commitment.

To further characterise their identity, we performed single-cell RNA sequencing to compare the global transcriptomic profile of SLCs along their differentiation trajectory with their in vivo counterparts from embryonic to adult testis. Pseudotime analysis revealed that the SLCs correspond to transcriptional signatures of early developmental stages of in vivo SCs. Together, this work provides a framework for generating and critically evaluating human SLCs in vitro, advancing the development of a fully human testicular research models, with implications for modelling IVS and potential future therapeutic applications in male infertility or DSD.

EVERYBODY KNOWS HOW TO MANAGE ED: THE DARK SIDE OF SEXUAL MEDICINE

Friday, 18 September 2026 - 11:30-12:30

A-0184 ED AS A SYMPTOM OF PSYCHIATRIC DISEASE

Cobi Reisman

The Netherlands

A-0211 ED AS A SYMPTOM OF OTHER SEXUAL DYSFUNCTIONS

Walter Vena

Italy

A-0043 PROLONGED-RELEASE SILDENAFIL IN ERECTILE DYSFUNCTION: A RANDOMIZED PLACEBO-CONTROLLED COMPARISON WITH A STANDARD SILDENAFIL

Andrey Morozov 1, Svetlana Bogatova 2, Valeriia Patrikeeva 2, Leonid Spivak 1

1 Institute for Urology and Reproductive Health, Sechenov University, Moscow, Russia; 2 Institute for Clinical Medicine, Sechenov University, Moscow, Russia

Introduction

Sildenafil citrate (Viagra) has been used for ED since 20th century, but its use is limited by frequent side effects such as headache or hemodynamic instability. Prolonged-release formulations like Vildegra may reduce the frequency and manifestation of side effects. This study evaluates the efficacy, safety, and side effect profile of a 4-week therapy with Vildegra compared to placebo and Viagra in patients with ED.

Materials and methods

Vildegra is a generic drug of Sildenafil, formulated within a Hypromellose shell. That ensures a gradual release of active substance, extending its effect up to 13 hours compared to up to 4 hours Viagra effect.

Triple cross-randomized open-label placebo controlled clinical trial was conducted to assess outcomes of treatment with the medicine "Vildegra" 50 mg in comparison with the medicine "Viagra" 50 mg. This clinical trial was perfomed after IRB approval in accordance with the Protocol, World Medical Association Declaration of Helsinki and Tripartite guideline on Good Clinical Practice (ICH GCP).

Patients: men aged 19 to 60 years with an established diagnosis of ED.

Methods included physical examination, ultrasound of penile blood flow during erection with Viagra, ECG, SEP, IIEF, IIEF-5, GAQ questionnaires, laboratory tests, patient diary.

In the experimental group, patients sequentially received Vildegra for 28 days, followed by a week-long wash-out period, placebo for 28 days, another one week wash-out period, and 28 days of Viagra. The control group followed a different sequence: Viagra-placebo-Vildegra. The patients took these drugs on demand, but not less than 6 tablets per course, and not more than 1 tablet per day.

Results

In 60 patients, Vildegra enhanced sexual activity according to questionnaires and patient diaries 1.6 times higher than Viagra and 2.4 times higher than placebo. IIEF-5 increase did not differ significantly: baseline

values for Vildegra vs Viagra were 14 ± 3.8 and 13.9 ± 3.5, respectively, and after 1 month rising to 20.9 ± 3 and 20 ± 3.4, p=0.678.

Adverse events were 1.6 times less common with Vildegra than Viagra. During the wash-out period after taking Sildenafil, the EF continued to improve, that indicates the aftereffect of Vildegra and Viagra.

As a result of 28-day therapy with Vildegra the EF assessment on the IIEF-5 scale increased significantly by 49.3%, p<0.05 versus placebo. According to the questionnaires in 96.6% of patients the erection lasted long enough to have successful sexual intercourse with ejaculation.

Placebo was used 1.5-2 times/week (75% of patients, presumably due to ineffectiveness), Viagra 2.25-4 times/week (81%), and Vildegra 4.25-7 times/week (59%), reflecting better efficacy and minimal adverse events rate.

No clinically significant ECG changes or laboratory abnormalities occurred with Vildegra.

Conclusion

Vildegra demonstrates non-inferior efficacy to Viagra, while it is better tolerated by patients. That influenced the higher frequency of Vildegra intake compared to Viagra. This improved safety profile indicates that prolonged-release Sildenafil is a valuable therapeutic option for ED.

A-0027 Living Together, Loving Apart: Health, Sexuality and Relationships in Middle-Aged and Older Men in Europe

Clotilde Sparano [1], Giammarco Alderotti [2], Giulia Rastrelli [3], Giovanni Corona [4], Linda Vignozzi [3], Leen Antonio [5], Freijo Felipe Casanueva [6], Aleksander Giwercman [7], Thang S Han [8], Ilpo T. Huhtaniemi [9], Chiara Lucchi [1], Terence O'neill [10], Jolanta Słowikowska-Hilczer [11], Jos Tournoy [12], Dirk Vanderschueren [5], Frederick Wu [13], Daniele Vignoli [2], Mario Maggi [1]

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Introduction

The landscape of modern family structures has become increasingly heterogeneous, leading to a diversification of adult partnership models. While extensive research has evaluated marital, cohabiting, and "Living Apart Together" (LAT) relationships regarding psychological health and quality, their specific sexual dynamics remain relatively under-explored. Theoretical perspectives suggest that co-residential unions may institutionalize sexuality differently than LAT arrangements, where physical distance might either attenuate or intensify sexual intimacy. This study aims to address this empirical gap by investigating sexual function and satisfaction among men across these three relationship categories, further exploring how hormonal profiles intersect with the social dynamics of contemporary society.

Methods

We conducted a cross-sectional analysis using data from the European Male Ageing Study (EMAS), a multicenter, observational cohort study involving eight European nations. The analytical sample included

3,259 men (aged 40–79) categorized by relationship status. Sexual function was quantified using the validated EMAS Sexual Function Questionnaire (EMAS-SFQ). In addition to sexual metrics, hormonal assays, lifestyle factors, and general health indicators were recorded. Statistical modeling, including ANCOVA and multivariable linear regression analyses, was employed to adjust for potential confounders such as age, educational background, lifestyle habits, and comorbidities.

Results
Within the cohort, 78.7% of participants were married, while LAT and cohabiting relationships accounted for 6.4% each. Men in LAT unions were characterized by younger age, higher educational attainment, and a greater prevalence of smoking compared to married men. Analysis revealed that men in LAT relationships exhibited significantly higher levels of free testosterone, increased sexual desire, and superior satisfaction compared to those in co-residential arrangements. Furthermore, LAT men reported a higher frequency of sexual intercourse, morning erections, and masturbation. Cohabiting men occupied a median position, whereas married men reported the lowest levels of sexual drive and activity.

Conclusions
Our results indicate that LAT relationships are associated with heightened sexual function and satisfaction. However, these advantages appear to exist independently of the broader benefits intrinsic to co-residence, such as emotional stability, shared domestic responsibilities, and partner-mediated health monitoring. These findings suggest a distinct trade-off between different partnership models. Further longitudinal research is necessary to clarify the complex interactions between relationship types, endocrine health, and overall well-being.

ROOM 3

PODIUM SESSION

Friday, 18 September 2026 - 08:00-09:00

A-0074 TESTICULAR ULTRASOUND INHOMOGENEITY: CLINICAL, SEMINAL, HORMONAL AND ULTRASOUND CORRELATES

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Testicular inhomogeneity (TI) is an ultrasound (US) feature associated in a few previous studies with sperm abnormalities and hypogonadism. However, no study investigated systematically the relationship between TI and its severity with clinical, seminal, hormonal and male genital tract US parameters. The aim of this study is to assess the prevalence of TI in males of infertile and fertile couples (MIC and MFC) and correlations of TI and its severity, defined according to the European Academy of Andrology (EAA) classification, with clinical, seminal, hormonal and US parameters. We evaluated (i)a cohort of 879 males (36.7±7.1 years) who attended our Unit from 2011 to 2021 (762 MIC and 117 MFC-healthy, fertile men with a live birth-), (ii)a subgroup of MIC (n=762) and (iii)a further subgroup of MIC without obstructive or genetic confounders (n=666). Since 2013, we also evaluated TI severity as “absent, mild, moderate, severe” using the EAA classification. Correlations of TI severity were therefore evaluated in (i)785 males (668 MIC and 117 MFC), (ii)a subgroup of MIC (n=668) and (iii)MIC without obstructive or genetic confounders (n=556). All subjects underwent, within the same day, medical history, physical examination, hormonal and glycolipid assessment, semen analysis, scrotal and transrectal US. Statistical analyses were adjusted for age and waistline whenever appropriate. Considering the entire cohort, TI prevalence was 37% and was significantly higher in MIC than in MFC (42.1% vs 2.6%; $p<0.0001$). Comparing subjects with and without TI, age was not different, but those with TI showed higher prevalence of history of cryptorchidism, orchiepididymitis, orchiectomy, testicular torsion, chemo-/radio-therapy; higher waistline, BMI, obesity and metabolic syndrome prevalence; lower average Prader- and US-testicular volume; lower sperm parameters and higher azoospermia prevalence; higher gonadotropins, lower total and calculated free testosterone, higher hypogonadism prevalence; higher chromosomal abnormalities frequency (all $p<0.005$). In a multivariate analysis including age, waistline and US-testicular volume as confounders, both TI and testicular volume were significantly and independently associated with low sperm parameters and

elevated gonadotropins, but only TI was associated with low testosterone levels. When TI severity was evaluated, absent, mild, moderate and severe TI were found in 70.5%, 20%, 8.5% and 1% of the sample, respectively, and were significantly higher in MIC than in MFC. Increasing TI severity was associated with increasing prevalence of history of cryptorchidism, orchiepididymitis, orchiectomy, testicular torsion; increasing prevalence of obesity and metabolic syndrome; progressive reduction in testicular volume; progressive worsening of sperm parameters and increase in azoospermia prevalence; progressive increase in gonadotropins, decrease in total and calculated free testosterone, increase in hypogonadism frequency (all $p<0.05$). All results were confirmed in the MIC subgroup and in the MIC sample without obstructive or genetic confounders. In conclusion, US-TI correlates with well-known conditions that cause a direct testicular damage, congenital or acquired, and, interestingly, with obesity and metabolic syndrome. TI severity correlates with a progressive worsening of testicular volume, sperm parameters, gonadotropins and testosterone levels. The association between TI, sperm and hormonal parameters is independent of testicular volume, highlighting the importance of US and TI assessment in evaluating the infertile male.

A-0025 Erectile dysfunction and physical comorbidities in young men, a population-based cohort study

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Background: Erectile dysfunction (ED) is a multidimensional disorder resulting from organic and psychological conditions. Although psychogenic causes typically account for ED in young men, evidence suggests that organic causes also are common in this group.

Objectives: To investigate a possible association between prescription of ED medication (EDM) and physical and psychological disorders among men <39 years of age.

Materials and Methods: We conducted a registry-based cohort study. We collected data from national health registries for all men aged 15–39 living in Sweden from 2006 to 2022. Men prescribed EDM were classified as exposed, and individuals without a prescription were classified as unexposed. The first EDM prescription defined the index date. Controls were randomly selected, 1:10 for each exposed individual. Matching criteria included the index date and year of birth. The 2 groups had comparable age distributions. ICD diagnosis and/or ATC prescription was considered "yes" if it occurred within 1 year prior to or after the index date, otherwise "No".

Results: 55783 men had at least 1 prescription of EDM. A prescription was significantly associated with the presence of physical and psychiatric disorders, and different medications compared to non-users. The highest OR was observed for endocrine (6.9% vs 1.4%, OR 5.38) and psychiatric disorders (21.2% vs. 5.0%, OR 5.15). Androgens/antiandrogens/gonadotropins were often used by men with EDM prescriptions (3,3% vs 0,3%, OR: 13.16), followed by psychoactive drugs (48.1% vs 14.8%, OR 5.34). Unmarried men and those with >9 years of education had the highest EDM prescription rate in comparison with other groups.

Discussion and Conclusion: Young men in Sweden using EDM have a high prevalence of physical and psychological disorders. Physicians should provide adequate medical consultation for men of all ages seeking help for ED to modify risk factors, prevent disease progression, and improve mental health.

A-0217 Super-resolution microvascular ultrasound is a novel and highly sensitive testicular functional imaging modality when tested in men with testicular dysfunction

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Background: Global sperm counts have halved since the 1970s. Neither serum testosterone nor semen analysis are highly predictive of fertility potential unless severely impaired. We describe a super-resolution ultrasound (SRUS) model-based technique to track microbubbles in contrast enhanced ultrasound (CEUS), allowing measurement of localized perfusion characteristics within the testes with a spatial resolution an order of magnitude higher than existing clinical ultrasound. We tested novel SRUS-derived biomarkers to detect testicular function, using hypogonadotropic hypogonadism (HH) as an experimental, treatable model of male infertility.

Methods: Ethics-approved, experimental study with blinded ultrasound and computational analysis in men with HH and serum testosterone <8nmol/L (230 ng/dl). Study A: HH (n=9) versus healthy-controls (n=10). Study B: HH without treatment (HH-alone; n=12); HH on testosterone replacement therapy (HH-TRT) (n=11); HH on gonadotrophins (HH-GnT) (n=9). Study C: Longitudinal follow up of men with HH on gonadotrophins (n=7) versus HH on TRT (n=7). All participants had reproductive hormones and semen testing, and CEUS with SRUS vascular mapping, drawing and quantitative microvascular parameter generation from the testicular region-of-interest (ROI) of each testis.

Results: Study A: Intratesticular vessel density (IVD), vessel diameter (VD) and vessel tortuosity (VT) were highly discriminatory between men with HH and controls [IVD (ratio:pixels of vessel area/ pixels of total ROI): 0.11 ± 0.06, controls; 0.04 ± 0.02, HH; p<0.01; VD (μm): 87 ± 16, controls; 71 ± 11, HH; p=0.01; VT (C/L): 3.17 ± 0.29, controls; 4.08 ± 0.69, HH; p<0.01]. Serum fasting morning testosterone was positively correlated with IVD (r=0.62, p<0.01) and VD (r=0.53, p=0.02), and negatively correlated with VT(r= -0.69, p<0.01). Similar, sperm concentration was positively correlated with IVD (r=0.72, p<0.001), VD (r=0.65, p<0.01), and negatively correlated with VT (r= -0.62, p<0.01). Study B: Compared with non-treatment and TRT, GnT was associated with increased IVD (0.08 ± 0.08, HH-alone; 0.07 ± 0.05, HH+TRT; 0.20±0.10, HH+GnT; p<0.001) and VD (77 ± 17, HH-alone; 79 ± 15, HH-TRT; 98 ± 20, HH-GnT; p=0.02) in men with HH. Study C: SRUS discriminated testicular activation more rapidly than testicular volume or inhibin B, in men with HH undergoing fertility treatment for 1 year.

Conclusion: SRUS is a novel imaging modality which can reliably distinguish men with normal versus impaired testicular function. Additionally, SRUS detects restoration of testicular function achieved with GnT therapy. SRUS is therefore an imaging modality with potential to provide accurate and clinically meaningful assessment of male reproductive function. Larger scale studies are warranted to determine the potential of ULM as a novel ‘male fertility scan’.

A-0068 Seminal plasma HSPA2 as a non-invasive biomarker to distinguish Sertoli cell-only syndrome from mixed testicular atrophy in non-obstructive azoospermia

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Introduction: Non-obstructive azoospermia (NOA) is the most severe form of male factor infertility and affects about 10% of male patients. Compared to obstructive azoospermia (OA), NOA originates from damage of testicular tissue and impairment of spermatogenesis whereas OA is linked to mere obstruction of the epididymides and/or vasa deferentia with mostly preserved spermatogenesis. NOA comprises heterogeneous testicular phenotypes with highly variable sperm retrieval outcomes: Whereas mixed testicular atrophy (MTA) is associated with higher retrieval success, maturation arrests or Sertoli cell-only phenotype (SCO) have virtually zero sperm retrieval success rates. Differentiation between phenotypes currently requires invasive biopsy as conventional clinical and hormonal parameters inadequately predict sperm retrieval. Seminal plasma proteomics has identified germ cell-derived proteins as potential non-invasive biomarkers. Heat shock 70 kDa protein 2 (HSPA2) is essential for spermatogenesis and has been proposed as a promising candidate biomarker reflecting the presence of germ cells in the testis.

Material and Methods: This retrospective observational study analyzed testicular tissue and seminal plasma samples from men with OA (intact spermatogenesis, serving as controls) and NOA patients diagnosed with MTA or SCO. Samples were collected during routine diagnostic and therapeutic procedures over several years at the Clinic for Urology, University Hospital Giessen. Seminal plasma samples were obtained from n=21 OA patients, n=22 NOA patients with successful sperm retrieval (MTA), and n=21 NOA patients with unsuccessful retrieval (SCO). Testicular biopsies from normal spermatogenesis (n=6), MTA (n=7), and SCO (n=6) were additionally analyzed. HSPA2 abundance was quantified by ELISA, testicular expression by RT-qPCR and immunohistochemistry. ROC analysis was performed to evaluate the predictive performance of ELISA.

Results: Testicular HSPA2 expression differed significantly between MTA and SCO samples ($p=0.002$), and between normal spermatogenesis and both NOA subtypes ($p\leq0.01$). Via immunohistochemistry, we localized HSPA2 predominantly to germ cells, with absent staining in SCO samples due to lack of germ cells in those samples. Seminal plasma HSPA2 concentrations were significantly lower in SCO compared to MTA ($p=0.001$), while levels in MTA overlapped with fertile controls. With ROC analysis, we demonstrated moderate discrimination for sperm retrieval outcome. Using a cut-off value of 50 ng/mL, HSPA2 ELISA achieved 86.0% specificity and 71.4% sensitivity for predicting positive sperm retrieval in NOA. The area under the curve was 0.78 for differentiation between MTA and SCO, indicating moderate predictive accuracy beyond chance.

Conclusion and future perspectives: Seminal plasma HSPA2 levels were significantly lower in SCO and showed a moderate predictive value for successful sperm retrieval in NOA patients. By this, seminal plasma HSPA2 represents a promising non-invasive biomarker to support clinical decision-making in fertility counselling. Until now, the study was performed with a rather low number of samples; a validation in larger prospective cohorts pending. After that, the use of HSPA2 – potentially also combined with other markers assessed from seminal plasma - as non-invasive biomarker could reduce unnecessary invasive procedures and improve patient counseling.

A-0089 High glucose supplementation aggravates experimental autoimmune epididymo-orchitis via localized Th17 skewing in the male reproductive tract

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Background: Chronic epididymo-orchitis contributes significantly to male infertility through immune cell infiltration and pro-inflammatory cytokine elevation. High glucose intake promotes Th17 differentiation and autoimmunity, but its effects on autoimmune testicular inflammation remain unclear.

Objectives: To determine whether chronic high glucose supplementation exacerbates experimental autoimmune epididymo-orchitis (EAEO), impairs spermatogenesis, and drives $CD4^+$ T-cell dysregulation, particularly Th17 polarization, in the reproductive tract.

Materials and Methods: EAEO was induced in male C57BL/6 mice with or without chronic 10% glucose supplementation in drinking water. Sperm parameters, ROS levels, apoptosis, and histopathology were evaluated. $CD4^+$ T-cell subsets (Th1, Th17, Treg, TNF-α^+ effector memory) were quantified by flow cytometry in testis, epididymis, spleen, and testicular lymph nodes (TLN) at days 30, 60, and 90 post-immunization.
Results: High glucose intake accelerated EAEO progression, markedly worsening sperm concentration and motility, elevating sperm ROS, increasing germ cell apoptosis, and causing severe histopathological damage. In the reproductive tract, glucose induced time-dependent $CD4^+$ T-cell accumulation and late-phase selective Th17 skewing (days 60–90), with significant IL-17A$^+$ $CD4^+$ Teff increases and accompanying TNF-α^+ $CD4^+CD44^+$ Teff upregulation, while IFN-γ^+ Th1 responses remained modest. These pro-inflammatory changes were strictly localized, with no significant Th17, Th1, Treg, or TNF-α alterations in spleen or TLN.

Discussion and Conclusion: Chronic high glucose exacerbates EAEO by intensifying oxidative stress, apoptosis, and tissue injury while promoting a late-phase, tissue-restricted Th17-biased $CD4^+$ T-cell

response in the testis and epididymis. These findings reveal a metabolic-immune axis that may accelerate immune-mediated male subfertility via localized Th17 mechanisms.

A-0279 Comparison of Functional and Anatomical Outcomes of Grafting and Combined (Graft and Tunical Shortening) Techniques in the Surgical Treatment of Peyronie's Disease

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Objective: This study aims to comparatively evaluate the preoperative characteristics, surgical preferences, and postoperative functional and anatomical outcomes of patients undergoing either grafting alone or a combined technique, consisting of grafting plus tunical shortening, for the treatment of Peyronie's disease (PD).

Materials and Methods: We retrospectively analyzed patients surgically treated for PD at Istanbul Medical Faculty between 1997 and 2025. Patients were divided into Group 1 (Graft Only, n=84) and Group 2 (Combined Technique, n=55). Graft materials used were IVP (n=134) and ITP (n=5). Exclusion criteria included isolated tunical shortening, penile prosthesis implantation, and preoperative medical treatment-resistant erectile dysfunction (ED). Patients with preoperative ED who were responsive to PDE5 inhibitors were included in the study. All patients were followed for at least 12 months. Postoperative ED was evaluated via a non-structured questionnaire, and recurrent curvature was defined as >20° during intracavernosal injection and stimulation (CIS) testing. Statistical analyses were performed using Mann-Whitney U, Chi-Square, and Fisher's Exact tests, with $p<0.05$ considered significant.

Results: Both groups were similar regarding age (54.2 vs 53.7, p=0.733), comorbidities, preoperative curvature degree (55.4° vs 57.3°, p=0.493), and curvature type (p=0.844). However, curvature direction significantly influenced surgical preference (p=0.014). While grafting alone was more frequent in dorsal curvatures (72.5% vs 49.1%, p=0.007), the combined technique was significantly more common in lateral curvatures (36.4% vs 16.2%, p=0.014). No significant differences were found between Group 1 and Group 2 regarding recurrent curvature rates (11.9% vs 20.0%, p=0.229) or the development of new medical treatment-resistant ED (39.2% vs 35.7%, p=0.812). Furthermore, postoperative complications such as penile shortening (16.7% vs 23.6%, p=0.382), hypesthesia (6.0% vs 5.6%, p=1.000), and suture knot palpation (2.4% vs 7.3%, p=0.213) occurred at similar rates in both groups.

Conclusion: Technique selection in PD surgery is primarily driven by curvature direction, with the combined technique being frequently preferred in cases of lateral asymmetry. Our data demonstrate that adding a shortening procedure to grafting does not adversely affect functional outcomes—including erectile function, penile length, or sensation—compared to isolated grafting. The combined approach remains a safe and effective strategy for the anatomical correction of challenging deformities over at least a one-year follow-up period.

A-0107 Global prevalence of recreational use of phosphodiesterase type 5 inhibitors: a systematic review and meta-analysis

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Phosphodiesterase type 5 inhibitors (PDE5i) are the gold standard for treating erectile dysfunction (ED). However, their use has increasingly shifted from strictly therapeutic to "recreational", defined as their consumption without a specific medical diagnosis or supervision to enhance sexual performance or confidence. This study provides the first systematic quantitative synthesis of the global prevalence of recreational PDE5i use among the male population.

Following PRISMA guidelines, a systematic search was conducted across PubMed, Scopus, Web of Science, and PsycINFO for studies published up to March 2026. Observational studies reporting quantitative prevalence of recreational PDE5i use in adult males were included. Random-effects models (DerSimonian-Laird) were employed to estimate pooled prevalence. Data were stratified by population:

men who have sex with men (MSM), young adults under the age of 30 years (YA), and general adult population (GAP) and recall period (lifelong vs recent use).

Out of 1,847 identified records, 20 studies met the inclusion criteria. The overall pooled lifelong prevalence was 23.1% (95% CI: 17.2–29.6%). Subgroup analysis revealed significant variations: prevalence was highest among MSM (38.6%), followed by the GAP (20.7%) and YA (11.9%). Sensitivity analysis excluding an outlier in the MSM subgroup adjusted the lifelong prevalence to 30.9%.

For recent use, the global pooled prevalence was 11.8% (95% CI: 5.6–19.8%). Again, MSM reported higher rates (18.1%) compared to YA (4.7%). Within the MSM recent use subgroup, a linear trend was observed as the recall period increased, from 12.0% at 1 month to 21.3% at 6 months. Common motivations included performance enhancement, managing performance anxiety, and curiosity. Significant heterogeneity was observed across most analyses ($I^2 > 90\%$), reflecting diverse study methodologies and populations.

In conclusion, recreational PDE5i use is a widespread phenomenon, particularly concentrated among MSM. The high prevalence in YA suggests a growing trend toward the medicalization of male sexuality. Clinicians, especially andrologists, urologists and psycho-sexology experts, should incorporate non-medical PDE5i use into routine pharmacological history-taking. Public health strategies are needed to address risks associated with unsupervised use, including potential psychological dependence, unsafe drug interactions (e.g., with nitrates or "poppers"), and the consumption of counterfeit products.

A-0132 Dietary associations with sperm DNA fragmentation in men from infertile couples: Swedish RUBIC data

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Sperm DNA fragmentation (SDF) is associated with infertility, poor embryo development, miscarriage and preeclampsia in the female partner. A longer abstinence time, a higher age and smoking have been reported to be associated with a higher degree of SDF, with increased production of reactive oxygen species as a suggested mechanism. The contribution of dietary factors is less studied, although more zinc and high intake of fruits and vegetables have been reported protective, and a high dietary glycaemic load and high B12 reported detrimental. We investigated whether answers to seven empirically chosen questions on dietary factors were associated with SDF in 599 men from infertile Swedish couples enrolled in the Danish-Swedish collaborative ReproUnion Biobank and Infertility Cohort.

Information on the dietary factors were obtained through a questionnaire. SDF was assessed using the Sperm Chromatin Structure Assay, expressed as the DNA fragmentation index (DFI), after which associations between dietary habits and DFI were examined.

Negative correlations (Spearman's rho) were found between intake of beef (whole/stew) ($\rho = -.11$, $p < .01$), pork (whole/stew) ($\rho = -.12$, $p < .01$), Brussels sprouts/broccoli ($\rho = -.09$, $p = .04$) and fried potatoes ($\rho = -.13$, $p < .01$), and DFI, whereas cooking yogurt ($\rho = 0.09$, $p = .04$) and muesli ($\rho = 0.11$, $p = .01$) correlated positively with DFI. Further, men who usually ate dinner had a lower DFI than men who usually did not ($p < .001$).

In conclusion, eating dinner, meat, fried potatoes and certain cabbage plants seemed protective against SDF, whereas cooking yoghurt and muesli seemed to increase it. The associations found would need confirmation in more extensively designed observational or interventional studies.

A-0154 Performance of an AI real-time detection model compared with computer-aided sperm analysis for sperm concentration

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The gold standard for the assessment of sperm concentration is the manual method recommended by WHO. The procedure is time-consuming and requires extensive training and continuous quality control to achieve reproducible results. Computer-aided sperm analysis (CASA) systems have been developed to improve the reproducibility, in addition to making semen analysis less time-consuming. The performance of CASA instruments is dependent on the nature of the semen sample, and for sperm concentration, it is a challenge to do the assessment in the ejaculate. This may result in a substantial variability in sperm concentration, especially at low or high sperm concentration and in the presence of debris and other cells.

Several AI-based approaches have been investigated for semen analysis. However, there are few studies where the AI performance has been compared with the manual gold standard as well as with CASA, also for sperm concentration. In our study, we compare the performance of a CASA system with an AI model, and both methods with the manual WHO assessment.

Images from 100 semen samples were collected using a Microptic Sperm Class Analyzer (SCA) and 2X-CEL chambers with magnetic clamp. The spermatozoa in each image were detected using the AI model Real-Time Detection Transformer, and counted. The model had been trained on another dataset (20 samples/videos). The sperm concentration was also determined by the SCA (n=70) and by manual assessment according to the WHO recommendations (n=18).

We found a strong correlation between the sperm concentration evaluated by CASA and the AI model (Pearson's r=0.961; 95% CI 0.938, 0.988). On average, the AI-model gave higher results with a mean bias of 6.6 mill sperm per mL (Bland-Altman, limits of agreement -15.7 to 29.0). A correlation was also seen when these results were compared with the manual assessments (r=0.977; 95% CI 0.936, 0.992), however the number of samples was low. Compared with the manual method, both the CASA and the AI values overestimate the concentration in the low range and underestimate at high values. A strength of the study is that the analysis by CASA and AI was performed on the same video. Furthermore, the use of 2X-CEL chambers avoids viscosity-dependent error. A limitation of the study is that only one frame was annotated for each sample by the AI model.

The AI model shows good performance compared with CASA. A manual re-annotation of the AI-annotated dataset with subsequent training of the AI model could improve the performance further. Testing on new material and inclusion of more samples assessed by the WHO manual method are necessary to evaluate the various approaches.

A-0143 Semen quality in transgender individuals seeking fertility preservation

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Background rnTransgender individuals assigned male at birth (AMAB) may choose to preserve their fertility prior to starting gender-affirming hormone therapy (GAHT). However, limited data exist regarding the baseline reproductive and hormonal characteristics of this population before and after GAHT. ObjectivesrnTo characterize semen quality and hormonal profiles in transgender AMAB individuals prior to GAHT and compare findings with cisgender men and with transgender individuals who discontinued GAHT for at least 3 months. Materials and methodsrnThis retrospective study included transgender AMAB individuals from two tertiary andrology centers who underwent sperm cryopreservation before GAHT initiation (treatment-naïve group). Clinical evaluation included anthropometric measures, testicular volume assessment, varicocele detection, and sex hormone measures. Semen parameters were analyzed according to WHO criteria and compared with those of cisgender men and previously treated transgender individuals. ResultsrnThirty-two treatment-naïve transgender AMAB individuals were included. Hormonal parameters were generally within reference ranges. Only 46.9% of treatment-naïve individuals met WHO criteria for normozoospermia, compared with 75.5% of cisgender controls. Compared with nine previously

treated individuals, estradiol levels were higher and seminal volume lower. A higher frequency of seminal abnormalities was observed, although not statistically significant. ConclusionsrnA substantial proportion of treatment-naïve transgender AMAB individuals exhibited impaired semen parameters prior to GAHT initiation. Prior GAHT exposure showed a trend toward poorer semen quality. These findings support early fertility counselling and preservation in transgender individuals prior to GAHT initiation.

EAA Centre Symposium

Friday, 18 September 2026 - 10:30-11:30

A-0231 Region-Specific Macrophage – Epithelial Crosstalk Shapes Epididymal Homeostasis

Tiziana Grimaldini [1]*, Julia König [2]*, Vishnu Kumar [3], Marek Bartkuhn [3], Sudhanshu Bhushan [1], Denise Messerer [4], Christian Schulz [5], Christiane Pleuger [1], Andreas Schlitzer [2]*, Andreas Meinhardt [1]*

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The epididymis comprises a single, highly coiled duct divided into initial segment, caput, corpus, and cauda, along which spermatozoa undergo progressive post-testicular maturation driven by region-specific epithelial secretory activity. The epithelium consists of principal cells, proton-secreting clear cells, basal cells with progenitor-like properties, and intraepithelial macrophages. Immunologically, the proximal and distal epididymis mount compartmentalized responses to infection, forming a characteristic inflammatory gradient along the organ. Flow cytometry and single-cell RNA sequencing identify macrophages as the dominant immune population within the tissue, positioning them as candidate regulators of this gradient. We therefore hypothesized that intraepithelial macrophages act as spatial regulators of epithelial homeostasis under both physiological and inflammatory conditions.

To interrogate macrophage–epithelial interactions, we achieved ~90% macrophage depletion throughout the epididymis for up to 30 days using the CSF1R inhibitor PLX5622. Immunofluorescence and morphometric analyses revealed a significant increase in tubule diameter (~10%) and basal cell ($KRT5^+$) projection length (~30%) exclusively in the initial segment, while no histological alterations in the caput, corpus, or cauda were detected. This striking regional specificity implicates macrophages as non-redundant regulators of epithelial architecture in the most proximal epididymal segment, where luminal conditioning is critical for downstream sperm maturation. To probe reciprocal epithelium-to-macrophage signaling, we employed a basal cell–specific CSF1 depletion model to destabilize the macrophage niche. This resulted in compensatory $CCR2^+$ monocyte-derived macrophage recruitment selectively to the cauda within 10 days, regulated by as of yet unknown signals. Together, these findings reveal that epididymal macrophage homeostasis is regionally autonomous, that epithelial CSF1 is a non-redundant niche factor in the cauda, and that distinct macrophage subpopulations are functionally specialized along the organ axis. Given the relevant role of epididymitis in male infertility, these data open a new framework for understanding how macrophage–epithelial crosstalk safeguards the luminal environment permissive for sperm maturation.

A-0232 Urinary metabolites of selected non-persistent endocrine-disrupting chemicals and sperm DNA fragmentation in young men from the general population

Renata Walczak-Jedrzejowska [1] , Joanna Jurewicz [2], Wojciech Hanke[3], Bartosz Wielgomas [4], Katarzyna Marchlewska [1], Jolanta Slowikowska-Hilczer [1]

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Environmental exposure to endocrine-disrupting chemicals (EDCs) is widespread, yet evidence regarding their impact on sperm DNA integrity remains inconclusive. We aimed to assess associations between ten urinary metabolites of selected non-persistent pesticide and phenolic endocrine disruptors and sperm DNA fragmentation index (DFI), analyzing each metabolite separately. The study included 306 young men recruited from the general population mainly residing in urban areas. On the study day, participants provided urine and semen samples, underwent a medical examination with anthropometric measurements, and completed standardized questionnaires. Urinary EDC metabolites were analyzed using gas chromatography–mass spectrometry (GC–MS). Urinary concentrations were corrected for specific gravity, and values below the limit of detection were imputed. Sperm DNA fragmentation was assessed using the Sperm Chromatin Dispersion (SCD) test. For statistical analysis, metabolites with higher detection frequency were analyzed as continuous variables and in tertiles, whereas those with lower detection frequency were evaluated as binary variables (detected vs non-detected). Associations were assessed using linear regression models with and without adjustment for potential confounders. The median age was 23 years. Most participants had normal BMI (68%); 70% were non-smokers, 53% reported drinking alcohol more than once per week, and 56% had used medication within three months prior to the visit. A small proportion reported varicocele (9%) or a history of cryptorchidism (4%). The median DFI was 15%, and 29% of participants had DFI values ≥20%. Detection frequencies ranged from approximately 20% to over 70%, indicating heterogeneous environmental exposure. Across continuous, categorical, and binary analyses, no statistically significant associations were observed between urinary EDC metabolites and DFI. No exposure–response trends were identified in tertile analyses. In conclusion, no association was observed between environmental exposure to the investigated non-persistent EDCs and sperm DNA fragmentation in this cohort of healthy young men.

A-0233 Artificial intelligence improves prediction of sperm retrieval success in non-obstructive azoospermia

Zsolt Kopa [1], József Bokor [2], Anett Szabó [1]

[1] Semmelweis University, Budapest, Hungary; [2] MTA SZTAKI, Budapest, Hungary

Introduction
Azoospermia represents the most severe form of male infertility and affects approximately 10–12% of infertile men. In non-obstructive azoospermia (NOA), the underlying cause is impaired testicular function. However, focal areas of spermatogenesis may still be present within the testes. In such cases, sperm can be retrieved using microdissection testicular sperm extraction (mTESE) for use in assisted reproductive techniques, although successful retrieval occurs in only about 50% of procedures. Therefore, the aim of this study was to develop a predictive model to identify patients who are most likely to benefit from mTESE.

Methods
A post hoc analysis was performed using preoperative clinical and hormonal data together with surgical outcomes to develop predictive models for sperm retrieval. Both logistic regression and a neural network approach were applied. The data were collected at Semmelweis University in Budapest, Hungary, between 2019 and 2022.

Results
Among the 196 procedures analyzed, sperm retrieval was successful in 117 cases. The principal variables included in the predictive model were follicle-stimulating hormone (FSH), luteinizing hormone (LH), total testosterone (tT), and anti-Müllerian hormone (AMH), initially modeled as linear and subsequently as non-linear functions. In the logistic regression model, total testosterone emerged as the most significant predictor, followed by AMH, AMH², FSH², and the AMH:LH interaction term. Less influential predictors included LH², the FSH:tT interaction, and the FSH:LH interaction. These variables were subsequently used to construct the neural network, which achieved a predictive accuracy of 86%.

Conclusion
The predictive accuracy achieved by the model is sufficiently high to support its potential use in the preoperative evaluation of patients with NOA. By estimating the individual probability of successful sperm retrieval, the model may assist patients and clinicians in making more informed decisions regarding mTESE surgery.

A-0013 Sub-Gluteal Ligation of the Internal Pudendal Vein for Management of Veno-Occlusive Erectile Dysfunction (Shaeer's Vein Ligation–I): The Cadaveric Study

Osama Shaeer [1,2], Ehab A. A. El-Shaarawy [3], Hossam Y. Emam [3], Islam Elsisi [1], Eslam Sokar [1], Kamal Shaeer [1]

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Vein ligation for veno-occlusive erectile dysfunction is being abandoned due to the recurrence rate. Among the reasons for failure is inability to ligate the deep system of veins; the internal pudendal vein. The vein exits the pelvis in the gluteal region, from the lesser sciatic foramen to the greater sciatic foramen, coursing over the ischial spine and sacro-spinous ligament, under the gluteus maximus. This work aims to verify feasibility of the first surgical procedure to ligate the internal pudendal vein through the gluteal approach. This cadaveric study involved five formalin-fixed cadavers. A surface anatomical landmark was designed to identify the ischial spine, at the intersection of two lines: a vertical line from posterior superior iliac spine to ischial tuberosity, and a horizontal line extending from sacro-coccygeal joint, laterally. An incision is cut encompassing the target point. Subcutaneous fat is dissected down to the gluteus maximus, which is split along the direction of its fibers. The vein can be found crossing over the ischial spine. “Shaeer’s Vein Ligation – I” appears to be surgically feasible. A protocol for a surgical study is registered at clinicaltrials.gov , and is open for participation.

Keywords: veno occlusive erectile dysfunction; venous leak; dorsal vein ligation; internal pudendal vein ligation; internal pudendal vein embolization

Posters

Highlighted posters 1

Thursday, 17 September 2026 - 15:30-16:00

A-0026 Divergent signaling of LH and hCG through the TSH receptor

Carmela Perri [1,2], Giada Mercanile [1], Clara Lazzaretti [1], Samantha Sperduti [1,3], Manuela Simoni [1,3,4], Giulia Brigante [1,4], Livio Casarini [1,3]

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Human chorionic gonadotropin (hCG) is the pregnancy hormone absent in the male and used as a drug for the treatment of male infertility. It shares a high structural similarity with thyrotropin (TSH) and can cross-activate the TSH receptor (TSHR), therefore sustaining thyroid hormone production during pregnancy. hCG also binds the luteinizing hormone receptor (LHCGR), which is shared with luteinizing hormone (LH) and mediates gametogenesis. However, whether LH can interact with TSHR remains unclear. In this study, we compared the effects of hCG and LH on TSHR-mediated signaling pathways.

COS7 cells transfected to overexpress human TSHR were exposed in vitro to increasing concentrations of LH and hCG (nM–µM range) to evaluate activation of Gs- and Gq-associated pathways. Intracellular cyclic adenosine monophosphate (cAMP) levels were measured using a bioluminescence resonance energy transfer (BRET) method, while inositol monophosphate (IP1) accumulation was quantified by homogeneous time-resolved fluorescence (HTRF) assay. cAMP response element (CRE)-dependent transcriptional activity was assessed through a luciferase reporter assay. Cells treated with 300 nM TSH served as a positive control. In addition, human thyroid organoids were used to validate the effects of hCG on thyroid tissue maintenance and responsiveness to TSH. All experiments were performed in quadruplicate (n=4), and statistical analyses were conducted using the Kruskal-Wallis test followed by Dunn’s post test ($p < 0.05$).

Exposure to 1 μM hCG significantly increased intracellular cAMP levels and enhanced CRE-dependent transcriptional activity, consistent with activation of the TSHR/Gs signaling pathway. In contrast, sub-μM concentrations of LH did not stimulate cAMP production or CRE activity. Interestingly, 1 μM LH inhibited these responses. Both LH and hCG failed to induce intracellular IP1 increase. However, co-administration of LH and TSH reduced TSH-induced IP1 levels, while it was not with hCG + TSH, suggesting the existence of an inhibitory effect of LH on TSHR-mediated Gq signaling.

The hCG-specific effects were confirmed in human thyroid organoids, where hCG treatment supported the cAMP responsiveness to TSH stimulation. These observations indicate that hCG selectively cross-activates TSHR-dependent cAMP/CRE signaling, in line with its physiological role during pregnancy. Conversely, LH appears to act as a functional antagonist at TSHR, negatively modulating both Gs- and Gq-mediated pathways.

Previous studies have shown that LH and hCG can elicit distinct signaling responses through LHCGR. Our findings further support the concept of differential signaling between these two gonadotropins and identify TSHR as a potential additional receptor partner. This interaction may represent a previously unrecognized mechanism contributing to the regulation of thyroid physiology and pathophysiology, and may have clinical implications in male patients treated with hCG.

A-0292 Role of Testosterone Decline in Determining the Symptoms of Functional Hypogonadism Related to Obesity

Bombardieri Alessio [1], Cotugno Ilaria [1], Corona Giovanni [2], O'Neill Terence W. [3], Bártfai György [4], Casanueva Felipe F. [5], Forti Gianni [6], Giwercman Aleksander [7], Han Thang S. [8], Slowikowska-Hilczer Jolanta [9], Punab Margus [10], Antonio Leen [11], Vanderschueren Dirk [11], Huhtaniemi Ilpo T., Wu Frederick C. W., Maggi Mario [6], Rastrelli Giulia [1]

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Introduction

Late-onset male hypogonadism (LOH) is a syndrome characterized by reduced testosterone levels associated with physical, psychological, and sexual symptoms. Of particular interest are the forms of functional hypogonadism, which are frequent in obese men and in those with chronic comorbidities. In such cases, it remains unclear whether the reduction in testosterone is the cause of the symptoms or rather an epiphenomenon of an overall compromised health status.

Objective

To assess the relative contribution of reduced testosterone levels and obesity to the development of typical LOH symptoms in a population of middle-aged or elderly European men without organic causes of hypogonadism, in whom obesity was a predisposing factor for testosterone reduction.

Methods

A total of 2,122 subjects from the EMAS study (aged 40–79 years) with baseline and follow-up data on total testosterone (TT), free testosterone (FT), and BMI were included. Sexual, physical, and psychological

function was evaluated using validated questionnaires (SFQ, SF-36, BDI, PASE) and objective tests (PPT, DSST, Camden Memory Test). A mediation analysis was performed to assess the role of TT (<10.5 and <8 nmol/L) and FT (<220 and <170 pmol/L) as mediators between obesity and the onset/worsening of symptoms, and vice versa.

Results
Obesity was a strong predictor of the onset of hypogonadism and was also associated with the appearance or worsening of physical symptoms (inability to bend, walk >1 km, or perform vigorous activities), reduced mental energy, erectile dysfunction, and decreased morning erections. However, only sexual symptoms were partially mediated by the decline in TT (with OR values between 1.04 and 1.07), whereas physical and psychological symptoms were more directly related to obesity, without significant mediation by androgen deficiency. Only the lower FT threshold (170 pmol/L) was found to mediate erectile dysfunction [OR: 1.05 (1.01–1.09)] and some physical symptoms [inability to bend OR: 1.07 (1.00–1.14); inability to perform vigorous activities OR: 1.04 (1.00–1.07)].

When the model was inverted, it was observed that the effects of TT decline on physical symptoms—particularly the inability to perform vigorous activities (OR: 1.09 for TT <10.5 nmol/L and OR: 1.15 for TT <8 nmol/L)—and of FT decline (for the three physical symptoms, ORs between 1.09 and 1.16) were mediated by obesity. Conversely, sexual symptoms were directly affected by the reduction in testosterone, only partially mediated by obesity.

Conclusions
Sexual symptoms are confirmed as the most specifically associated with hypogonadism, whereas physical and psychological symptoms appear less distinctive. A TT threshold of 10.5 nmol/L correctly identifies hypogonadism-related symptoms associated with obesity, while for FT, a threshold of 170 pmol/L appears more appropriate.

A-0237 Low bone mineral density in males from infertile couples - association with reproductive parameters and body mass index. Data from ReproUnion Biobank and Infertility Cohort (RUBIC)

Alexandra Kondic [1,2]*, Sofie Fagerström [1,2]*, Maria Dahlberg [1,2], Karin Odlén [1,2], Anders Juul [4], Anja Bisgaard Pinborg [5], Henriette Svarre Nielsen [6,7], Sandra Søgaard Tøttenborg [8], Johan Malm [3], Aleksander Giwercman [1,2], Angel Elenkov [1,2]

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Osteoporosis is a common disease that increases the risk of fractures. In Sweden, it is estimated that one in two women and one in five men will suffer an osteoporosis-related fracture after the age of 50. Many fractures, especially vertebral and hip fractures, cause significant suffering, disability, increased mortality and large costs for the healthcare system and society. Secondary osteoporosis, implying severely reduced bone mass or deterioration of bone microarchitecture due to factors other than age and menopause, is more common in men than in women. A z-score below -2.0, without an obvious explanation, often indicates need for extended investigation to find the underlying cause and start preventive measures. The aim of this study was to compare prevalences of z-score < -2 in males and females from infertile couples. We also wanted to investigate if low bone mineral density (BMD), in those relatively young men, was associated with, body mass index (BMI), total testosterone levels or sperm concentration.

In the ReproUnion Biobank and Infertility Cohort (RUBIC) study, infertile couples apart from a fertility examination underwent several other examinations including a Dual-energy X-ray Absorptiometry (DEXA) scan. Data was obtained from the Swedish part of RUBIC. Nine hundred and ninety-two men underwent a DEXA scan with Lunar iDXA. GE Healthcare uses standards from the National Health and Nutrition Examination Survey (NHANES) to enable comparison of bone mineral content (BMC) and BMD with a representative population.

We found 8.3% of 992 men presenting with hip and/or lumbar spine z-score < -2.0. The corresponding percentage among their female partners was 2.9 (Fisher's exact test, p<0.0001).

Fasting morning blood samples and semen samples were provided at the same time. Linear regression analysis was applied to test association between mean sperm concentration (two samples) and z-score, separately for hips and lumbar spine (n=807). Similar strategy was applied for BMI and total testosterone which were, however, tested in the same model.

Fos mean sperm concentration we found borderline statistically significant (p=0.06) negative association with lumbar spine but not hip z-score. In a bivariate model, both BMI and P-testosterone were statistically significantly (p=0.04 and p=0.009, respectively) positively associated with hip z-score. For the lumbar spine, the positive association with P-testosterone was borderline (p=0.05) statistically significant, whereas for BMI a negative association (p=0.018) was seen. However, ROC analysis showed that BMI, P-testosterone and sperm concentrations were not good predictors of low BMD (AUC < 0.6).

Among men from infertile couples, the prevalence of z-score below -2.0 and that of t-score below -1 seem to be statistically significantly higher among men than in their female partners. Although, impaired testicular function, as expressed by low sperm concentration and/or testosterone levels might, at least partly, explain the unexpectedly high prevalence of men with BMD z-score below – 2.0, none of these markers seems applicable for identifying high-risk patients.

A-0162 Cardiovascular Risk in Klinefelter Syndrome: Clear Benefits of Testosterone Therapy Despite Residual Excess Mortality - a Swedish register-based Study

Mats Holmberg* [1,2], Ida Nordgren* [3,4], Simon Chang [5,6], Claus Højbjerg Gravholt [5,6,7], Giorgio Tettamanti [3,8]

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Background:
Klinefelter syndrome (47,XXY) is the most common sex chromosome disorder in men, affecting approximately 1 in 600 live male births. It is characterized by primary hypogonadism, testosterone deficiency, and increased risks of metabolic and cardiovascular disease, contributing to reduced life expectancy. Testosterone replacement therapy (TRT) is widely used to treat hypogonadism and improve well-being, but its long-term cardiovascular effects have only been evaluated in one previous Danish study.
Methods:
We conducted a matched cohort study of cardiovascular outcomes in men with Klinefelter syndrome (KS) identified from the Swedish National Patient Register, comparing those treated with testosterone to untreated men with KS, as well as to age- and sex-matched individuals from the general population (25 individuals per KS case). Information regarding testosterone treatment was obtained from the Prescribed Drug Register and is available from July 2005. Analyses were adjusted for baseline comorbidities, including diabetes, prior venous thromboembolism, and autoimmune disorders.

Results:
We identified 1,278 men with KS in the National Patient Register; 834 had received at least one testosterone prescription, while 444 had not. Testosterone replacement therapy (TRT) was associated with a significantly reduced risk of cardiovascular death (HR 0.57 (0.36-0.92), major adverse cardiovascular events (MACE) (HR 0.47, 95% CI 0.31–0.73), and stroke (HR 0.40, 95% CI 0.17–0.96). No difference in all-cause mortality was observed between treated and untreated KS men and rates of myocardial infarction, atrial arrhythmia, pulmonary embolism and heart failure were similar between the two groups. Compared with matched comparisons from the general population, men with KS who received TRT exhibited increased mortality, and had higher risks of pulmonary embolism and atrial arrhythmia.

Conclusion:
We confirm previous results that TRT for hypogonadism in KS is beneficial and implies lower risks of

cardiovascular death and MACE compared with untreated men with KS. However, compared with the general population, men with KS who received TRT still exhibit increased mortality.

Limitations:
Our dataset lacks ATC codes for certain cardiovascular medications (e.g., statins, antiplatelet agents, antihypertensives), limiting our ability to adjust for these treatments.

A-0141 Impact of FSHB and FSHR genes polymorphisms on hormonal profile and sperm retrieval outcome in men with Klinefelter syndrome: a clinical–genetic predictive study

Andrea Graziani [1,2], Giuseppe Grande [2], Maria Santa Rocca [2], Massimo Iafrate [3], Riccardo Selice [2], Antonella Di Mambro [2], Alberto Ferlin [1,2]

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Background: Genetic variability within the follicle-stimulating hormone (FSH) signalling pathway might contribute to phenotypic heterogeneity in patients with Klinefelter syndrome (KS), yet its clinical impact on sperm retrieval remains unclear.

Objectives: To investigate the association between FSHB c.211 G>T and FSHR polymorphisms (c.2039 A>G and c.29 G>A) and hormonal parameters, as well as their potential role in predicting sperm retrieval rate (SRR) in patients with KS undergoing testicular sperm extraction (TESE).

Materials and methods: A retrospective cohort of patients with KS was analysed. Only subjects not receiving testosterone replacement therapy were included (n=417). Hormonal, anthropometric, and clinical variables were compared across genotypes using non-parametric tests. Additive genetic models were applied to evaluate allele-dose effects. Multivariable logistic regression was performed to identify independent predictors of SRR. Predictive performance of clinical and combined clinical–genetic models was assessed using ROC curve analysis.

Results: FSHB and FSHR polymorphisms were associated with variations in serum FSH concentrations, confirming a modulatory role of the FSH pathway. In additive modelling, the FSHR c.29 G>A polymorphism emerged as an independent predictor of SRR (adjusted OR 0.29, 95% CI 0.09–0.97), whereas FSHB c.211 G>T showed a non-significant trend. The inclusion of genetic variants modestly improved predictive accuracy compared with clinical variables alone, as demonstrated by ROC analysis.

Discussion and conclusion: Genetic variants within the FSH signalling pathway might contribute incremental information for the prediction of sperm retrieval in patients with KS. While the overall predictive gain remains moderate, additive genetic modelling highlights a potential allele-dose effect of FSHR c.29 G>A on reproductive outcome, supporting a role for integrated clinical–genetic assessment in this population, although external validation remains required.

A-0152 Ultrasound evaluation of testicular volume and correlation with seminal and hormonal parameters in indonesian infertile men: the first Indonesian andrological report

Seso Sulijaya Suyono [1,2], Rossy Sintya Marthasari [3], Francesco Lotti [4,5]

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It is well known that testicular volume (TV) is associated positively with sperm parameters and testosterone levels and negatively with gonadotropins levels. These relationships have been reported in infertile and fertile men considering both Prader orchidometer-assessed and ultrasound (US)-derived TV. Correlations between mean TV, sperm and/or hormonal parameters have been reported in men of Western countries and Asian populations. However, in our knowledge, a study on this topic is lacking in the Indonesian population. The aim of this study is to evaluate the mean US-TV of Indonesian infertile men and its associations with sperm and hormonal parameters.

We evaluated 63 consecutive Indonesian males of infertile couples attending two infertility clinics (Sadewa IVF Centre and Gladiool IVF Centre) between May 2024 and August 2025. All subjects underwent medical history, physical examination and scrotal US according to the European Academy of Andrology methodology and the European Society of Urogenital Radiology recommendations. Moreover, all subjects underwent, within the same day, sperm analysis according to the WHO 2021 Manual, and hormonal evaluation including Follicle Stimulating Hormone (FSH) and total testosterone levels. The Indonesian infertile men evaluated had a mean age of 32.75±4.84 years old and showed a mean US-TV of 8.87±2.24 ml. Mean FSH and testosterone levels were 5.62±3.01 IU/L and 471.78±209.05 ng/dl, respectively. Considering sperm parameters, mean US-TV was positively correlated with sperm concentration ($r=0.269$, $p<0.05$) and showed a statistical trend toward a positive association with sperm progressive motility ($r=0.159$, $p=0.212$). In addition, mean US-TV was negatively associated with FSH levels ($r = -0.425$, $p<0.001$) and positively with testosterone levels ($r=0.290$, $p<0.05$). The previous associations between mean US-TV, sperm concentration, FSH and testosterone levels were confirmed after adjusting for age (all $p < 0.05$).

Although TV varies among populations (Caucasian males show larger testis compared to Asian counterpart), infertile men show smaller TV than fertile men. Our results is in line with previous studies since overall our study population had mean TV values lower than the lower reference limit of TV reported in European healthy fertile men. However we can not make any further conclusion since TV reference ranges for Indonesian fertile men population is still lacking and in our study we had no control group of fertile men. Our study demonstrated in Indonesian infertile men that mean US-derived TV was associated positively with sperm concentration and serum testosterone level, while it was associated negatively with FSH level, in line with previous studies performed in other ethnic groups. In conclusion, in our knowledge this is the first study performed in Indonesian infertile men assessing TV with US and its correlations with sperm and hormonal parameters. Mean TV of Indonesian infertile men seem slightly smaller than that reported in wide cohorts of infertile men of European countries. The relationships between US-TV and sperm and hormonal parameters are in line with those reported in previous studies on non-Indonesian populations.

A-0269 Relationship between dynamic penile duplex ultrasound and retinal microcirculation in patients with suspected vascular erectile dysfunction

Matteo Nardin [1], Paolo Facondo [2], Claudia Rossini [3], Luigi Mori [3], Massimo Salvetti [3], Alberto Ferlin [4,5], Carolina De Ciuceis [3], Andrea Delbarba [7,8]

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Background. Vascular erectile dysfunction (ED) is closely associated with increased cardiovascular risk and may precede major cardiovascular events. Dynamic-penile duplex ultrasound (D-PDU) is the gold standard for discriminating between vascular and non-vascular etiologies of ED. Retinal microcirculation, assessed non-invasively by adaptive optics, reflects systemic microvascular remodeling and may provide an early marker of vascular damage. However, no studies have specifically evaluated retinal microvascular morphology and function in patients with ED, either vascular or non-vascular. We inquired the relationship between retinal microcirculatory parameters can differentiate between vascular and non-vascular ED and support cardiovascular risk stratification.

Methods. This is a prospective longitudinal observational study including adult males with clinically diagnosed ED undergoing D-PDU. Clinical history, ongoing treatments, and IIEF-5 score will be recorded. D-PDU will include cavernous artery caliber and course, peak systolic velocity (PSV), end-diastolic velocity (EDV), and cavernous intima-media thickness (IMT). Such parameters were collected separately, right and left side, and their mean was used for the analysis. We used a cutoff of ≤35 cm/s for PSV, >5 cm/s for EDV, and ≥0.3 mm for IMT to consider impairment. Retinal microcirculation assessment was performed using adaptive optics, with measurements of inner and outer diameters, wall thickness, wall cross-

sectional area (WCSA), and wall-to-lumen ratio (WLR); flicker light stimulation was used to measure vasodilatory responses.

Results. A total of 98 males have been included, showing an overall mean IIEF-5 score of 12.0±6.7. Among them, 33 (34%) presented a mean PSV ≤35 cm/s. Subjects with low PSV were older (p<0.001) and had higher body mass index (p=0.034) and waist circumference (p=0.001), a higher prevalence of hypertension (p=0.019), hypercholesterolemia (p<0.001), diabetes mellitus (p=0.024), and family history of coronary artery disease (p=0.037). In relation to D-PDU evaluations, patients with impaired PSV displayed a higher rate of impaired EDV compared to those without (39.4% vs 15.4%, p=0.008) and of impaired IMT (34.6% vs. 7.4%, p=0.004). No significant differences were found in WCSA and WLR according to PSV impairment. No differences were appreciated in the vasodilatory response after flicker light stimulation according to PSV impairment. Conversely, we observed a reduced vasodilatory response to flicker light stimulation among males with impaired EDV compared to those with normal EDV (0.32 vs. 1.64 percentage of vasodilatation, p=0.042). Regarding the structural, high IMT at D-PDU was associated with a higher WLR (0.24 vs. 0.26, p=0.016). Correlation analysis confirmed a significant relationship between IMT and WLR (r 0.274, p=0.017) and between EDV and percentage of vasodilatation (r 0.353, p=0.017). In backward stepwise multivariable linear regression analysis, IMT (beta = -0.527, p<0.001) and hypercholesterolemia (beta = -0.227, p=0.014) were identified as independent predictors of mean PSV.

Conclusions. These preliminary findings suggest a novel link between D-PDU abnormalities and retinal microcirculation in men with ED. In particular, IMT was associated with WLR, while impaired EDV was associated with a reduced vasodilatory response. These data support the hypothesis that penile and retinal microcirculation may share a common vascular substrate. Further in-depth studies are warranted to confirm the findings and assess their diagnostic and prognostic relevance.

A-0213 In-depth genetic and phenotypic characterisation of CFTR-associated and CFTR-independent congenital bilateral absence of the vas deferens

Ina Klockner [1,2], Johanna Steingröver [1,2], Jann-Frederik Cremers [3], Sabine Kliesch [3], Isabell Schumann [2], Frank Tüttelmann [1,2]

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Congenital bilateral absence of the vas deferens (CBAVD) is a distinct form of obstructive azoospermia with variable phenotypic manifestations. It is most commonly caused by variants in the CFTR gene, encoding the cystic fibrosis transmembrane conductance regulator expressed in epithelial cells of multiple organs, including the lungs and male reproductive tract. CFTR mutations are inherited in an autosomal recessive manner and can result in a spectrum of clinical presentations ranging from classical cystic fibrosis to isolated CBAVD. CBAVD is frequently associated with heterogeneous CFTR variants that impair protein function, disturbing fluid homeostasis. However, a proportion of cases remains genetically unexplained. Furthermore, associated anomalies such as epididymal abnormalities or unilateral renal agenesis suggest that broader developmental mechanisms may contribute to the phenotype in some patients.
Despite over three decades of research on CFTR and related disorders, important questions regarding the genetic and phenotypic spectrum of CBAVD remain unresolved. In particular, the contribution of variants in coding regions in combination with intronic variants, such as the T allele and associated TG repeats within the CFTR gene, remains understudied.

In this study, we established a highly selected cohort of azoospermic men with surgically confirmed CBAVD and performed comprehensive clinical and molecular characterisation. Diagnosis was confirmed during testicular sperm extraction (TESE). Clinical assessment included semen analysis, reproductive hormone profiling, and histological assessment.

Among more than 3,800 men undergoing TESE, CBAVD was identified in 93 patients (2.4%). A genetic cause was identified in 70% of these cases. Consistent with previous reports, the most frequent variants included F508del and R117H in combination with the T5;TG12 allele. In addition, approximately 35% of patients carried rarer CFTR variant combinations.

In-depth genetic analyses were conducted to investigate genotype–phenotype correlations and to expand current knowledge of both CFTR-associated and CFTR-independent CBAVD. This systematic approach aims to refine diagnostic yield, improve genetic counselling, and support informed reproductive decision-making.

A-0266 CCR6 DYSREGULATION CONTRIBUTE TO MALE INFERTILITY BY DISRUPTING SPERMATOZOON–OOCYTE BINDING

Jin-Chuan Liu [1,3], Chen Yang [1,2], Xiaofeng Liu [1], Siyu Wang [2], Xiaoyu Zhang [2], Yong Liu [2], William Shu-Biu Yeung [1,3], Jean-Pierre Allam [4], Yong-Gang Duan [1]

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Background: To determine whether CCR6 expression on spermatozoa is dysregulated under inflammatory conditions and whether Ccr6 deficiency leads to male infertility.

Methods: The expression level of CCR6 on human spermatozoa was assessed by flow cytometry and immunofluorescent staining. Fertilization and early embryonic development were investigated with in vivo and in vitro experiments using C57BL/6 Ccr6 knockout (KO) mice.

Results: We observed that CCR6 expression was significantly upregulated in spermatozoa from healthy individuals following induction of acrosome reaction (AR). In contrast, in men with chronic inflammation of genital tract, the CCR6 expression showed no significant change post-AR, and the CCR6 levels were uncorrelated with sperm concentration or progressive motility. Using Ccr6 KO mice and in vitro fertilization experiments, we demonstrated that CCR6 deficiency in spermatozoa impaired spermatozoon-oocyte binding, leading to infertility. This effect was specific to spermatozoon-oocyte binding process and not attributable to other events of fertilization.

Conclusion: The results establish CCR6 as a critical mediator of spermatozoon-oocyte interaction, and link CCR6 dysregulation to inflammation-induced male infertility.

HIGHLIGHTED POSTERS 2

Friday, 18 September 2026 - 10:00-10:30

A-0264 LONGITUDINAL CHANGES IN BONE MINERAL DENSITY (BMD) IN PATIENTS WITH KLINEFELTER SYNDROME (KS): A PROSPECTIVE OBSERVATIONAL STUDY IN A REAL-WORLD SETTING

Filippo Vignali [1], Francesco Costantino [1,2], Daniele Renda Livraghi [1], Veronica Maria Denichelis [1], Sara De Vincentis [1], Vincenzo Rochira [1,2]

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INTRODUCTION: Patients with Klinefelter syndrome (KS) are at risk of low bone mineral density (BMD) and fragility fractures. Therefore, guidelines recommend regular DXA assessments at the lumbar spine and femur, along with fracture risk evaluations. While BMD loss is largely attributed to hypogonadism, additional mechanisms independent of serum testosterone may also play a role. This study aimed to evaluate the longitudinal changes in BMD in KS.

METHODS: This prospective, observational, longitudinal, real-world study included KS who underwent at least two evaluations of bone status and pituitary–gonadal axis. Osteoporosis, osteopenia and age-adjusted low BMD were defined by DXA (Hologic) according to WHO and ISCD criteria.

RESULTS: 56 patients with KS were included. At baseline, the mean age was 36.25 ± 14.86 years with mean total testosterone levels of 4.09 ± 2.90 ng/mL. The initial DXA revealed an average BMD of 0.98 ± 0.14 g/cm² at the total femur (TF), 0.88 ± 0.14 g/cm² at the femoral neck (FN) and 0.98 ± 0.12 g/cm² at the

lumbar spine (L1–L4). The prevalence of osteoporosis, osteopenia, and low BMD for age were 3.6%, 10.7%, and 14.3%, respectively. Only two fragility fractures were observed and no patient had ever received osteoactive therapies. Follow-up DXA scans were available after a remarkable interval of 7.68 ± 6.24 years. No significant differences in BMD were detected at FN, TF, or L1–L4 when comparing baseline and follow-up measurements. Regarding testosterone replacement therapy (TRT), 23 KS initiated TRT during follow-up, 16 were already on TRT at baseline and 14 never received TRT. Longitudinal BMD changes were not significantly different among TRT initiators, those already treated at baseline and TRT-naïve. Respectively, the average BMD percentage changes were −3.69%, -2.24% and +3.07% at TF; +1.82%, +5.42% and +3.07% at FN and −5.42%, −3.73% and −1.30% at L1–L4.

CONCLUSIONS: Despite the widespread belief that bone involvement is highly prevalent in Klinefelter syndrome, our longitudinal data indicate a low prevalence of reduced BMD and osteoporosis, along with preserved skeletal stability over time. Importantly, BMD trajectories appear largely independent of testosterone replacement therapy, with neither significant bone loss nor relevant gains at sites typically associated with fragility fractures. These findings challenge the current perception of skeletal fragility in KS and suggest that bone risk may be overestimated. Prospective studies incorporating fracture outcomes are needed to refine bone health assessment and management strategies in this population

A-0286 Persistent Sexual and Psychological Symptoms after Finasteride Discontinuation: A Cross-Sectional Observational Study (under peer review)

Paweł Jędrzejczyk, Tomasz Ząbkowski, Jarosław Ratajski, Kamil Ciechan, Tomasz W. Kaminski, Patryk Uciechowski, Tomasz Syryło

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Introduction:
Persistent sexual and psychological symptoms following finasteride discontinuation have been increasingly reported; however, determinants of symptom persistence remain poorly defined.

Objective(s):
To evaluate associations between finasteride treatment duration, therapeutic indication, and patient age with persistent sexual and psychological symptoms after treatment discontinuation.

Material & Method(s):
A cross-sectional observational study was conducted in 129 adult men with prior finasteride exposure. Sexual function was assessed using the International Index of Erectile Function (IIEF), depressive symptoms with the Patient Health Questionnaire-9 (PHQ-9), and anxiety with the Generalized Anxiety Disorder-7 (GAD-7). Data on treatment duration, cumulative exposure, indication (male pattern hair loss vs. benign prostatic hyperplasia), and demographic characteristics were collected. Logistic regression analyses were performed to identify predictors of persistent symptom severity.

Result(s):
Median treatment duration was 24 months, and median time since discontinuation was 8 months. Erectile dysfunction persisted over time, with no significant improvement in IIEF scores (15.2 at baseline vs. 15.4 at 6 months). Depressive and anxiety symptoms showed partial improvement but remained clinically relevant (PHQ-9: 12.4 to 9.1; GAD-7: 3.29 to 2.54). Longer cumulative finasteride exposure and older age were significantly associated with greater severity and persistence of sexual and psychological symptoms. Therapeutic indication independently influenced symptom profiles. Hormonal parameters, including testosterone levels, were not significantly associated with symptom severity.

Conclusions:
Persistent sexual and psychological symptoms after finasteride discontinuation are associated with treatment duration, cumulative exposure, and patient age. Symptoms may persist despite normalization of hormonal parameters. These findings highlight the importance of careful patient selection and counseling prior to initiating finasteride therapy and underscore the need for prospective longitudinal studies.

A-0111 From Methylation to Metabolism: Epigenetic Regulation of IDH3A and Its Significance in Prostate Cancer — An In Silico Perspective

Rachid Ezzahra [1,2], Adli Othmane [3], Kbirou Adil [4], Aboutaieb Rachid [3,4], Abboussi Oualid [2]

[1] Science & Engineering of Biomedicals, Biophysics and Health Laboratory, Higher Institute of Health Sciences, Hassan First University of Settat, Morocco; [2] Physiology and Physiopathology Team, Faculty of Sciences, Genomic of Human Pathologies Research Centre, Mohammed V University, Rabat, Morocco; [3] Laboratory of Sexual and Reproductive Health, Faculty of Medicine and Pharmacy, Hassan II University, Casablanca, Morocco; [4] Department of Urology, Ibn Rochd University Hospital Center, Casablanca, Morocco

Introduction and Objectives
Metabolic reprogramming and epigenetic dysregulation are hallmarks of prostate cancer (PCa) progression and therapeutic resistance. The mitochondrial enzyme isocitrate dehydrogenase 3 alpha (IDH3A) plays a crucial role in the tricarboxylic acid (TCA) cycle, maintaining redox balance and energy metabolism. Aberrant DNA methylation may silence IDH3A expression, contributing to metabolic shifts that favor tumor survival and proliferation. This study aimed to investigate the methylation status and expression profile of IDH3A in prostate cancer and to explore its association with clinicopathological features and patient outcomes.

Methods
An in-silico analysis was performed using the Shiny Methylation Analysis Resource Tool (SMART) and The Cancer Genome Atlas (TCGA-PRAD) dataset. IDH3A promoter methylation levels and mRNA expression were compared between prostate tumor and normal tissue samples. Correlations were evaluated between IDH3A expression, methylation status, and clinical parameters such as Gleason score, disease stage, and biochemical recurrence. Survival analyses were conducted to assess prognostic relevance.
Results
Preliminary analyses revealed significant promoter hypermethylation of IDH3A in prostate tumor tissues compared to normal controls. This epigenetic alteration was accompanied by marked downregulation of IDH3A expression. Higher methylation levels were associated with advanced disease stage, elevated Gleason grade, and poorer survival outcomes. These findings suggest that IDH3A silencing may impair mitochondrial oxidative metabolism, promoting the metabolic reprogramming typical of aggressive and treatment-resistant PCa.

Conclusions
Our data identify IDH3A as a potential epigenetically regulated biomarker in prostate cancer. Promoter hypermethylation–mediated silencing of IDH3A appears to contribute to metabolic adaptation and disease aggressiveness. These results warrant further validation in experimental and clinical settings to assess the therapeutic potential of targeting IDH3A-related metabolic pathways

A-0150 Artificial intelligence–based semen analysis versus expert manual spermogram: a comparative study

Iurii Arian, Andrei Chiriac, Victoria Nofit, Anastasia Ambros, Victoria Ghenciu, Ion Dumbraveanu

Lab of Urology, Andrology and Reproductive Medicine, Nicolae Testemitanu State University of Medicine and Pharmacy, Chisinau, Republic of Moldova

The increasing use of artificial intelligence in laboratory diagnostics has created the need to validate automated semen analysis systems against established manual reference methods. Ensuring concordance between automated systems and expert evaluation is essential for maintaining the clinical reliability of semen analysis.

This study aimed to compare semen parameters obtained by an automated artificial intelligence–based analyzer with those obtained by expert manual spermogram performed by a specialist with more than 30 years of experience in andrology. A total of 31 semen samples from patients with visually normal semen parameters on initial microscopic screening were included. Each sample was analyzed simultaneously by both methods under identical laboratory conditions. The mean analysis time was approximately 8 minutes for the automated method and 60 minutes for the manual assessment.

Automated semen analysis was performed using the LensHooke 12PRO system (Bonraybio, Taiwan), an artificial intelligence–based computer-assisted semen analysis platform that combines microfluidic technology, optical imaging, and deep learning algorithms to automatically quantify sperm concentration, motility parameters, and morphology from standardized disposable chambers.

Manual semen analysis was performed according to WHO criteria (6th edition). Sperm concentration was determined using standard dilutions and hemocytometers with improved Neubauer ruling under phase contrast microscopy. Motility assessment was performed at 37°C within 30–60 minutes after ejaculation using phase contrast microscopy, with duplicate assessments evaluating at least 200 spermatozoa. Morphology was evaluated using Tygerberg strict criteria after Papanicolaou staining, with at least 200 spermatozoa examined under oil immersion microscopy (1000×). Statistical analysis included Pearson and Kendall correlation coefficients, with statistical significance set at $p<0.05$.

Mean sperm concentration measured by the automated system was 55.0 ± 40.2 million/ml compared with 66.7 ± 42.3 million/ml by manual evaluation, demonstrating strong correlation between methods (Pearson $r=0.92$, $p<0.001$). Total motility showed moderate agreement, with mean values of 50.7 ± 18.7% for automated analysis and 55.4 ± 17.4% for manual assessment ($r=0.69$, $p<0.001$). Rapid progressive motility averaged 16.6 ± 11.2% using automated analysis and 22.3 ± 13.1% manually ($r=0.57$, $p=0.002$). Morphology showed lower concordance, with normal forms averaging 7.2 ± 4.5% in automated analysis and 3.8 ± 2.6% in manual assessment ($r=0.41$, $p=0.028$).

Automated semen analysis demonstrated strong agreement with expert manual evaluation for sperm concentration and moderate agreement for motility parameters, while morphology showed lower concordance. Automated analysis significantly reduced examination time while maintaining clinically relevant correlation with manual reference methods. These findings highlight the potential role of artificial intelligence–based systems in improving standardization and reducing operator-dependent variability in routine semen analysis.

A-0085 Clinical Hormonal Outcomes of Clomiphene Citrate in Mexican Men With Functional Hypogonadism and Infertility: A Three-Month Paired Analysis

Andrés Mora Topete [1], Moises Abraham Adel Domínguez [2]

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Clomiphene citrate has emerged as a therapeutic option for men with functional hypogonadism who wish to avoid exogenous testosterone exposure. However, data on short-term hormonal response in Mexican patients remain limited. The aim of this study was to assess hormonal changes associated with clomiphene citrate treatment in Mexican men with functional hypogonadism and infertility, and to determine whether these changes remained significant after adjustment for age and body mass index (BMI).
A retrospective paired analysis was conducted in 133 Mexican men who underwent treatment with clomiphene citrate for three months. Hormonal assessment included total testosterone, free testosterone, and estradiol at baseline and follow-up. Changes in the proportion of patients achieving normal-range hormonal values after treatment were evaluated using McNemar's test. An ANCOVA model was used to assess whether the main hormonal outcome remained significant after adjustment for age and BMI.

At baseline, a substantial proportion of patients had subnormal hormonal values. After three months of treatment, the proportion of patients with free testosterone within the reference range increased from 45.6% to 79.7% ($p=0.00015$). Significant improvement was also observed in patients with initially low total testosterone and estradiol levels, with post-treatment normalization rates showing statistical significance ($p=0.0087$ and $p=7.3\times10^{-7}$, respectively). In adjusted analyses, the improvement in free testosterone remained significant after controlling for age and BMI. Age showed only a modest negative association with response, whereas BMI did not materially alter the main treatment effect.

These findings support the short-term hormonal effectiveness of clomiphene citrate in Mexican men with functional hypogonadism and infertility. The observed improvement in key hormonal parameters, particularly free testosterone, and the persistence of this effect after adjustment for age and BMI suggest that clomiphene citrate may represent a useful fertility-conscious therapeutic option in appropriately selected patients. Further studies with longer follow-up and broader clinical outcomes are warranted to define its long-term role in andrological practice.

A-0248 Oxidative stress and DNA integrity alterations induced by methotrexate in human spermatozoa and the protective effect of metformin: an in vitro experimental study

Amira Sallem [1,2], Amira Dhaoui [1,2], Manel Boussabbeh [1], Faouez Boujelben [1], Narjes Trabelsi [1], Ranin Selmi [1,2], Elhem Ghodhbane [1], Meriem Mehdi [1]

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Methotrexate (MTX) is widely used in the treatment of autoimmune diseases and certain malignancies. However, its potential toxicity on male reproductive function remains a concern. Oxidative stress is considered a key mechanism underlying MTX-induced cellular alterations. This study aimed to evaluate the impact of MTX on oxidative stress and DNA integrity in human spermatozoa, as well as the potential modulatory effect of metformin.

An in vitro experimental study was conducted on semen samples obtained from a cohort of men with normal semen parameters. Semen samples were obtained by masturbation in the department of reproductive biology (Monastir, Tunisia) and were included in the current study when patients gave informed consent so that their samples can be used in research purpose after spermogram analysis. Each semen sample was divided into four aliquots: control, MTX (0.5 µM), metformin (80 µM), and MTX + metformin. After 4 hours of incubation at 37°C under 5% CO_2, oxidative stress was assessed using the DCFH-DA assay. DNA integrity was evaluated using acridine orange staining and the comet assay.

A total of 140 provided from 35 men were assessed during the current study. MTX exposure was associated with increased ROS levels (fluorescence units, FU) , with values of 9506 in the MTX group compared to 5331 in the control group (p), while intermediate values were observed in the MTX + metformin group (5934).

In addition, DNA denaturation was higher in the MTX group (33.9%) compared to the control group (15.4%) (p), with intermediate levels observed in the MTX + metformin group (25.3%) ($p<0.001$).

Similarly, DNA fragmentation was increased in the MTX group (38.5%) compared to controls (12%), whereas the MTX + metformin group showed a value of 16.8% ($p<0.001$).

Methotrexate is associated with increased oxidative stress and alterations in sperm DNA integrity. Metformin is associated with reduced oxidative and genetic damage, suggesting a potential protective effect on human spermatozoa. These findings could help in dealing with sperm quality impairment in patients under a methotrexate-based treatment through the association with metformin.

A-0050 A spatiotemporal multimodal atlas of human genital tubercle reveals fine cellular organization and cell-type-specific sex-biased genes associated with disorders of sex development

Antonio Suglia [1], Bertrand Evrard [1], Laurianne Lesné [1], Maryne Toupin [1], Aurélie Lardenois [1], Paul Rivaud [1], Alain Chédotal [2,3,4], Terje Svingen [5], Antoine Dominique Rolland [1], Séverine Mazaud-Guittot [1], Frédéric Chalmel [1]

[1] Irset (Institut de recherche en santé, environnement et travail), Rennes, France; [2] Institut de la Vision, Paris, France; [3] Institut de pathologie groupe hospitalier Est, hospices civils de Lyon, France; [4] University Claude Bernard, Lyon, France; [5] Technical University of Denmark, Lyngby, Denmark

Malformations of the external genitalia are among the most common congenital anomalies worldwide. Hypospadias alone affects approximately 1 in 250 male newborns. Although several genetic factors have been identified, a large proportion of disorders of sex development (DSD) remain unexplained, and increasing evidence suggests that fetal environmental exposures may contribute to their etiology. The penis and clitoris arise from the genital tubercle (GT), an embryonic structure that undergoes androgen-dependent sexual differentiation during the first trimester. However, the cellular and molecular programs governing external genitalia development remain poorly defined.

Here, we present a comprehensive multimodal spatiotemporal atlas of the human GT spanning 5-12 weeks post-conception (PCW), including both XY and XX samples encompassing the key developmental stage of early differentiation (9-12 PCW). By integrating multiomic (RNA-seq and ATAC-seq on matched nuclei) with spatial transcriptomics data, we identified and reconstructed the spatial localization of the major mesenchymal and epithelial cell populations within the GT as well as their sex-related transcriptional program.
Among the major findings, we confirmed in humans the existence of several cell populations that had previously been observed only in rodents. In addition, we identified a previously undescribed cell population, characterized by a distinct localization and transcriptional signature, which may play a role in distal urethral patterning and closure.

Following the onset of sexual differentiation, we identified more than 1,600 sex-biased genes across GT cell populations. Importantly, 55 of these differentially expressed genes are known or candidate DSD-associated genes, including genes that are marker of cell populations, highlighting the specific cellular contexts in which pathogenic mechanisms may arise. Sex-specific transcriptional programs were particularly enriched in peri-urethral and ventral mesenchyme as well as urethral and stratified epithelial regions potentially involved in urethral tubularization. Functional analyses further revealed strong associations between sex-biased gene signatures and phenotypes linked to genital anomalies, including displacement of the urethral meatus, abnormal penis morphology, and micropenis, conditions frequently observed in hypospadias and related DSD.

We also included in the atlas a GT sample exposed in utero to multiple recreational drugs, revealing altered transcriptional signatures characterized by activation of stress-response pathways and suppression of developmental programs in mesenchymal populations critical for genital morphogenesis.

In conclusion, this atlas reveals the detailed cellular composition and spatial organization of the developing GT, providing cellular and mechanistic insights into the molecular basis of DSD. It establishes a valuable resource for dissecting the genetic and environmental factors that shape external genital differentiation and may help improve the understanding of unknown DSD etiologies.

POSTER SHIFT 1 (UNTIL THU 10:00)

A-0001 COMPARISON OF DEMOGRAPHIC AND CONTRACEPTIVE PATTERNS IN MEN UNDERGOING VASECTOMY BEFORE AND AFTER THE COVID-19 PANDEMIC IN BARCELONA, SPAIN

Massimiliano Raffo, Josvany Rene Sanchez Curbelo, Maurizio De Rocco Ponce, Luis Malca Caballero, Jose Antonio Arrus Soldi, Ricardo Contreras Garcia, Sebastian Dumas, Gabi Adalid, Eduard Ruiz-Castane, Doron Vantman Luft

Fundacio Puigvert, Barcelona, Spain

Introduction and Aims
Vasectomy is the only permanent method of contraception available for men. The aim of this study was to compare whether there is a difference in the demographic characteristics of patient undergoing a vasectomy between the pre- and post-COVID-19 pandemic periods.

Materials and Methods
This retrospective study evaluated 1,774 sexually active men undergoing vasectomy between 2016 and 2024 at a single tertiary-referral center. Baseline data, including age, number of children, and contraceptive methods were collected. Patients were divided into two groups. Group 1 represented men undergoing vasectomy pre-COVID-19 pandemic period between 2016 and 2020; and group 2 represented patients undergoing surgery post-COVID-19 pandemic period, between 2021 and 2024. Statistical analysis utilized nonparametric tests and multivariate logistic regression to identify potential associations between the two groups ($p < 0.05$).

Results
Group 1 included 1202 patients with a median age of 42 years old (IQR 18-65) and a median number of children of 2 (IQR 0-7). 336 (28%) men did not use any contraceptive method, 610 (51%) used condoms, 144 (12%) oral contraceptives, 99 (8%) intrauterine devices and 13 (1%) injectable contraceptives. Group

2 included 572 patients with a median age of 42 years (IQR 18-67) and a median number of children of 2 (IQR 0-5). 159 men (27.8%) did not use any contraceptive method, 274 (47.9%) used condoms, 82 (14.4%) oral contraceptives, 51 (8.9%) intrauterine devices, and 6 (1%) injectable contraceptives. At the multivariable analysis, no statistically significant differences were found in terms of age, number of children, and contraceptive methods when comparing the two groups of patients ($p>0.05$).

Conclusions
The COVID-19 pandemic did not significantly influence the demographic profile or contraceptive patterns of men undergoing vasectomy at a tertiary-referral center in Barcelona, Spain.

A-0002 Assessing vasectomy outcomes and follow-up patterns in relation to contraceptive use: a retrospective analysis

Massimiliano Raffo, Josvany Rene Sanchez Curbelo, Maurizio De Rocco Ponce, Luis Malca Caballero, Jose Antonio Arrus Soldi, Ricardo Contreras Garcia, Sebastian Dumas, Gabi Adalid, Eduard Ruiz-Castane, Doron Vantman Luft

Fundacio Puigvert, Barcelona, Spain

Introduction and Aims

Vasectomy is the only permanent method of contraception available for men. The aim of this study was to assess the success rate of the vasectomy procedure, the compliance of patients to the follow up and which contraceptive method could be a predictor of patient compliance to post-vasectomy semen analysis (PVSA).
Materials and Methods

1,774 men undergoing vasectomy between 2016 and 2024 at a single tertiary-referral center in Barcelona, Spain, were retrospectively evaluated. Data including the PVSA three months after the procedure and contraceptive methods were collected. Descriptive statistics was used to determine the cohort. A chi-square test of independence was applied to assess the association between contraceptive methods and azoospermia status at PVSA (significance: $p < 0.05$).

Results
1,516 men (86%) achieved azoospermia at the first PVSA. 80 (4%) still had spermatozoa present, and 178 (10%) did not show up for the PVSA. A new PVSA was scheduled for all patients who did not achieve azoospermia or were absent at follow-up (n 258, 14%). 93 (5%) achieved azoospermia, 27 (1%) still had spermatozoa, and 138 (8%) did not show up for second PVSA. 495 (27.9%) men did not use any contraceptive method, 880 (50%) used condoms, 226 (12.7%) oral contraceptives, 150 (8.4%) intrauterine devices and 19 (1%) injectable contraceptives. By analyzing the association between contraceptive method and follow-up PVSA, the chi-square test of independence yielded a χ^2 value of 23.2 ($p=0.0031$), indicating a statistically significant association between men that did not use a contraceptive method and not attending to the PVSA follow up.

Conclusions
Succes rate in PVSA was 99%. 8% of patients did not do follow up (2 scheduled PVSA). The absence of contraceptive method use appears to be associated with reduced adherence to recommended PVSA.

A-0005 Corporoplasty for Peyronie's disease and congenital penile curvature: real world effectiveness, safety, and patient reported outcomes at a single tertiary-referral center

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Introduction and aims
Peyronie's disease (PD) and congenital penile curvature (CPC) significantly impacts erectile function, sexual quality of life, and psychological well-being of men. Surgical intervention continues to represent the definitive therapeutic approach in stable cases presenting with clinically meaningful anatomical deformity.

This study aims to evaluate the real-world effectiveness, safety, and patient-reported outcomes of various corporoplasty techniques at a tertiary referral center.

Materials and methods
Data from 324 sexually active men surgically treated for penile curvature between 2020 and 2025 at Fundació Puigvert in Barcelona were retrospectively analyzed. Preoperative data, surgical techniques, and clinical characteristics were collected. Patient-reported outcomes were assessed by telephone 6 to 48 months postoperatively using the Global Assessment Questionnaire (GAQ), and supplementary satisfaction questions (SQ). Descriptive statistics was applied.

Results
Of 324, 274 (84.6%) patients presented with PD and 50 (15.4%) with CPC, respectively. The median age of the entire population was 51 (IQR 18–72) years. Surgical techniques were 16-dot plication performed in 159 patients (49%), Yachia in 81 (25%), modified Nesbit in 65 (20%) and lengthening procedures with Surgisis patch in 19 (6%), respectively. According to the GAQ, 211 patients (65%) reported an improvement in sexual quality of life following surgery, whereas 55 (17%) experienced no change and 58 (18%) reported a deterioration. Based on GAQ-2 responses, 159 individuals (49%) noted enhanced ability to achieve/maintain erections, 100 (31%) reported no change, and 62 (19%) experienced worsening. Regarding the SQ, 249 (77%) patients expressed satisfaction with the surgical outcomes, 259 (80%) considered the penile curvature to be adequately corrected, and 256 (79%) indicated they would recommend the procedure to others. Overall, complications occurred in 67 (20.7%) patients. Complication rates were highest following the Yachia procedure, with 23 patients (28.4%) affected; the most frequent adverse events included edema in 7 cases (30.4%) and hematoma in 6 (26%). Among those who underwent 16-dot plication, 28 patients (17.6%) experienced complications, most commonly edema (7 cases, 25%), infection (7 cases, 25%), and hematoma (6 cases, 21.4%). After modified Nesbit surgery, 12 patients (18.5%) reported adverse outcomes, including edema in 6 cases (50%) and decreased penile sensitivity in 4 (33.3%). Regarding grafting procedures, complications were observed in 4 patients (21%) treated with Surgisis patch, with decreased sensitivity and hematoma each occurring in 2 cases (10.5%).

Conclusions
Corporoplasty for PD and CPC demonstrated robust real-world effectiveness with acceptable postoperative morbidity.

A-0009 Partnership status and contraceptive method distributions in vasectomy patients with and without children: a single-center analysis

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Introduction and aims
Men without children represent a distinct subset of vasectomy candidates, yet their demographic characteristics and contraceptive behaviors compared to patients with children remain poorly characterized. This study aimed to compare demographic and behavioral characteristics between vasectomy candidates with no children and those with ≥1 child, focusing on age, partnership status, and contraceptive method distributions.

Materials and methods
This retrospective study analyzed 1,732 sexually active men who underwent vasectomy between 2016 and 2024 at a single tertiary center in Barcelona, Spain. Baseline data—age, number of children, and contraceptive methods—were collected. Patients were grouped by child status: group 1 (no children), group 2 (≥ children). Descriptive statistics summarized the cohort; two-proportion z-tests and chi-square were used to compare partnership prevalence and contraceptive method distributions between groups.

Results
Group 1 included 117 men (6.7%) with a median age of 41 years (IQR 37–45). Among them, 38 (32.4%) were not in a relationship, while 79 (67.6%) were in a relationship. Contraceptive methods were distributed as follows: 52 men (44.4%) didn’t use contraceptive methods, 65 (55.6%) used contraceptive methods. Of them, 45 (69.2%) condoms, 13 (20%) oral anticontraceptives, 6 (9.2%) intrauterine disposables, and 1

(1.6%) injectable anticontraceptives. Group 2 included 1615 men (93.3%) with a median age of 42 years (IQR 36-46). Of them, 194 (12%) were not in a relationship, while 1421 (88%) were in a relationship. Of Group 2, 416 men (25.8%) did not use contraceptive methods while, 1199 (74.2%) used contraceptive methods. Of them, 823 (68.6%) used condoms, 209 (17.4%) oral anticontraceptives, 144 (12%) intrauterine disposables, and 18 (2%) injectable anticontraceptives. No differences in terms of age were found between groups (p=0.58).

Partnership prevalence was significantly lower in Group 1 than in Group 2 (64.96%, 76/113 vs 87.99%, 1421/1610; $p < 0.001$); contraceptive method distributions also differed between groups (chi-square $p < 0.001$), and sensitivity analysis showed greater use of no contraceptive method in Group 1 compared with Group 2 ($p < 0.001$). Among patients without a partner and using or not contraceptive methods, the distribution of having children versus contraceptive method shows 18 (9.3%) patients without children using no contraceptive method and 8 (4.1%) using condoms. While among those with children, 49 (25.3%) did not use any method and 119 (61.3%) used condoms. The chi-square test shows a statistically significant difference in distribution ($p = 0.00016$), indicating contraceptive method choice is significantly associated with having children in this subgroup.

Conclusion
Vasectomy patients with 0 children have lower prevalence of being in a relationship compared to men with ≥ child. Group 1 demonstrated a distinct contraceptive method pattern compared with Group 2. These differences support tailored pre procedural counseling based on partnership status and contraceptive behaviors.

A-0010 Permanent contraception without regret: outcomes of vasectomy in childless men from a tertiary referral center

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Introduction and aims
Vasectomy is a highly effective, minimally invasive, and permanent contraceptive method for men. This study aimed to describe the demographics, semen analysis outcomes, regret rate and the adherence to follow up of a cohort of men with no children undergoing vasectomy.

Materials and methods
This retrospective study evaluated 117 sexually active men undergoing vasectomy between 2016 and 2024 at a single tertiary-referral center in Barcelona, Spain. Baseline data, including age, BMI, relationship status, contraceptive methods used, follow up semen analysis, complications, re-vasectomy, testicular sperm extraction (TESE), regret rate and post-surgery pain were collected. Descriptive statistics was used to analyze the entire cohort.

Results
The median age was 41 years (IQR 37-45) with a median BMI of 26 (IQR 20-32). Among them, 38 (32.4%) were not in a relationship, while 79 (67.6%) were in a relationship. Contraceptive methods were distributed as follows: 52 men (44.4%) didn't use contraceptive methods. 65 (55.6%) used contraceptive methods. Of them, 45 (69.2%) condom, 13 (20%) oral anticontraceptives, 6 (9.2%) intrauterine disposables, and 1 (1.6%) injectable anticontraceptive. In the first semen analysis, 102 patients (87.1%) were azoospermic, 2 (1.7%) had still spermatozoa and 13 (11.2%) did not show at the follow up. At the second assessment of the 15 patients, the 2 patients (13.3%) with spermatozoa at the previous control, achieved azoospermia and 13 (86.7%) again did not show at the analysis. Moreover, 0 patients reported regret with the procedure; no complications, re-vasectomies or TESEs were observed and no chronic pain was reported at follow-up.

Conclusion
Vasectomy can be considered a safe and effective procedure, with early azoospermia observed in most patients and no recorded early or late complications. 11% of these patients did not show at the post-vasectomy semen analysis. In this cohort of childless men who received structured preoperative counselling, none expressed post procedure regret, underscoring the value of thorough decision support in

electing permanent contraception, and should be considered an option in men that don't want to have children.

A-0011 Patient Satisfaction After Penile Prosthesis Implantation in Octogenarians: A Retrospective Comparative Study

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Introduction:
Erectile dysfunction (ED) is highly prevalent in aging men and increases in both frequency and severity with advancing age. In older populations, ED is commonly refractory to first- and second-line therapies, including phosphodiesterase type 5 inhibitors and intracavernosal injections, making penile prosthesis implantation (PPI) the definitive treatment for restoring penetrative sexual function and improving quality of life.

Although PPI has demonstrated high long-term reliability, functional outcomes, and patient satisfaction in the general population, the procedure remains underutilized in very elderly men. This reluctance is largely driven by concerns regarding perioperative risk, postoperative complications, and perceived limited benefit in advanced age, rather than evidence-based contraindications.

Objective:
To evaluate patient satisfaction following penile prosthesis implantation (PPI) in men aged 80 years and older, and to compare outcomes with those of patients aged 75–79 years.

Materials and Methods:
Retrospective observational study conducted including men aged ≥75 years who underwent PPI for medically refractory erectile dysfunction between January 2015 and September 2025. Patients were stratified into two age groups: ≥80 years and 75–79 years. The primary outcome was patient satisfaction, assessed using a 5-point ordinal scale during postoperative follow-up. Secondary outcomes included functional device use and postoperative complications. Satisfaction scores were compared using the Mann–Whitney U test.

Results
Total of 36 patients included, of whom 13 were aged ≥80 years and 23 were aged 75–79 years. Mean patient satisfaction scores were 4.54 ± 1.13 in the ≥80-year group and 4.78 ± 0.52 in the 75–79-year group, with median scores of 5.0 in both groups. Satisfaction score of 5 was reported by 76.9% of octogenarians and 82.6% of patients aged 75–79 years. There was no statistically significant difference in satisfaction between groups ($p = 0.67$). Full functional device use was reported by 84.6% of patients aged ≥80 years and 95.7% of those aged 75–79 years. Overall complication rates were comparable between groups.

Conclusions:
PPI in men aged 80 years and older is associated with high patient satisfaction, preserved functional outcomes, and acceptable complications. Satisfaction in octogenarians are comparable to those in slightly younger elderly patients, suggesting that advanced chronological age alone should not be considered a barrier to meaningful surgical benefit.

A-0014 Obesity alters sperm function and testicular extracellular vesicle localisation, with frozen tissue limiting vesicle extraction

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Obesity is a major contributor to male infertility, adversely affecting spermatogenesis and sperm function. Extracellular vesicles (EVs) are key mediators for intercellular communication, carrying bioactive molecules such as proteins, lipids and nucleic acids. Increasing attention has been given to EV-mediated communication within the testes, particularly in relation to testosterone production and spermatogenesis. Despite this, a significant knowledge gap remains regarding the effects of obesity on EV production, localisation and communication. Therefore, this study aimed to investigate the effects of obesity on sperm

function, testicular EV localisation, and to assess the feasibility of EV extraction from frozen testis tissue. This study utilised a genetic mouse model of obesity (ob/ob) against C57BL/6 control mice to examine sperm function, testicular EV localisation, and extraction. Male mice were assigned to control (n=20) or obese (n=15) groups and were euthanised at 16 weeks of age via isoflurane gas. Epididymides were harvested to extract sperm. Sperm motility (total and progressive motility, and immotility) kinematic parameters (straight line velocity (VSL), curvilinear velocity (VCL) and amplitude of lateral head displacement (ALH)), as well as morphology were assessed via Computer-Aided Sperm Analysis (CASA). Additionally, testes were harvested and processed for immunohistochemistry (IHC) and immunofluorescence (IF) analyses, targeting common EV biomarkers (CD63, CD9 and ANXA1) to assess obesity's impact on their localisation. The remaining tissues were frozen for EV extraction whereby testes were dissociated, digested and underwent differential centrifugation. Thereafter, subsequent western blotting (WB) was performed targeting CD63, CD9 and ANXA1 to confirm EV isolation. Obese mice presented with significantly reduced total ($P<0.0001$) and progressive ($P=0.009$) motility, with increased immotile spermatozoa ($P=0.0004$) when compared to controls. Kinematic measurements revealed decreased VSL ($P=0.0064$), VCL ($P<0.0001$) and ALH ($P<0.0001$). Sperm morphological investigations indicated that obese mice had significantly increased abnormal sperm cells ($P<0.0001$), with increased head ($P<0.0001$) and midpiece ($P=0.0089$) defects when compared to controls. IHC staining of seminiferous tubules showed visible differences in EV localisation between control and obese testes for CD63, CD9 and ANXA1, with IF staining indicating a significant decrease in CD63 intensity in obese testes ($P=0.0345$). WB analysis of extracted EVs (CD63, CD9 and ANXA1) from frozen testes were unquantifiable, indicating technical limitations from frozen tissue. Results from this study showed obesity-induced alterations in sperm parameters. Additionally, assessments of EV localisation using CD63, CD9 and ANXA1 revealed marked differences between control and obese testes, representing a novel observation within seminiferous tubules under obese conditions. This may suggest altered EV production and release under an obese context, potentially affecting communication necessary for spermatogenesis and sperm function. In contrast, EV extraction from frozen testicular tissue provided technical challenges, with weak or absent EV marker detection via WB. This emphasises the need for further investigations to overcome these limitations. Together, these findings highlight gaps in understanding the effects of obesity on testicular EV biology and extraction from frozen tissue.

A-0016 Serum 17-Hydroxyprogesterone and Intratesticular Testosterone as Predictors of Micro-TESE Outcome in Azoospermic Men

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Background:Azoospermia represents one of the most challenging conditions in andrology practice. Microdissection testicular sperm extraction (micro-TESE) remains the cornerstone for sperm retrieval in non-obstructive azoospermia; however, reliable preoperative predictors of success are still lacking.

AIM: To evaluate the predictive value of serum 17-hydroxyprogesterone (17-OHP) and intratesticular testosterone for micro-TESE outcome.

Methods: This cross-sectional analytic study included 89 azoospermic men undergoing micro-TESE. Preoperative assessment included serum FSH, total testosterone, and 17-OHP. Intratesticular testosterone was measured from testicular tissue obtained during micro-TESE. TESE outcome was classified as positive or negative sperm retrieval. Correlation analysis, group comparisons, and ROC curve analysis were performed.

Results: Serum 17-OHP showed a strong positive correlation with intratesticular testosterone ($r = 0.928$, $p < 0.001$) and a moderate positive correlation with serum testosterone ($r = 0.432$, $p < 0.001$), while demonstrating a negative correlation with FSH ($r = -0.239$, $p = 0.026$). However, no statistically significant differences were found between positive and negative TESE outcomes regarding serum 17-OHP, serum testosterone, intratesticular testosterone, or FSH. ROC curve analysis revealed poor predictive performance for all hormonal parameters.

Conclusion: Although serum 17-OHP correlates strongly with intratesticular testosterone, it is not a reliable predictive factor for micro-TESE outcome.

A-0017 Age-specific Prevalence of Male Infertility in Germany: A Comparative Analysis of Infertility criteria based on Self-Reports and Health Insurance Claims Data

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Background: Male infertility affects up to 50% of infertile couples globally, being the sole factor in 20-30% of cases. Beyond reproductive impact, infertile men demonstrate higher morbidity and mortality compared to the fertile population. Accurate prevalence assessment is therefore critical for healthcare planning. However, population-level prevalence is difficult to assess, as semen analyses are typically unavailable for broader samples. Prevalence estimates rely on self-reports or physician diagnoses via ICD-10 codes in health insurance data. Both methods face substantial limitations from data quality variations, underreporting, and evolving clinical definitions, resulting in widely varying prevalence estimates (2-12.5%) globally, with a global lifetime prevalence of 17.5% in WHO assessments.
Methods: We conducted a cross-sectional analysis of baseline data from the German National Cohort (NAKO), Germany's largest population-based cohort study, with baseline visits conducted between 2014 and 2019. We analyzed 39,694 male participants aged 20-75 years with available health insurance claims data. A subset of 946 participants (2.4%) also completed infertility-specific questionnaires. Health insurance diagnostic codes (ICD-10 N46.X) were available for the period 2010-2022, providing comprehensive coverage of male infertility diagnoses across multiple years. Main variables included ICD-10 code N46.X from insurance data and self-reported infertility (attempting conception >12 months) from questionnaires. Covariates included age, marital status, number of children, desire for paternity, and reported causes of infertility. We calculated and compared age-specific prevalences of both proxies. Primary and secondary infertility were defined using restrictive criteria combining N46.X codes with reproductive history.

Results: Overall prevalence of N46.X codes was 2.1% (n=824), peaking at 5.0% in ages 30-39 years, then declining. Among men with N46.X codes, 70.5% were married or in relationships, 42.0% had no children, and 20.9% currently desired paternity. Secondary infertility (1.1%) was more prevalent than primary infertility (0.6%). Self-reported infertility prevalence was substantially higher at 8.5% (n=80), with highest rates in ages 40-49 years (13.2%), yet only 11.2% had corresponding N46.X codes in insurance records.

Among self-reported infertile men, similar patterns emerged: 73.8% were married or in relationships and 23.8% had no children, while only 7.5% currently desired paternity, indicating predominantly retrospectively reported infertility in older age groups. Furthermore, 71.3% sought medical assistance, 28.8% attributed infertility to poor sperm quality. Discrepancies were particularly pronounced by age: in infertility-specific questionnaires, N46.X codes concentrated in younger ages (37.5% at 30-39 years) despite absence of reported semen quality problems, while older men reporting poor sperm quality (concentrated at ages 40-49 and 50-59 years) showed zero N46.X coding from age 50 onwards.

Conclusion: Substantial underreporting in diagnostic coding (89% of self-reported cases lack N46.X codes) likely represents a widespread European and global challenge, underestimating male infertility burden and comorbidity risks. Findings highlight urgent need for improved coding practices, standardized international definitions, comprehensive destigmatization across clinical, societal, and policy levels, and multi-source data integration.

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A-0018 mTESE outcome in a patient with Klinefelter syndrome and chromosome Y microdeletion

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Herein, we present the outcome of microdissection testicular sperm extraction (mTESE) in a 31-year-old man with primary infertility associated with Klinefelter syndrome (47,XXY) and chromosome Y microdeletion. The patient was somatically healthy, with normal pubertal development, no significant comorbidities, and no relevant family history. The couple was non-consanguineous. Physical examination revealed normal secondary sexual characteristics, height of 182 cm, and weight of 78 kg. Azoospermia was confirmed on two semen analyses. Hormonal evaluation showed elevated FSH (13 IU/L), LH (7.3 IU/L), testosterone at the lower limit of normal (12.1 nmol/L), and reduced inhibin B (31), consistent with primary spermatogenic failure. Testicular ultrasound demonstrated bilaterally small testes.

Genetic evaluation confirmed Klinefelter syndrome and a partial AZFc microdeletion of the Y chromosome (GR/GR subtype). Y-chromosome analysis showed the presence of marker sY1191 and the absence of sY1291, consistent with GR/GR deletion. Although this partial deletion is not invariably associated with infertility, it increases the risk of impaired spermatogenesis. The AZFc region contains genes essential for spermatogenesis, including the DAZ gene family; GR/GR deletion reduces their copy number, compromising germ cell development. Clinical manifestations range from oligozoospermia to azoospermia, as observed in this patient. Klinefelter syndrome (typically 47,XXY) is characterised by hypergonadotropic hypogonadism and progressive degeneration of seminiferous tubules. The coexistence of chromosomal aneuploidy and AZFc microdeletion represents a dual genetic risk factor for severe spermatogenic impairment. Mosaicism, often present in Klinefelter syndrome, may allow small populations of 46,XY spermatogenic cells to persist, enabling possible sperm retrieval via mTESE.

Histological examination of bilateral testicular biopsies revealed predominantly “Sertoli cell only” syndrome, with seminiferous tubules lined exclusively by Sertoli cells and absence of spermatogenic cells, typical of severe spermatogenic failure. A minority of tubules showed hypospermatogenesis, including immature spermatogenic cells and rare mature spermatids. Isolated tubules containing mature spermatids and spermatozoa confirmed focal preservation of spermatogenesis, characteristic of mixed atrophy, supporting the indication for mTESE. The interstitium showed normal-to-hypertrophic Leydig cells, focal vascular intimal thickening, and hyalinization, consistent with chronic degenerative changes. PLAP and OCT ¾ immunostaining was negative, excluding germ cell neoplasia in situ (GCNIS). Overall, histology indicated markedly reduced but not completely absent spermatogenic activity.

In conclusion, this patient’s primary infertility resulted from combined genetic abnormalities: Klinefelter syndrome and partial AZFc (GR/GR) deletion. Despite severe impairment, focal spermatogenesis provided an opportunity for sperm retrieval by mTESE, followed by intracytoplasmic sperm injection (ICSI). Comprehensive genetic counselling is essential due to the risk of transmitting Y-chromosome microdeletions to male offspring.

A-0019 SPINAL CORD INJURY: DOES IT AFFECT THE TESTIS FUNCTION AND SPERM RETRIEVAL?

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Spinal cord injury (SCI) in men is frequently associated with profound reproductive dysfunction and represents a major cause of infertility in this population. Between 85% and 97% of affected men experience permanent loss of ejaculatory capacity, primarily due to disruption of the complex neural pathways that coordinate emission and ejaculation. While assisted techniques such as penile vibratory stimulation and rectal probe electroejaculation enable semen retrieval in many cases, the underlying impact of SCI on intrinsic testicular function and spermatogenesis is less commonly emphasised and warrants detailed consideration.

Although the testes are considered immuno-privileged organs with a relatively protected microenvironment, evidence suggests that spinal cord injury can significantly impair spermatogenic activity. In our cohort of 490 patients treated surgically for azoospermia, eight men were identified with prior spinal injury, predominantly involving thoracic segments (Th5–Th12) and one cervical lesion (C6).

Despite normal or only slightly reduced testicular volumes, endocrine evaluation revealed a pattern suggestive of primary testicular dysfunction: follicle-stimulating hormone (FSH) and luteinizing hormone (LH) levels were elevated in most cases, whereas total testosterone concentrations remained within normal limits. This hormonal profile indicates partial failure of the seminiferous tubules, with preserved Leydig cell steroidogenic function.

Genetic etiologies were excluded, as none of the patients demonstrated abnormal karyotypes or Y-chromosome microdeletions, further supporting the concept that spinal cord injury itself may contribute directly or indirectly to impaired spermatogenesis. Histopathological examination of testicular tissue obtained via testicular sperm extraction (cTESE) revealed heterogeneous but consistently abnormal findings. Five patients exhibited hypospermatogenesis, characterised by reduced but ongoing spermatogenic cell development, including the presence of spermatozoa. Two showed maturation arrest or mixed atrophy of the seminiferous tubules, while one was diagnosed with Sertoli cell-only syndrome, representing the most severe impairment of the seminiferous epithelium. These structural alterations demonstrate that SCI-related infertility extends beyond ejaculatory dysfunction and involves intrinsic changes within the testicular parenchyma.

The mechanisms underlying this relationship are likely multifactorial, including autonomic denervation, altered scrotal thermoregulation due to immobility, recurrent urinary tract infections (noted in three cases), systemic inflammation, and oxidative stress. Disruption of neuroendocrine signalling and impaired local testicular blood flow may further compromise the delicate balance required for spermatogenesis. When non-invasive semen retrieval methods fail or semen parameters are severely compromised, combined cTESE with intracytoplasmic sperm injection (ICSI) provides an effective therapeutic option.

In conclusion, spinal cord injury cannot only abolish ejaculatory function but can also profoundly disturb testicular physiology and spermatogenesis, underscoring the need for comprehensive andrology evaluation and individualised reproductive management in this patient population.

A-0020 A PROSPECTIVE STUDY ON THE IMPACT OF MALE BMI ON INTRA-UTERINE INSEMINATION OUTCOME IN COUPLES WITH UNEXPLAINED OR MILD MALE INFERTILITY

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Introduction: While the detrimental effects of female obesity on fertility are well documented, emerging evidence suggests that male obesity similarly impairs reproductive outcomes. However, the specific influence of paternal body mass index (BMI) on intrauterine insemination (IUI) success remains understudied.
Objective: To evaluate the association between male BMI and reproductive outcomes in couples undergoing natural-cycle IUI for unexplained or mild male infertility, across repeated treatment cycles.

Methods: This monocentric prospective study was conducted at Antwerp University Hospital (Belgium). Couples with ≥12 months of infertility undergoing up to four natural-cycle IUI attempts were included. Primary outcome was live birth (≥22 weeks gestation). Secondary outcomes included clinical pregnancy (ultrasound-confirmed fetal heartbeat) and miscarriage (<22 weeks gestation). BMI was categorized to normal weight (18.5–24.99 kg/m²) or overweight/obesity (≥25 kg/m²) for analysis. Associations between BMI categories and outcomes were assessed using generalized estimating equations (GEE) to account for multiple cycles per couple, with adjustment for female age, female BMI, and infertility duration.

Results: Ninety-two couples were included. Live birth occurred in 24 couples (27%), corresponding to 11.6% of cycles in the normal-weight group, 10.7% in the overweight group. No live births were observed couples of which men had obesity (BMI ≥30 kg/m²). Clinical pregnancy occurred in 32 couples (36%), corresponding to 15.5% of cycles in the normal-weight group, 13.1% in the overweight group, and 4.0% in the obese group. In GEE analyses, male BMI was not significantly associated with clinical pregnancy, live

birth, or miscarriage in unadjusted or adjusted models. Longer infertility duration was independently associated with lower odds of live birth in adjusted analysis. Although odds ratio (OR) suggested a trend toward lower pregnancy and live birth rates with increasing BMI, confidence intervals were wide, reflecting limited statistical power.

Conclusion: In couples undergoing natural-cycle IUI for unexplained or mild male infertility, male BMI was not an independent predictor of clinical pregnancy, live birth, or miscarriage. While a non-significant trend toward reduced reproductive outcomes with increasing BMI was observed, the small number of obese participants limits definitive conclusions. An important strength of this study was the use of multiple IUI cycles per couple. Larger prospective studies with greater representation of obese men are warranted to better define the relationship between paternal adiposity and IUI outcomes.

A-0021 Seminal plasma cfDNA signatures derived from mitochondrial and germ-cell DNA track spermatogenic function in azoospermic men

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Introduction
Seminal plasma contains cell-free DNA (cfDNA) derived from the male reproductive tract. Although cfDNA fragment size and concentration have been associated with semen quality, the biological drivers of cfDNA heterogeneity remain unclear. Tissue-of-origin approaches suggest testicular contributions, yet it is unknown which cfDNA features dominate inter-individual variation and whether these reflect spermatogenic activity. In azoospermia, reliable non-invasive biomarkers distinguishing residual sperm production from complete testicular failure are lacking, leading to invasive diagnostic procedures and uncertainty regarding TESE.

Objective
To determine whether seminal plasma cfDNA contains mitochondrial and nuclear testis-derived signatures associated with spermatogenic activity and clinically relevant heterogeneity in azoospermic men.

Methods
Retrospective exploratory analysis of seminal plasma cfDNA from men with normozoospermia (n=9), cryptozoospermia (n=7), oligozoospermia (n=8), obstructive azoospermia (n=2) and non-obstructive azoospermia (n=9), including azoospermic patients with known TESE outcome (n=5). cfDNA was sequenced using low-coverage paired-end short-fragment optimised libraries. Gene-level cfDNA profiles were reconstructed using Fourier transform based pseudotranscriptomic analysis and summarised by principal component analysis (PCA). Mitochondrial DNA abundance was quantified as normalised chrM coverage and validated by digital PCR. Nuclear tissue-of-origin similarity was assessed by correlating nuclear cfDNA pseudotranscriptomes with Human Protein Atlas transcriptomes after exclusion of mitochondrial genes.

Results
PCA revealed a dominant molecular axis explaining 79.8 % of total variance. This axis was strongly associated with mitochondrial DNA abundance ($R^2 = 0.53$), indicating that mitochondrial cfDNA was a major contributor to inter-sample heterogeneity. Digital PCR confirmed marked differences in mitochondrial copy number, correlating with sequencing-derived chrM estimates (Spearman rho = 0.74, $p < 0.0001$, $n = 22$).

Samples aligned along this mitochondrial axis. Sertoli-cell-only and TESE-negative azoospermic patients were located toward the high chrM end, whereas normozoospermic, cryptozoospermic, oligozoospermic and TESE-positive samples clustered toward the low chrM end. After exclusion of mitochondrial genes, nuclear cfDNA profiles showed highest similarity to testis compared with other tissues. This nuclear testis similarity score was enriched toward the low mitochondrial end of the axis ($R^2 = 0.13$), consistent with increasing germ-cell contribution.

Conclusions
Seminal plasma cfDNA integrates mitochondrial abundance and nuclear testis-derived signals into a quantitative molecular axis tracking spermatogenic activity. This non-invasive molecular framework

captures biological heterogeneity in azoospermia and aligns with TESE outcome, supporting further validation in larger prospective cohorts.

Funding
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A-0028 Patient Perspectives on Klinefelter Syndrome: A Nationwide Survey in Germany on Diagnosis, Treatment, and Care

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Background: Klinefelter syndrome (KS) is the most common sex chromosome aneuploidy in males and affects approximately 1 in 500 births. Despite its relatively high prevalence, KS is frequently diagnosed only in adulthood. This delayed diagnosis can postpone appropriate medical care, endocrine treatment, fertility counseling, and psychosocial support.

Objective: The present study aimed to explore the experiences and perspectives of individuals with KS regarding their diagnostic pathway, healthcare management, and treatment.

Methods: A study-specific electronic questionnaire developed by the authors was distributed to patients at our clinic and through patient self-help groups throughout Germany. Data were collected using the REDCap (Research Electronic Data Capture) platform. The survey captured quantitative and qualitative information on diagnostic pathways, access to medical care, therapeutic interventions, and the perceived impact of KS on education, employment, treatment, and psychosocial well-being.

Results: A total of 101 individuals with KS responded to the survey; 84 met the inclusion criteria and were included in the analysis. The results suggest a bimodal diagnostic pattern, with diagnoses occurring either early in life, often following developmental concerns or prenatal testing, or later in adulthood. This pattern indicates persistent gaps in early recognition and highlights missed opportunities for timely diagnosis and coordinated care.

Conclusion: Improving awareness and interdisciplinary collaboration may facilitate earlier recognition of KS and contribute to optimized long-term management and quality of life.

A-0029 Testicular ultrasound as a screening method for testicular dysfunction in men born with hypospadias: Cross-sectional case-control study

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Introduction
Hypospadias is the most common congenital penile anomaly, affecting 0.5–1% of newborn males. Emerging evidence shows that testicular dysfunction, manifesting as subfertility and subclinical hypogonadism, is more common than previously recognized. Yet, testicular morphology, particularly parenchymal inhomogeneity and microcalcifications, remains poorly understood. This study uses ultrasound to screen testicular morphology in adolescents and adults born with hypospadias, comparing them with peers and examining pubertal changes and associations with clinical, prenatal, and lifestyle factors.

Methods
Testicular ultrasound was performed hypospadias cases (n=244; Tanner 1: n=17; Tanner 2-4: n=27; Tanner 5: n=200) and controls (n=51; all Tanner 5) by three physicians with varying experience in ultrasonography (junior to senior). Microcalcifications and homogeneity were scored on a 4-point and 3-point Likert scale, respectively. Associations were sought with clinical data and maternal, dietary and substance factors.

Results
Substantial interobserver agreement was found between physicians (microcalcifications: κ=0.735; inhomogeneity: κ=0.647). Testicular volumes were similar between hypospadias cases and controls, though considerably smaller in complex compared to isolated hypospadias (p<0.001). Testicular inhomogeneity was more common hypospadias (p=0.004), especially in severe (p<0.001) and complex hypospadias (p<0.001). Prevalence of heterogeneity increased with puberty progression. Associations were found with semen quality, surgical outcomes, adult penile length, anthropometry at birth and lifestyle. The positive and negative predictive value of inhomogeneity for oligozoospermia and azoospermia were 29.6% and 84.8%, respectively. Prevalence of testicular microcalcifications was similar to controls (p=0.599).

Discussion
Testicular parenchymal inhomogeneity was more prevalent in men born with hypospadias and correlated with testicular, surgical, and prenatal factors. These findings suggest that testicular inhomogeneity may serve as a relevant, non-invasive marker to identify individuals who could benefit from further andrological evaluation. Screening may be particularly valuable from late puberty onward, when the first morphological changes typically appear.

A-0031 How Reliable is Ultrasonography in Suspected Penile Fracture? Surgical Correlation From A Single-Center Experience

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Background
Penile fracture is typically diagnosed clinically, but ultrasonography (USG) is frequently used to confirm the diagnosis and localize tunical defects. However, the true diagnostic reliability of USG remains debated.

Methods
We retrospectively evaluated patients who underwent surgical exploration for suspected penile fracture at a tertiary referral center between 2011 and 2026. Demographic characteristics, clinical findings, imaging results, and intraoperative findings were reviewed. The diagnostic performance of USG was assessed using intraoperative findings as the reference standard. Continuous variables were analyzed using the Mann–Whitney U test, and correlations were evaluated with Spearman's rho.

Results
Twenty-seven patients were included (mean age 40.4 ± 14.9 years). Sexual intercourse was the most common etiology (66.7%), followed by masturbation (33.3%). Penile hematoma was present in all cases and penile edema in 92.6%. Penile fracture was confirmed intraoperatively in 20 patients (74.1%). USG demonstrated a sensitivity of 90.0%, specificity of 57.1%, positive predictive value of 85.7%, negative predictive value of 66.7%, and an overall diagnostic accuracy of 81.5%. Agreement between USG and intraoperative findings was moderate (κ = 0.49). USG correctly identified the side of the tunical defect in 83.3% of confirmed fractures. No significant correlation was observed between USG-measured defect size and intraoperative defect size (ρ = 0.19, p = 0.428). Patients with confirmed fractures presented earlier and underwent surgery sooner than patients without fracture (p = 0.011 and p = 0.025).

Conclusions
Ultrasonography shows high sensitivity but only moderate agreement with surgical findings and limited accuracy in estimating defect size. In patients with strong clinical suspicion, USG should be considered an adjunctive diagnostic tool rather than a replacement for surgical exploration.

A-0033 The Relationship Between Testosterone and Brain Health and Mental Health in Young Men

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Introduction: Sex hormones are associated with brain and mental health in both men and women. Non-invasive brain stimulation techniques such as transcranial direct current stimulation (tDCS) increase cortical excitability to improve mental health. However, the relationship between sex hormones and cortical

excitability is currently unknown. Importantly, research examining this relationship has largely focused on female participants. As such, this study aimed to determine, in young men, the relationships between cortical excitability (indexed by response to tDCS), mental health, and circulating levels of testosterone (total and free), oestradiol and the T:E2 ratio, as well as sex-hormone binding globulin (SHBG), prolactin, luteinising hormone (LH), follicle-stimulating hormone (FSH), and albumin.

Methods: Using a between-subjects design, 60 males aged 18–40 attended the testing site prior to 9 am to provide blood samples for hormone quantification, and completed measures of mental health (negative affect, anxiety, and emotion regulation) both before and after either real tDCS (N = 30) or sham tDCS (N = 30).

Results: Prior to tDCS (i.e. at baseline), emotion regulation and negative affect were associated with the T:E2 ratio, as well as circulating levels of testosterone and oestradiol, with the strongest associations observed for T:E2 ratio. Furthermore, tDCS-induced improvements in these symptoms were positively associated with circulating the T:E2 ratio and both testosterone and oestradiol levels.

Conclusions: These findings highlight the importance of testosterone (as well as oestradiol) for healthy brain function and mental health in men. Future research should examine whether circulating sex hormone levels influence responsiveness to non-invasive brain stimulation as a mental health intervention over extended periods.

A-0034 Loss of Y chromosome in male infertility

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Introduction: When alterations in semen parameters cannot be explained, the contribution of as-yet-unidentified genetic factors is advocated. The Y chromosome (chrY) includes many genes essential for spermatogenesis. It is susceptible to age-related mitotic errors that may lead to chrY loss in some of the germ cells, negatively impacting fertility. Loss of chrY has been associated with several pathological conditions, but its potential role in the pathogenesis of male infertility remains poorly investigated. This study aims to explore the association between chrY loss and male infertility.

Methods: Male partners of infertile couples attending our Unit were prospectively enrolled and divided into cases (semen parameters <5th WHO percentile) and controls (semen parameters >5th WHO percentile). In addition to semen analysis, all participants underwent blood testing for follicle-stimulating hormone (FSH), luteinizing hormone (LH), total testosterone, estradiol, prolactin and prostate-specific antigen (PSA) levels. To quantify the percentage of chrY loss, genomic DNA was extracted from peripheral blood, and X and Y chromosome copy numbers were assessed using droplet digital-PCR. Target genes on chromosome X (AMELX and androgen receptor gene, AR) and Y (AMELY and USP9Y) were selected. The analysis was performed in technical duplicate by counting the copies of AMELY and AMELX, USP9Y and AR genes by TaqMan assays, and calculating the percentage loss of chrY using the equation: (copies of Y/copies of X)*100.

Results: This ‘interim analysis’ included 114 patients: 73 cases (64.0%) and 41 controls (36.0%). Age (38.0±5.7 vs 35.3±5.0 years) and FSH levels (7.8±5.7 vs 4.9±2.5 IU/mL) were significantly higher in cases than in controls (p=0.014 and p=0.004, respectively, Mann–Whitney’s U-test). No differences were observed for BMI (p=0.921), LH (p=0.252), and estradiol levels (p=0.730). The AMELY/AMELX ratio was 0.983±0.034 in cases and 0.999±0.001 in controls (p=0.003, Welch’s t-test). Comparable results were obtained considering the USP9Y/AR ratio.

Discussion: Patients with altered semen parameters had higher FSH levels, consistent with impaired spermatogenetic function. At the same time, they had greater loss of chrY compared with controls. Although these patients were also significantly older than controls, the average age of both groups remains deeply below 50, the age threshold after which the loss of chrY has been widely demonstrated in the literature. For this reason, our finding may represent a factor directly associated with poorer semen quality.

Conclusions: These results suggest that chrY loss assessment may represent a new potential etiology of male infertility, possibly independent of ageing.

A-0037 New, non-hormonal contraceptive strategies for all genders: The collaborative research project PREVENT

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Currently, men have very limited contraceptive options, namely condoms and vasectomy. This poses a significant setback for male reproductive autonomy, gender equality and public health. To meet the need for alternative methods, non-hormonal, reversible options targeting sperm production, maturation, function or fertilization potential are explored. Sperm-specific targets should limit adverse effects.

The Precision REproductive and contraceptiVE target discovery NeTwork (PREVENT) is supported by the German Federal Ministry of Education and Research's guideline for promoting contraceptive research for all genders. Aim of this project is to establish a pipeline testing novel targets for male and female contraception. Biochemists, pharmacologists, developmental biologists and andrologists are involved in the project.

Target proteins are identified utilizing murine knockout models, in vitro pull-down experiments and literature search. Next, target proteins are recombinantly produced and screened against libraries of small molecules in order to design highly selective inhibitors: chemical probes. Developed chemical probes will be tested on human sperm as well as in murine in vitro fertilization. Sperm fertilization potential, viability, motility, morphology and DNA integrity will be examined. To rule out unwanted growth promoting effects, testicular germ cell tumor (TGCT) cell lines will be used to test cell viability, cell cycle progression and apoptosis.

One target protein is ACTL7A. Due to the fact that women with antibodies against ACTL7A present with infertility, we will raise nanobodies against ACTL7A. Analysis of effects on sperm and fertilization as well as identification of the epitope will guide in finding fitting chemical probes.

A-0038 Unexplained Male Infertility and Hyperspermia: Integrating Our Clinical Experience with Current Literature Insights

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Introduction:
Male infertility is a common clinical problem, and in nearly one-third of affected men no identifiable cause can be determined. Hyperspermia, defined as an ejaculate volume exceeding 6 mL, is a relatively neglected semen abnormality, and its role in unexplained male infertility remains unclear. This study aimed to evaluate the prevalence of hyperspermia and to investigate its potential association with impaired fertility outcomes.

Materials and Methods:
A retrospective analysis was performed on 44,510 semen analyses conducted at a tertiary andrology center between 2013 and 2023. Semen samples with a volume >6 mL were classified as hyperspermic. Clinical characteristics, semen parameters, hormonal profiles, and imaging findings were reviewed. Patients with known male or female infertility factors were excluded. Fertility outcomes were obtained through clinical records and follow-up. Statistical analyses were conducted using non-parametric tests, with a significance threshold of $p < 0.05$.

Results:
Hyperspermia was identified in 4,052 cases (9.1%). After exclusion of confounding factors, 105 hyperspermic patients were eligible for fertility outcome analysis. Mean semen volume was 6.89 ± 0.99

mL, while sperm concentration, motility, and morphology showed moderate impairment. Spontaneous pregnancy occurred in 36.2% of patients, and 13.3% achieved fertility through assisted reproductive techniques, whereas 50.5% remained infertile. Subgroup analysis across increasing semen-volume categories demonstrated no consistent deterioration in hormonal profiles, testicular volumes, or most semen parameters, although a dilutional effect on sperm concentration was observed in selected comparisons.

Discussion:
Hyperspermia was found to be relatively common and was associated with unfavorable fertility outcomes after exclusion of other etiological factors. These findings suggest that hyperspermia may represent an overlooked contributor to unexplained male infertility, warranting further prospective investigation.

A-0039 Successful Micro-TESE Following Intratesticular Stromal Vascular Fraction Therapy: A Case Report

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Introduction:In non-obstructive azoospermia(NOA) the success rate of sperm retrieval decreases with repeated m-TESE procedures. This rate is particularly lower in patients with histopathological findings such as Sertoli cell-only syndrome and maturation arrest; therefore, repeated m-TESE procedures are generally not recommended in these patients. Stromal vascular fraction (SVF) is a heterogeneous cell mixture derived from adipose tissue containing multiple cell types. By promoting tissue repair, immune modulation, and angiogenesis, SVF has emerged as a promising therapeutic option in regenerative medicine. Case Presentation: A 46-year-old male presented to the andrology clinic with a six-year history of primary infertility. His medical history revealed that he had previously undergone two m-TESE procedures at another center. Histopathological reports from these procedures indicated “severe hypospermatogenesis, hyaline sclerosis in 20% of seminiferous tubules, interstitial fibrosis, and a few spermatids in the lumen of 3–4 tubular sections,” and “maturation arrest at the spermatid stage.” On physical examination, the right testicular volume was 12 cc and the left testicular volume was 10 cc. Hormonal evaluation showed: FSH: 22.4 mIU/mL (reference: 1.5–12.4 mIU/mL) Total testosterone: 4.47 ng/mL (reference: 2.18–9.06 ng/mL). Karyotype analysis revealed a normal 46,XY pattern with no AZF microdeletions. Intratesticular SVF injection was planned. During the procedure, 30 cc of adipose tissue was aspirated from the right periumbilical region. The harvested adipose graft was placed into a tissue dissociation device (GCELL tissue dissociation device®) containing special blades capable of fragmenting tissue to 35 microns along with a filtration system. The tissue was mechanically processed for 12 minutes at 100 rpm, producing a 100% autologous SVF preparation.The processed adipose tissue was then centrifuged at 400 rpm for 10 minutes with the tube positioned at a 90-degree angle to separate fat from the cellular component. The cellular fraction at the bottom of the tube was passed through a 0.45-micron filter to remove residual fat cells. Subsequently, the preparation underwent photo-activation for 10 minutes using a device operating at a wavelength range of 635–900 nm with LED penetration, non-thermal light emission, and a 25-degree light dispersion. The cellular suspension was injected under ultrasound guidance by a radiologist, delivering 0.3 cc into each rete testis and the remaining volume into the testicular parenchyma, for a total of 2.5 cc. Four months after the procedure, semen analysis showed a volume of 3.22 mL, azoospermia, and a negative pellet result. One year later, bilateral m-TESE was performed. Histopathological evaluation reported: “right testis: diffuse tubular atrophy and hyalinization, germ cell aplasia in 95% of tubules with spermatocytes present in 5%; left testis: maturation at the spermatid level in 20% of tubules, tubules containing spermatogonia in 35%, and tubular atrophy and hyalinization in 45%.” During the m-TESE procedure, six spermatozoa were retrieved and intracytoplasmic sperm injection (ICSI) was performed. Four oocytes from the patient’s partner underwent ICSI, resulting in the development of one embryo, which was subsequently transferred. Conclusion:This case is noteworthy as it represents the first report of successful sperm retrieval following intratesticular SVF therapy in a patient with NOA and two previous unsuccessful m-TESE procedures.

A-0040 A Case of Non-Obstructive Azoospermia Associated with a Novel X–Autosomal Reciprocal Translocation: 46,XY,t(X;1)(p22.1;p36.1)

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Introduction: Approximately 50% of infertility cases in couples are attributed to male factors. The primary diagnostic test in the evaluation of male infertility is semen analysis (SA). Karyotype analysis and AZF microdeletion testing are recommended for all men with a sperm concentration below 5 million/mL. The prevalence of chromosomal abnormalities in men presenting with infertility is reported to be approximately 5.8%. These abnormalities are classified as numerical, such as trisomy, or structural, such as inversion, deletion, or translocation. Among these abnormalities, approximately 4.2% involve sex chromosomes, whereas 1.5% involve autosomes.A review of the literature revealed that only one X–autosomal translocation involving chromosome 1 has been reported in infertile men. In this study, we report a previously unreported case of non-obstructive azoospermia in which karyotype analysis revealed a 46,XY,t(X;1)(p22.1;p36.1) translocation.

Case Presentation:A 35-year-old male presented to the andrology outpatient clinic with an eight-year history of primary infertility.The anatomical and endocrine evaluations of his partner were normal.On physical examination, the penis appeared normal and both testes were located in the scrotum with a volume of 18 mL bilaterally.Two consecutive semen analyses performed two weeks apart demonstrated azoospermia with negative pellet findings. The semen volumes were 5.37 mL and 5.24 mL, respectively. Hormonal evaluation revealed an FSH level of 3.71 mIU/mL, total testosterone level of 4.65 ng/mL within the reference ranges. Karyotype analysis demonstrated 46,XY,t(X;1)(p22.1;p36.1), and AZF microdeletion analysis was negative.Following evaluation, the patient underwent bilateral microscopic testicular sperm extraction (m-TESE). No spermatozoa were detected in the retrieved testicular tissue. Histopathological examination revealed maturation arrest at the spermatid stage in 95% of the tubules in the right testis with a Johnsen score of 7, and in the left testis maturation arrest at the spermatid stage in 80% of the tubules and at the spermatocyte stage in 15% of the tubules, also corresponding to a Johnsen score of 7.

Discussion:Balanced translocations involving the X chromosome in men are generally clinically silent; however, they may have a significant impact on spermatogenesis and are often detected during infertility evaluation. In this study, we report a novel type of X–autosomal translocation in an infertile male patient with azoospermia.To best of our knowledge, only one balanced reciprocal translocation between the X chromosome and chromosome 1 has previously been reported in men. Karaer et al. reported a 31-year-old infertile male with a karyotype of 46,Y,t(X;1)(p22.3;q25) in 2010. The patient underwent m-TESE due to non-obstructive azoospermia, and motile spermatozoa were successfully retrieved. Although both cases involve a translocation between the X chromosome and chromosome 1, the breakpoint locations differ. This suggests that different chromosomal segments are involved and may affect different gene regions, potentially leading to variable clinical outcomes.

Conclusion:This case demonstrates a rare cause of non-obstructive azoospermia in a patient with maturation arrest resulting from a balanced reciprocal translocation between the X chromosome and chromosome 1. The potential role of genetic abnormalities in the etiology of primary infertility should not be overlooked. Karyotype analysis and AZF microdeletion testing enable the identification of underlying genetic causes in such patients.

A-0041 Defining the impact of protamine alterations on sperm epigenetics

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Spermiogenesis is characterized by extensive chromatin remodeling. In this process, canonical histones are replaced by protamines, resulting in 90-99% of the paternal genome (85%–95% in humans) being packed with protamines, leading to two differential chromatin domains: the nucleo-histone domain and the nucleo-protamine domain.

In mice and human, sperm chromatin contains two types of protamines: protamine 1 (P1) and the protamine 2 family (P2). While P1 is translated as a mature protein, P2 is synthesized as a precursor (Pre-

P2) that, upon DNA binding, is sequentially cleaved to generate the mature form. The ratio of mature protamines is species-specific, approximately 1:1 in human and 1:2 in mice; alterations of the ratio are linked to subfertility and infertility.

In addition to protamines, in sperm 1-10% of specific histones carrying histone post-translational modifications (PTMs) remain bound to the DNA in mice (5-15% in human). Research suggests a nonrandom binding of the nucleo-histone DNA, being mainly enriched in intergenic regions, repetitive DNA, and developmentally important genes. In contrast, infertile men show a random histone retention and a diminished association of specific modified histones at genes required for early development.

Despite their essential role in sperm chromatin compaction, the mechanisms by which the two chromatin domains interplay with each other remain poorly understood. To address this question, different protamine-deficient mouse lines are being used (dHET: Prm1+/− Prm2+/−; Prm1+/−; Prm1−/−; Prm2+/−; Prm2−/−). Acid urea western blot and immunofluorescence staining were performed to assess histone levels in epididymal and caput sperm, respectively. Western blot analysis of WT, dHET, Prm1+/-, Prm2+/-, Prm1-/- and Prm2-/- sperm nuclear protein extracts showed that dHET samples exhibit the highest levels of histone retention, with amounts comparable to the histone levels observed in WT testis. Moreover, although to a lesser extent, histone retention was also increased in Prm1+/-, Prm1-/- and Prm2-/- sperm. In contrast, no obvious differences were observed in Prm2+/− sperm compared to WT samples. This supports an association between defective protamine incorporation, impaired histone removal, and compromised fertility.

Beyond the overall levels of histone retention, alterations in the histone variants and their PTMs may play a critical role not only in sperm chromatin organization but also in the transmission of epigenetic information to the embryo. Therefore, to gain deeper insight into how protamines could interplay with the molecular nature of retained histones, we are currently establishing a proteomics pipeline to characterize the histone variants and associated PTMs. This approach will be particularly valuable to identify epigenetic signatures associated with protamine deficiency that may not only underlie infertility but also impact embryo development.

A-0048 Molecular Network Signatures of Genome Instability in Azoospermia and Oligoasthenoteratozoospermia

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Male infertility is a complex and heterogeneous condition. Genetic factors are considered an important cause, particularly in cases of severe male infertility. Nevertheless, more than 60% of affected men are still classified as having idiopathic infertility. Increasing evidence suggests that the genomes of infertile men may be exposed to elevated molecular stress, which can lead to genome instability and disturbances in cellular regulatory processes. However, studies that combine exome sequencing with gene network–based analyses are still relatively limited.

The aim of this study was to investigate genetic variants associated with genomic instability in two severe male infertility phenotypes—non-obstructive azoospermia (NOA) and oligoasthenoteratozoospermia (OAT)—and to construct and analyse gene ontology networks.

In this study, exome sequencing was conducted on 27 azoospermia and 63 OAT samples using the Twist Comprehensive Exome Panel. Gene networks were analysed with Cytoscape software, employing the CytoHubba and ClueGO plugins. Genome instability was assessed using the CINMetrics R package. As a control group, 141 exomes from proven fertile men the Genome Database of the Latvian Population were used.
Five spermatogenesis and DNA repair associated similar network pairs sharing at least one common gene or Gene Ontology (GO) function were identified both in NOA and OAT samples. Networks unique to the OAT group included genes involved in late spermatogenesis, such as sperm glucose metabolism, sperm motility, and fertilization. In contrast, NOA-specific networks were enriched for genes related to membrane transport, signalling, and early spermatogenesis. CINMetrics results confirmed increased chromosomal

instability in infertility patient groups. No specific DNA repair or spermatogenesis networks were identified in fertile controls.

Our findings suggest that NOA and OAT pathogenesis share common processes, including altered DNA repair, ribosomal biogenesis and chromosomal assembly factors that can explain at least a part of idiopathic severe male infertility.

A-0051 TRANSCRIPTION PROFILES OF MITOCHONDRIAL DYNAMICS MARKERS ARE ALTERED IN MEN WITH TERATOZOOSPERMIA

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Mitochondria are important for spermatozoa development, homeostasis and functionality as well as for the process of fertilization.

Our study investigated the transcriptional profiles of human spermatozoa mitochondrial dynamics markers and seminal plasma hormone levels in relation to spermiogram types.

The samples were collected from men participating in the national assisted reproductive technology program. The study design and procedures were approved by the Ethical Committee. The normozoospermic (N), teratozoospermic (T), asthenoteratozoospermic (AT), and oligoasthenoteratozoospermic (OAT) spermatozoa were separated from seminal plasma. Testosterone, cortisol, estradiol and nitrites were measured in seminal plasma. Real-time PCR analyses were conducted to obtain transcriptional profiles of mitochondrial dynamics markers in human spermatozoa.

The results showed that the levels of hormones and nitrites in seminal plasma, as well as transcriptional patterns of mitochondrial dynamics markers in spermatozoa displayed high individual variations. A significant correlation between nitrite levels in seminal plasma and mitochondrial dynamics markers in spermatozoa from normozoospermic and teratozoospermic samples were observed. In teratozoospermic samples, correlation was also detected between gelatinous clamps and transcripts for PPARGC1A and MFN1 in spermatozoa. A significant increase in the levels of PPARGC1A, TFAM, MFN2 and OPA1 transcripts were observed in teratozoospermic spermatozoa comparing to normozoospermic. In the same samples, the levels of NFE2L2 and MFN1 transcripts decreased. MFN1/MFN2 increased in asthenoteratozoospermic spermatozoa. In oligoasthenoteratozoospermic spermatozoa, a significant increase of PPARGC1B, NRF2, MFN1/MFN2 and OPA1 transcripts were detected, while the level of TFAM transcripts decreased. Receiver-operating characteristic (ROC) curve analyses confirmed that TFAM, NFE2L2, MFN1, MFN2 and OPA1 have potentially good diagnostic parameters for teratozoospermia: TFAM (AUC = 1.00, p = 0.0112); NFE2L2 (AUC = 1.00, p = 0.0112); MFN1 (AUC = 0.9524, p = 0.0304), MFN2 (AUC = 1.00, p = 0.0143), OPA1 (AUC = 1.00, p = 0.0201), whereas nitrites were not informative (AUC = 0.52, p = 0.67.

Accordingly, the above-mentioned markers could provide a solid foundation for the development of a Mito-Sperm-Signature - a potential new prognostic/diagnostic tool to assess male infertility, as semen quality and fertility are important fundamental markers of reproductive and overall health.

The authors have nothing to disclose. The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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A-0052 Long-term effects of chemotherapy on sperm DNA fragmentation and sperm-borne miRNAs in testicular cancer survivors

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Introduction: Current treatment protocols for testicular cancer achieve a 5-year survival rate of over 90%, shifting the clinical focus toward long-term quality of life, including reproductive health. Exposure of young men to chemotherapy has raised concerns about its consequences on fertility and reproductive safety. Although spermatogenesis often recovers within two years after treatment, the persistence of genetic and epigenetic alterations in spermatozoa remains poorly understood. Limited data are available on the long-term modulation of sperm DNA integrity and sperm-borne microRNAs following chemotherapy.

Aim: The aim of this study was to evaluate the long-term effects of chemotherapy on semen parameters, sperm DNA fragmentation (SDF), and sperm-borne miRNAs in testicular cancer survivors.

Materials and Methods: Patients diagnosed with testicular cancer were recruited at the Laboratory of Seminology–Sperm Bank “L. Gandini”, Sapienza University of Rome. Semen samples were collected before chemotherapy (T0), at 24 months after treatment (T24), and at long-term follow-up beyond 24 months (T>24). Semen analysis was performed according to WHO criteria (2010, 2021). SDF was assessed using the TUNEL assay. The expression of miR-34c and miR-449b was evaluated by droplet digital PCR (ddPCR).

Results: Thirty-two patients were evaluated at baseline and 24 months after chemotherapy. The mean age at diagnosis was 28.9 ± 6.0 years. Histological examination revealed seminoma in 53% of cases, mixed germ cell tumors in 31%, and embryonal carcinoma in 19%. Semen parameters remained comparable to baseline values at 24 months after chemotherapy, except for a slight reduction in semen volume (p=0.04). A significant decrease in SDF was observed at follow-up, declining from 12.43 ± 7.05% at baseline to 8.45 ± 5.07% at 24 months (p=0.01). Similarly, miR-34c expression decreased significantly from 27.35 ± 16.54 at baseline to 15.45 ± 10.88 at T24 (p<0.01), while miR-449b levels remained unaltered (22.28 ± 17.43 vs 22.27 ± 17.82; p=0.96). We also evaluated a group of 16 patients with long-term follow-up beyond 24 months (median follow-up: 4 years). Histology revealed seminoma in 62.5%, mixed germ cell tumors in 25%, and embryonal carcinoma in 12.5%. Semen parameters remained comparable to baseline values. SDF was significantly lower at long-term follow-up, decreasing from 11.66 ± 6.55% at baseline to 6.25 ± 3.29% at T>24 (p=0.01). In addition, miR-34c expression decreased from 14.26 ± 10.72 to 6.94 ± 6.67 (p=0.05), and miR-449b from 15.88 ± 19.26 to 4.20 ± 3.59 (p=0.05).

Discussion and Conclusion: Our findings suggest that chemotherapy affects both genetic and epigenetic integrity of spermatozoa. While spermatogenesis and DNA integrity may recover within two years after chemotherapy, epigenetic alterations in germ cells may persist for a longer period. Evidence on the long-term modulation of sperm-borne miRNAs after chemotherapy in humans is limited. MiR-34c and miR-449b play an important role in spermatogenesis and early embryonic development, in particular, higher levels of miR-34c compared with miR-449b increase the probability of obtaining viable embryos in assisted reproductive technologies. Further longitudinal studies are needed to clarify whether these miRNA alterations represent transient adaptive responses or persistent epigenetic modifications.

A-0053 Exome evaluation by Next Generation Sequencing in sibling pairs with testicular cancer

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Introduction: Testicular cancer accounts for 1–2% of male cancers and is the most frequent neoplasia in men aged 15–39 years. Its etiopathogenesis remains unclear, although several predisposing factors have been identified, including family history. In particular, siblings of patients with testicular cancer have an 8–10-fold increased risk of developing the disease, a significantly higher risk compared with other cancer types, where the risk typically ranges from 2 to 4.

Aim: The aim of this study was to investigate potential genetic factors associated with the occurrence of testicular cancer in siblings pairs.

Materials And Methods: Sibling pairs diagnosed with testicular cancer were recruited at the Laboratory of Seminology–Sperm Bank “Loredana Gandini”, Department of Experimental Medicine, “Sapienza” University of Rome, where they underwent sperm cryopreservation after cancer diagnosis. Total DNA was extracted from peripheral blood using the Wizard Kit Genomic DNA Purification Kit (Promega, Madison, WI, USA) according to the manufacturer's instructions. Massive sequencing of selected genomic regions was carried out using Exome Panel (45 Mb) and Nextera DNA Flex LPK Enrichment kit on the NextSeq2000 System platform. Bioinformatic analysis was performed using DragenEnrichment_Setting software v.3.8.4, while eVai software v2.3 (EnGenome) was used for annotation and variant filtering. Clinical charts were reviewed and a structured questionnaire was administered to collect information on potential risk factors, including perinatal and maternal factors, lifestyle habits, occupation and dietary patterns.

Results: Five pairs of siblings affected by testicular cancer were recruited. The mean age at diagnosis was 30.5 ± 6.55 years. All subjects were born at term with physiological birth weight. All patients had testes in site at birth, except one who had cryptorchidism and underwent orchidopexy at 6 months of age. None of the patients reported smoking habits. Their occupations included student, employee, merchant, technician, factory worker, geometrician, and railroad worker. Histological examination mainly revealed mixed germ cell tumors and seminoma. The mean maternal age at pregnancy was 27.6 (± 4.50) years; none of the mothers smoked and their occupations included housewife, secretary, employee. The majority of subjects had lived in urban areas, while 40% had moved to the rural areas during the last five years. Whole-exome sequencing did not identify rare pathogenic variants with high penetrance.

Discussion And Conclusion: Since several pairs of brothers affected by testicular cancer were referred to our laboratory, we investigated both genetic susceptibility and potential environmental risk factors. The analysis of clinical data and questionnaire information allowed us to exclude several commonly reported risk factors, including perinatal factors, maternal factors, smoking habits, high-risk occupations, and specific dietary patterns. Whole-exome sequencing analysis did not identify rare pathogenic variants with high-penetrance predisposing to testicular cancer, in agreement with previous studies. These findings suggest that testicular cancer does not follow a classical Mendelian pattern of inheritance but rather represents a genetically complex disease. According to the effect size model, our results suggest that testicular cancer is probably an oligogenic disease characterised by multiple variants with small or moderate effects and incomplete penetrance.

A-0054 Machine Learning and Deep Learning Models for Predicting Testicular Sperm Retrieval in Men with Azoospermia: Development of a Clinical Decision Support Tool

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Background:
Predicting successful sperm retrieval in men with azoospermia undergoing testicular sperm extraction (TESE) or micro-dissection TESE (micro-TESE) remains clinically challenging. Accurate prediction could improve patient counseling and optimize surgical decision-making. This study evaluated the performance of interpretable machine learning (ML) and deep learning (DL) models for predicting sperm retrieval outcomes using routine clinical and hormonal parameters.

Methods:
A retrospective analysis was conducted on a dataset of 599 men diagnosed with azoospermia who

underwent TESE or micro-TESE at a University hospital and an affiliated fertility center. The primary outcome was successful sperm retrieval (positive vs. negative). The dataset included 18 clinical variables, encompassing clinical history, testicular examination findings, ultrasound findings, and hormonal parameters (FSH, LH, prolactin, total testosterone, and estradiol). Two predictive models were developed. An interpretable Decision Tree (DT) classifier using the CART algorithm was trained with a stratified 80/20 train-test split, with a maximum depth of five and class-weight balancing to address class imbalance. A Deep Learning model based on a multi-layer perceptron (MLP) architecture with three hidden layers (64, 32, and 16 neurons) was implemented using TensorFlow/Keras. Standard preprocessing included feature scaling, categorical encoding, and missing-data imputation. Model performance was evaluated using accuracy, precision, recall, and F1-score.

Results:
Out of a total of 599 patients, 127 (21.2%) achieved successful sperm retrieval. TESE resulted in successful sperm retrieval in 60 of 445 cases (13.5%), whereas micro-TESE yielded positive results in 67 of 154 cases (43.5%). The Decision Tree model achieved 73% overall accuracy, with a recall of 0.75 for successful retrieval cases, demonstrating strong sensitivity for identifying candidates likely to benefit from surgery. Key predictors included surgical type (micro-TESE vs TESE), FSH levels, total testosterone, and clinical history such as undescended testis. The Deep Learning model achieved 80% test accuracy, with a recall of 0.58 and an F1-score of 0.48 for the positive class after applying imbalance-aware training.

Conclusions:
Both ML and DL models demonstrated potential for predicting sperm retrieval in men with azoospermia. The Decision Tree provided clinically interpretable decision rules, while the Deep Learning model captured complex nonlinear relationships between hormonal and clinical variables. Integrating such predictive tools into clinical workflows may enhance preoperative counseling and patient selection for TESE procedures.

A-0056 Reproductive implications of Y chromosome microdeletions

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Microdeletions in the AZF regions of the Y chromosome are well-recognized as a recurrent genetic cause of male infertility. Studies have shown higher risk of oligo- and azoospermia in men carrying Y microdeletions, but large-scale investigations of Y microdeletions and association to other reproductive traits like reproductive hormones, testicular histology, use of fertility treatment, number of children and offspring karyotypes are lacking.

The objective of this study is to investigate fertility parameters in men from infertile couples with and without Y microdeletions. Results from a PCR based Y microdeletion analysis and semen analyses were obtained from 8101 men referred for andrological workup at the Department of Growth and Reproduction, Rigshospitalet, from 1997 until 2024. Furthermore, results from reproductive hormone analyses (n = 8100), testicular sperm extractions (n = 371), and testicular biopsies (n = 1173) were obtained from a subset of men. Finally, Danish National Registries were accessed to retrieve information on relevant clinical information such as fertility treatment, hormonal treatment, karyotype, paternity status, and aneuploidies in the offspring. Collectively this gives a unique possibility to link Y microdeletions to a large range of clinical outcomes related to fertility.

In total, 597 men with a Y microdeletion and 7504 men without a Y microdeletion were included. Preliminary analyses indicate differences in semen parameters, reproductive hormones, and a higher risk of a testicular histopathology linked to impaired spermatogenesis in men with specific Y microdeletions. The preliminary analyses also indicate that there is no difference in risk of having children with aneuploidies but inclusion of additional data from registries are still pending. Finally, initial subgroup analyses of different types of Y microdeletions (gr/gr (n = 288), b2/b3 (n = 181), b2/b4 (n = 45), AZFbc (n = 18), b1/b3 (n = 16), AZFabc (n = 12), and other deletions (n = 37)) appear consistent with previous studies suggesting that some Y microdeletions are more detrimental than others. By elucidating the clinical

significance of Y microdeletions, this study will improve genetic counselling of patients and contribute to future refinements of clinical guidelines.

A-0058 Selected markers of oxidative stress in semen of men with low sperm motility

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The ability of sperm to exhibit progressive motility is one of the key parameters enabling sperm to reach and fertilize the oocyte. It is estimated that low sperm motility is observed in more than 80% of men struggling with infertility. The etiopathogenesis of asthenozoospermia usually is associated with structural, functional, and molecular changes in spermatozoa, which may be determined by both genetic and non-genetic factors. In particular, the role of factors related to oxidative stress (OS) is semen has been highlighted. It is worth noting that OS has harmful effects on the sperm membrane lipids, proteins, and DNA. For example, peroxidation of membrane phospholipids leads to a decrease in membrane integrity, sperm viability and motility. Therefore, our study was designed to verify the distribution and risk of OS in semen, as well as low total antioxidant capacity (TAC) of seminal plasma in group of asthenozoospermic men, and to assess linear associations between basic semen parameters, normalized static oxidation–reduction potential (snORP), and TAC.

For the study, 91 male participants were enrolled. In this population, two groups were established: (1) 51 men with low sperm motility (<30%), and (2) 40 men with proven fertility and results of basic semen parameters within the range defined by the World Health Organization (2021). Routine semen analysis was performed according to the recommendations provided by the WHO. Oxidative stress in semen (based on snORP measurements expressed as mV/106 sperm cells/mL) was assessed using the MiOXSYS® electrochemical system, while TAC of seminal plasma (expressed as µM of Trolox equivalent) was evaluated using an antioxidant assay kit and reagents by colorimetric method.

Comparison of the groups showed that men with abnormal sperm motility were significantly older and had lower sperm count, morphology, motility, and vitality. However, no differences were observed in ejaculate volume or in the concentration of peroxidase-positive cells (leukocytes) in semen. Analysis of oxidation–reduction potential in semen revealed that nsORP was significantly higher in men with abnormal sperm motility than in fertile men (medians: 6.13 vs. 0.57). Furthermore, the first group showed a significantly higher incidence of oxidative stress in semen (related to nsORP > 1.37) compared with fertile men (80% vs. 16.63% of subjects). Consistently, the odds ratio (OR) for oxidative stress in the low sperm motility group was more than 21-fold higher (OR = 21.21). Additionally, values of nsORP significantly negatively correlated with sperm count, morphology, motility and vitality as well as positively with teratozoospermia index (parameter reflects multiple structural defects of spermatozoa). On the other hand, our study did not demonstrate differences between the compared groups in TAC levels, the frequency and OR of low TAC (≤ 1950 µM Trolox equivalent). Moreover, TAC was not significantly correlated with the studied parameters.
The obtained data indicate that oxidative stress in semen, assessed by nsORP, is strongly associated with impaired sperm motility and overall semen quality. Therefore, we can assume that oxidative imbalance may play an important role in the pathophysiology of reduced sperm motility.

A-0059 Short-term sexual abstinence as a meaningful and easily modifiable factor affecting sperm DNA integrity

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It is estimated that infertility affects 15–20% of reproductive-age couples. Importantly, a male factor is diagnosed in 30–50% of cases. Poor semen quality, especially disturbed sperm chromatin, is considered one of the major causes of male infertility. According to the World Health Organization (WHO) guidelines, the recommended period of sexual abstinence before routine semen analysis is 2–7 days. This time is required for sperm maturation. However, the European Society of Human Reproduction and Embryology

(ESHRE) recommends a shorter abstinence period of 3–4 days. Interestingly, some evidence suggests that semen quality may improve after a very short period of sexual abstinence. Therefore, the aim of this study was to evaluate the effects of short-term sexual abstinence on standard semen parameters and sperm chromatin status.

The effects of short-term sexual abstinence were evaluated using semen samples obtained from adult men (n = 95) who were partners in couples with reproductive failure. The men were asked to provide two semen samples on the same day. The first sample was obtained after 2–7 days of sexual abstinence, and the second after one hour of abstinence. Semen analysis included the assessment of basic semen characteristics performed according to the World Health Organization (2021) guidelines and a comprehensive evaluation of sperm chromatin status. For this purpose, three complementary assays were performed: (1) the sperm chromatin dispersion test (SCDt) to evaluate sperm DNA integrity, (2) the aniline blue test (ABt) to identify spermatozoa with abnormally retained histones, and (3) the toluidine blue test (TBt) to detect spermatozoa with decondensed chromatin.

Comparison of the samples using the Wilcoxon signed-rank test showed that one-hour abstinence significantly reduced ejaculate volume and total sperm count (median: 3.50 mL vs. 1.50 mL and 78.30 $\times 10^6$ vs. 29.00 $\times 10^6$ sperm cells, respectively). No significant differences were observed for the remaining standard semen parameters. However, short-term abstinence significantly improved sperm DNA integrity as assessed by SCDt (median SDF index: 19.00% vs. 16.50%). It is worth noting that an SDF index >20% is considered clinically relevant in the assessment of male fertility, as it distinguishes between fertile and infertile men. Our data showed a significant decrease in the proportion of participants with an SDF index >20% after short-term abstinence (44.18% vs. 24.46%). Consistently, the odds ratio for SDF index >20% was significantly reduced (OR = 0.4568). No significant differences were observed in ABt and TBt results. Based on the presented data, we can assume that a short period of sexual abstinence appears to negatively affect ejaculate volume and total sperm count, which is expected. However, it has a positive influence on sperm DNA integrity (SDF index). Therefore, these findings suggest that short-term sexual abstinence may be a useful approach for improving sperm DNA integrity, one of the key semen parameters.

A-0060 Age-related decline in testicular volume: a population-based UK Biobank study

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Background:
Testicular volume reflects seminiferous tubule mass and serves as a structural correlate of spermatogenic capacity and gonadal function. Although histological studies describe age-related degeneration of the seminiferous epithelium, large population-based studies quantifying structural testicular aging and its endocrine correlates remain limited. Magnetic resonance imaging (MRI) enables precise volumetric assessment at scale.

Methods:
We analyzed MRI-derived testicular volumes from the UK Biobank (n = 33,280). Age at imaging was derived from assessment year and year of birth. Linear regression models analyzed associations between age and testicular volume. Multivariable models were adjusted for BMI. Bioavailable testosterone (BAT) was calculated using a Vermeulen mass-action approach from baseline total testosterone, SHBG, and albumin, and included to evaluate endocrine contributions (n = 28,220 complete cases). In subsets with Olink proteomics data (NPX log2; FSHB: n = 2,870; LHB: n = 3,709), associations between age, testicular volume, and circulating gonadotropin subunits were examined.

Results:
Testicular volume declined significantly with age (β = -0.31 mL/year; p = 2.6e-180), corresponding to an average reduction of -3.1 mL per decade. The association persisted after BMI adjustment (β = -0.29 mL/year; p = 8.7e-158). In models additionally including BAT, age remained independently associated with smaller testicular volume (β = -0.27 mL/year; p = 8.8e-110), while higher BAT was independently associated with larger testicular volume (β = +0.38 mL per nmol/L BAT; p = 2.0e-9). In proteomic analyses, age was associated with higher circulating FSHB levels (β = +0.00509 NPX/year; approximately +3.6% per decade; p = 4.8e-8), whereas LHB showed no significant age trend.

Conclusion:
In this large MRI-based population study, testicular volume demonstrates a continuous age-related decline of approximately 3 mL per decade, independent of BMI and BAT. The observed rise in circulating FSHB alongside smaller testicular volume supports adaptive hypothalamic–pituitary signaling in response to structural gonadal aging, consistent with intrinsic testicular aging accompanied by endocrine remodeling.

A-0061 Plasma proteomics reveals a circulating signature of quantitative spermatogenesis

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Background:
Reliable blood-based biomarkers that reflect quantitative spermatogenesis in adult men are limited. Bi-testicular volume is a well-established proxy for spermatogenic tissue mass and mirrors sperm production. Circulating gonadotropins and other peptides have been used to obtain indirect information about intrinsic spermatogenic tissue integrity and sperm production. High-throughput plasma proteomics offers an opportunity to identify circulating protein signatures that track structural variation in the adult testis and, by extension, quantitative spermatogenesis.

Methods:
We analyzed men with magnetic resonance imaging (MRI)-derived bi-testicular volume and plasma proteomic profiles measured using the Olink Explore platform. Across 2,908 circulating proteins, associations with testicular volume were tested using linear regression adjusted for age at imaging and body mass index (BMI). Effect sizes were expressed per 10 mL increase in testicular volume. False discovery rate (FDR) correction was applied and partial R^2 was calculated to quantify variance uniquely attributable to testicular volume. Across proteins, sample sizes ranged from 2,841 to 3,850 participants. Non-linearity was explored using spline models. Predictive utility was assessed using an 80/20 train–validation split.

Results:
A broad circulating proteomic signature was associated with MRI-derived testicular volume after adjustment for age and BMI. The strongest positive correlates included testis-enriched proteins, such as TEX101 (+0.088 NPX per 10 mL; p = 9.4e-35; partial R^2 = 0.052) and ACRV1 (+0.105 NPX per 10 mL; p = 3.9e-18). The strongest inverse associations were observed for gonadotropin-related and metabolic proteins, including CGA (−0.098 NPX per 10 mL; p = 1.2e-54; partial R^2 = 0.062) and FSHB (−0.056 NPX per 10 mL; p = 4.2e-31; partial R^2 = 0.046). Spline analyses demonstrated that the inverse association between circulating FSHB and testicular volume was most pronounced at lower volumes and attenuated at medium-to-higher volumes, consistent with compensatory hypothalamic–pituitary feedback primarily operating in men with smaller testicular volume. A 10-protein panel selected from significant proteins predicted testicular volume beyond age and BMI in the validation group (R^2 = 0.231).

Conclusions:
Plasma proteome profiling identifies a reproducible and biologically interpretable circulating signature of MRI-derived testicular volume, a proxy for quantitative spermatogenesis, in adult men. Positive associations reflect testis-enriched structural and germ cell–related proteins, whereas inverse associations capture compensatory gonadotropic signaling and metabolic state. These findings suggest that plasma proteomics may provide a scalable blood-based window into quantitative spermatogenesis in population-based studies of male reproductive health.

A-0063 Magnetic Activated Cell Sorting (MACS) and molecular profile analysis of male gamete

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Introduction - Sperm selection techniques aim to reproduce in vitro the physiological processes occurring in the female reproductive tract, enabling the recovery of spermatozoa with higher fertilizing potential. The most commonly used methods are Swim-Up and Density Gradient Centrifugation (DGC). Recently, the WHO (2021) included Magnetic Activated Cell Sorting (MACS), an immunomagnetic separation technique that removes apoptotic spermatozoa by binding annexin V to phosphatidylserine exposed on the plasma membrane. Although this method allows the selection of spermatozoa with improved quality and chromatin integrity, phosphatidylserine externalization may also represent a physiological event associated with sperm capacitation, thus representing a potential limitation of this technique.

Aim - The aim of this study was to characterize the molecular profile of apoptotic and non-apoptotic sperm fractions from a pathophysiological perspective. We evaluated sperm DNA fragmentation (SDF%) and the expression levels of sperm-borne miRNAs (miR-34c/449b) to identify the molecular pathways involved in apoptosis induction.

Materials & Methods - Ten patients aged 40–60 years with no significant comorbidities were recruited. Semen analysis was performed before and after sperm selection according to WHO criteria (2021). Samples underwent DGC, and spermatozoa were recovered from the 90% pellet fraction. This fraction was then processed using MACS to obtain the non-apoptotic (annexin V-negative) fraction. The apoptotic fraction (annexin V-positive) was collected after the removal of the magnetic field. Expression of miR-34c/449b was evaluated by Droplet Digital PCR and SDF% was assessed using the TUNEL assay. These analyses were performed on whole semen, the 90% DGC pellet fraction, and the non-apoptotic and apoptotic fractions.

Results: Pre-selection sperm analysis - Semen parameters were in the 25th percentile of reference values. Sperm chromatin integrity - We found a statistically significant increase in SDF% in the apoptotic fraction compared with the non-apoptotic fraction ($p < 0.001$). Expression of miR-34c and miR-449b - We observed lower expression levels of miR-34c/449b in the apoptotic fraction (miR34c 3.05±6.84; miR449b 2.4±8.03), compared with non-apoptotic fraction (miR34c 14.9±78.1; miR449b 11.5±101), although the difference was not statistically significant. A significant positive correlation between miR-34c and miR-449b was observed across all analyzed fractions ($p < 0.001$).

Discussion & Conclusion - Sperm maturation is regulated by miR-34c/449b, which modulate the expression of proteins involved in distinct stages of spermatogenesis. Depending on the cellular context, these miRNAs may promote apoptotic processes or contribute to defining the phenotype of differentiated gametes. In this study, the apoptotic fraction was analyzed for the first time. The increase in SDF% in the apoptotic fraction indicates that MACS can effectively discriminate between apoptotic and non-apoptotic sperm and confirms the role of SDF% as a marker of sperm quality. Although not statistically significant, the observed changes in miR-34c/449b expression suggest a potential role for these molecules as biomarkers for more accurate assessment of male infertility. Furthermore, the significant positive correlation between miR-34c/449b across all fractions suggests coordinated regulation of the two miRNAs, indicating a possible joint involvement in the molecular mechanisms underlying sperm quality.

A-0064 Obesity and Assisted Reproduction: molecular study of male gametes

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Introduction – Obesity significantly contributes to male reproductive dysfunction. The pathophysiological mechanisms linking obesity to male infertility are complex and multifactorial. Current literature suggests that paternal obesity may negatively influence the outcomes of ART by affecting semen parameters. Nevertheless, the molecular mechanisms by which it affects sperm quality and early embryonic development are not fully understood.

Aim – To investigate cytological, molecular, and epigenetic features of sperm in patients stratified according to BMI, for clarifying the biological pathways through which obesity may impair male reproductive competence.

Results – normal-weight vs overweight/obese: 61 men, partners of couples undergoing ICSI, were stratified into BMI< 25 kg/m² and BMI≥ 25 kg/m².

Semen quality: semen analysis (WHO2021), showed parameters within the 25th percentile in both groups. After sperm-selection, overweight/obese exhibited a significantly higher percentage of atypical sperm forms (p= 0.026). SDF% (TUNEL assay) was significantly increased in overweight/obese (p= 0.023). Although not reaching statistical significance, a trend towards higher expression of miR-449b-5p (Droplet Digital PCR) was observed in normal-weight (p = 0.073). Moreover, in obese patients (BMI≥ 30 kg/m²) SDF% was significantly higher than BMI< 30 kg/m² (p= 0.032).

ART outcome: Overweight/obese showed a lower fertilisation rate than normal-weight (72.5% vs 91.2%), although this difference didn't reach statistical significance. In particular, semen samples with NO fertilization in BMI≥ 25 kg/m² had significantly higher proportion of atypical forms (p = 0.0132) and increased SDF% (p = 0.008). In BMI≥ 30 kg/m² reduced miR-34c-5p levels were significantly associated with an increased proportion of low-quality-embryos (p = 0.040). Increased SDF% was also significantly associated with slower embryonic development and blastocyst formation (day 6) (p = 0.020).

Discussion and conclusion – Obesity has frequently been linked to impaired semen quality, although the available evidence remains inconsistent. In this study, no significant differences were observed in baseline cytological semen parameters between groups. After sperm selection, a significantly higher proportion of atypical sperm forms was detected in BMI≥ 25 kg/m². These findings suggest that conventional cytological semen parameters may remain apparently preserved in overweight/obese. BMI-related male infertility may not be fully explained by standard semen analysis alone, highlighting the importance of investigating molecular sperm features. SDF%, widely recognised as a male fertility-potential biomarker, was significantly higher in BMI≥ 25 kg/m² and further increased in BMI≥ 30 kg/m². Increased SDF% was associated with slower embryo development, suggesting that paternal genomic damage may contribute to impaired early developmental processes. Literature data show sperm-borne miRNAs as potential regulators of early embryogenesis: miR-34c-5p has been positively associated with blastocyst quality and embryo viability, miR-449b-5p remains less clearly defined. In this study, lower miR-34c-5p levels were significantly associated with higher proportion of low-quality embryos, independently of BMI. Overall, these findings support the hypothesis that molecular sperm alterations may influence embryo development and ART outcomes. Further studies involving larger cohorts are required to clarify paternal metabolic status - sperm epigenetic - reproductive outcomes relationship. Identifing novel molecular biomarkers may improve clinical male infertility evaluations and contribute to personalised reproductive strategies.

A-0067 Developmental Impact of Testosterone on the Brain–Testis Axis in Rats with Hypogonadism: Preliminary Results

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Introduction: Infertility affects nearly 15% of couples worldwide, with male factors responsible for 40–50% of cases. Hypogonadism is present in up to 40% of men seeking infertility treatment and is often associated with impaired spermatogenesis. Although testosterone replacement therapy improves clinical symptoms, exogenous testosterone suppresses spermatogenesis and thus limits fertility. The hypothalamic–pituitary–gonadal (HPG) axis plays a central role in male reproduction, and kisspeptins

(Kiss1) are key regulators of puberty and fertility through HPG modulation. Sox9 is expressed in Sertoli cell precursors, which are essential for normal testicular morphology and germ cell development.

Aim: The aim of this study was to investigate the effects of testosterone on the brain–testis axis under ethane dimethanesulfonate (EDS)-induced hypogonadism at prepubertal, pubertal, and adult stages. Specifically, we examined GnRH-related neuronal activity, Kiss1–Sertoli cell interactions, and developmental outcomes including apoptosis, proliferation, and maturation, together with ultrastructural morphology and sperm parameters.

Methods: Sprague Dawley rats were divided into four groups: vehicle controls (cyclodextrin, DMSO), ethane dimethanesulfonate (EDS)-treated, and EDS+testosterone replacement. EDS (or DMSO) was administered on PND 5 and 14 to suppress Leydig cells, while testosterone replacement began on PND 6. Animals were dissected at Postnatal day (PND) 28, 35, and 60 to represent prepubertal, pubertal, and adult stages. Testis tissues were processed for routine morphological evaluation using Hematoxylin-Eosin (H&E) and Masson's Trichrome staining. Hypothalamic tissues were stained with thionin for neuroanatomical assessment. The expression of Kiss1 was evaluated by immunofluorescence in both the hypothalamus and testis, while Sox9 expression was analyzed in testicular tissues. Testicular ultrastructure was examined using transmission electron microscopy (TEM). Additionally, at PND 60, sperm count and morphology were assessed by light microscopy to evaluate fertility.

Results: In the EDS group, seminiferous tubules at PND 28 and 35 showed reduced cell numbers, and their lumens contained elongating spermatids compared to PND 60. Testosterone replacement restored tubule morphology, stage VII–VIII tubules were more at PND 60. The proliferation index was high in all groups, while the apoptotic index was lowest in the EDS group. Sperm count was reduced in both EDS ($p<0.05$) and EDS+T groups at PND 60. Hypothalamic morphology appeared normal in all groups. Kiss1 expression in hypothalamic tissue was highest in cyclodextrin controls at PND 28, increased in the EDS group at PND 35 but decreased with testosterone replacement and PND 35, and was elevated again in the EDS+T group at PND 60. In testicular tissue, Kiss1 and Sox9 expression levels were high in control groups but decreased in EDS and EDS+T groups. TEM findings were consistent with light microscopy observations.
Discussion: EDS-induced hypogonadism caused age-dependent changes in Kiss1 expression and testicular morphology, while hypothalamic structure remained intact. Testosterone replacement partially restored testicular organization and sperm parameters, particularly at PND 60 These findings highlight the developmental sensitivity of the brain–testis axis to testosterone and Kiss1 signaling under hypogonadal conditions, suggesting a compensatory role of testosterone in sustaining reproductive function.

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A-0072 Early detection of relapsing testicular germ cell tumours using miR-371a-3p and miR-373-3p

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Testicular germ cell tumours (TGCT) are the most common malignancies among young men and accounts for approximately 95% of all testicular cancers. TGCTs are broadly divided into two subtypes: seminomas and non-seminomas. About 75% of patients are diagnosed with clinical stage I disease localised to one or both testes, yet 20–30% experience relapse within five years. Although survival rates exceed 95%, the young age at diagnosis and the prolonged surveillance period impose a substantial psychological and clinical burden. Until recently, all patients followed the same surveillance regimen after a stage I TGCT, which relies on classical serum tumour markers (STMs): alpha-fetoprotein, human chorionic gonadotropin, and lactate dehydrogenase, together with repeated imaging. However, these STMs have limited diagnostic sensitivity. Less than 50% of patients present with elevated STMs at initial diagnosis, and only about one-third exhibit elevated levels at relapse. For stage I seminoma, elevated markers are found in only 25% of relapses, and merely 8% are detected by STMs alone. Consequently, there is an unmet need for novel,

sensitive and non-invasive biomarkers that can identify relapse earlier and improve the precision of follow-up.

Circulating microRNAs (miRNAs) have emerged as highly promising biomarkers for TGCTs. In particular, miR-371a-3p, and to a lesser extent miR-373-3p, have demonstrated excellent sensitivity and specificity (both >0.9) for detecting viable TGCT components. Studies suggest that these miRNAs outperform classical STMs both at primary diagnosis and during surveillance, potentially allowing earlier identification of relapse and enabling more personalised follow-up strategies.

In the present study, we have analysed 41 serum samples from 24 patients with stage I TGCT before they were diagnosed with a relapse. All samples were collected within one year prior to relapse diagnosis. Preliminary analysis indicates elevated levels of miR-371a-3p and/or miR-373-3p in 15 cases, indicating that miR-371a-3p and miR-373-3p frequently become detectable before a clinically recognised relapse. These promising early findings strongly suggest that miRNAs may enable earlier, more accurate relapse detection than current clinical markers.

By extending these analyses to a larger cohort, we will determine how early miRNAs become detectable before clinical relapse and assess their overall accuracy across tumour subtypes. In addition, we will explore how incorporating miRNA measurements could enhance the accuracy and reduce the burden of follow-up for men with stage I TGCT.

A-0073 METABOLIC PARAMETERS AS PREDICTIVE FACTORS FOR A FULL-TERM PREGNANCY IN MALES OF INFERTILE COUPLES: PRELIMINARY DATA FROM PATIENTS ATTENDING A NEW SERVICE FOR INFERTILITY TREATMENT

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Introduction and objectives: Although a male factor is reported in 40-50% of cases in infertile couples, the causes associated with male infertility are often underestimated and underinvestigated. The primary aim of this study was to assess the prevalence of cardiometabolic risk factors in a population of male partners of infertile couples. The secondary aim was to characterize these factors, evaluating their relationship with parameters usually considered as predictors of male infertility, such as the seminal ones, and their accuracy in predicting the couples’ reproductive outcome.

Methods: 239 male partners of couples consulting for infertility were included in a prospective study; they underwent anamnestic, physical and biochemical examination, as well as semen analysis and bioimpedenziometric analysis of body composition. For a subgroup of 119 patients, data regarding the achievement of a full-term pregnancy were also collected.

Results: Our study population showed a moderate prevalence of dysmetabolic conditions: 12.1% obesity, 6.7% blood hypertension, 8.4% dyslipidemia, 6.3% metabolic syndrome and 0.8% diabetes mellitus. Therefore, we investigated the association between metabolic disorders and the achievement of spontaneous full-term pregnancies, which was obtained in 11 cases out of 119. Differently from seminal parameters, Luteinizing Hormone levels showed a significant difference between subjects of group 1 (pregnancy yes) and group 2 (pregnancy no), as well as body composition. In particular, group 1 showed a significantly lower level of visceral fat, obesity, and metabolic age, compared to group 2. Furthermore, using ROC (Receiver Operating Characteristic) curve analysis, the highest predictive efficacy for a spontaneous full-term pregnancy was observed for metabolic age, with an area under the curve (AUC) of 0.757±0.068 (p=0.0001) and a cut-off of 25.5 years (specificity 77.7%; sensitivity 71.4%) in predicting pregnancy. Furthermore, we found that metabolic age increased in function of LDL cholesterol and insulin

levels, systolic and diastolic blood pressure, while it decreased in function of Total Testosterone, calculated Free Testosterone and Sex Hormone Binding Globulin levels.

Conclusions: Our data highlight the importance of the male partner in the infertility management, and how fundamental it is to evaluate “not conventional” parameters potentially associated with infertility, such as the metabolic ones, which proved to be important predictors of pregnancy.

A-0075 The role of the radiologist in the evaluation of male infertility: recommendations of the European Society of Urogenital Radiology Scrotal and Penile Imaging Working Group (ESUR-SPIWG) for scrotal imaging

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The Scrotal and Penile Imaging Working Group (SPIWG) of the European Society of Urogenital Radiology (ESUR) aimed to produce recommendations on the role of the radiologist in the evaluation of male infertility focused on scrotal imaging. The authors independently performed an extensive literature Medline search and a review of the clinical practice and consensus opinion of experts in the field. Levels of evidence (LoE), grade (GoR) and strength of the recommendations are reported and discussed. Scrotal ultrasound (US) is useful in investigating male infertility. US abnormalities related to abnormal sperm parameters (sperm concentration, total count, motility, and morphology) are low testicular volume (TV), testicular inhomogeneity (TI), cryptorchidism, testicular microlithiasis (TML), high-grade varicocele, bilateral absence of vas deferens, bilateral dilation and echotexture abnormalities of the epididymis. The proposed ESUR-SPIWG recommendations for imaging in the evaluation of male infertility are therefore: to measure TV; investigate TI; perform annual (US) follow-ups up to age 55 in men with a history of cryptorchidism/orchidopexy and/or in men with TML plus “additional risk factors” or with “starry sky” TML; perform scrotal/inguinal US in men with nonpalpable testis; perform scrotal US in men with abnormal sperm parameters to investigate lesions suggestive of tumors; evaluate varicocele in a standardized way; evaluate the presence or absence of vas deferens; investigate the epididymis to detect indirect signs suggesting obstruction and/or inflammation. The ESUR-SPIWG recommends investigating infertile men with scrotal US focusing on TV, inhomogeneity, localization, varicocele, vas deferens, and epididymal abnormalities. Cryptorchidism, TML, and lesions should be detected in relation to the risk of testicular tumors. MRI is recommended when the US study of the vas deferens is doubtful/inconclusive or to evaluate the intra-abdominal course of the vas deferens. The ESUR-SPIWG recommendations on scrotal imaging in the assessment of male infertility are useful to standardize the US examination, focus on US abnormalities most associated with abnormal semen parameters in an evidence-based manner, and provide a standardized report to patients.

A-0077 Semen quality in acute leukaemia patients: a retrospective study

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Background: Acute leukaemia frequently affects men of reproductive age. Improvements in survival rates thanks to advances in oncologic therapies have increased attention toward fertility preservation and future fatherhood. However, the impact of acute leukaemia itself on semen quality prior to treatment remains poorly characterized.

This study aims to evaluate semen parameters in patients with acute leukaemia before chemotherapy and to assess post-treatment semen outcomes, fertility-related information, and the feasibility of semen cryopreservation in this population.

Methods: We conducted a retrospective study including patients with acute leukaemia referred to the Laboratory of Seminology – Sperm Bank “Loredana Gandini”, Sapienza University of Rome, between 1996 and 2023 for semen cryopreservation prior to oncologic treatment. Clinical and reproductive data were collected, including semen analysis parameters, information on paternity desire and childbearing, and feasibility of semen cryopreservation procedures. Semen parameters were analysed according to standard laboratory procedures. A subgroup of patients was also evaluated after completion of oncologic therapies.

Results: A total of 56 patients with acute leukaemia were included: 36 with acute lymphoblastic leukaemia (ALL) and 20 with acute myeloid leukaemia (AML). Median age at diagnosis was approximately 27 years in both groups. Prior to cancer treatment, median semen parameters were generally within normal reference ranges and above the 25th percentile. No significant differences were observed between ALL and AML patients in terms of semen volume, total sperm number, progressive motility, or sperm morphology. Leukocytes and germ cells were rarely detected in semen samples. Semen cryopreservation was feasible in the majority of patients referred to the sperm bank. A subgroup of patients was evaluated after oncologic therapies, showing changes in semen quality in some individuals. Information on reproductive outcomes and paternity was also collected during follow-up. Over the years, an increasing number of patients were referred for fertility preservation before treatment.

Conclusions: In patients with acute leukaemia, semen quality prior to treatment is frequently preserved, suggesting that the disease itself may not severely impair spermatogenesis at diagnosis. These findings highlight the importance and feasibility of offering semen cryopreservation before the initiation of gonadotoxic therapies. Early referral for fertility preservation should therefore be considered an integral part of the multidisciplinary management of reproductive-age men diagnosed with acute leukaemia.

A-0078 Gender-Affirming Hormone Therapy: Impact on Fertility and Testicular Function

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Background: Feminizing gender-affirming hormone therapy (GAHT) in transgender individuals assigned male at birth (AMAB) induces profound endocrine changes that may adversely affect spermatogenesis and testicular function. However, longitudinal clinico-laboratory data integrating seminal, epigenetic, and

ultrasound parameters remain limited. This study aimed to evaluate the early impact of GAHT on fertility potential through a comprehensive longitudinal clinico-laboratory assessment.

Methods: We performed a prospective observational study in transgender AMAB individuals initiating feminizing GAHT, evaluated at baseline and, when available, at 3 and 6 months. The clinico-laboratory assessment included WHO 2021 semen analysis, sperm DNA fragmentation (TUNEL), sperm microRNA profiling (RT-qPCR), testicular ultrasound in a subgroup, comprehensive hormonal profiling (TT, E2, FSH, LH, PRL), and correlation analyses between endocrine, seminal, epigenetic, and ultrasound parameters (Spearman's ρ).

Results: Thirty-nine transgender AMAB individuals (mean age 25.0 ± 6.5 years) underwent baseline seminal evaluation; 10 and 6 completed follow-up at T3 and T6, respectively. GAHT was associated with a significant early decline in semen volume (p=0.0059) and total sperm concentration (p=0.027), with a trend toward reduced progressive motility. Morphological abnormalities increased over time (p=0.009 at T6). SDF significantly increased at T3 compared with baseline (median 11.8% vs 38.2%, p=0.0156), suggesting early genomic instability of spermatozoa. Two cases of azoospermia were observed during follow-up. Testosterone significantly decreased while estradiol increased, with a marked reduction in the TT/E2 ratio, correlating with worsening seminal parameters. Preliminary epigenetic analyses revealed dynamic modulation of sperm microRNA expression during GAHT. Ultrasound assessment showed a trend toward reduced testicular volume without major structural abnormalities.

Conclusions: Feminizing GAHT induces early testicular dysfunction, reflected by declining semen quality, increased sperm DNA fragmentation, and epigenetic changes, closely linked to androgen–estrogen imbalance. These findings highlight the importance of early fertility counseling and support sperm cryopreservation before initiation of GAHT, as hormonal treatment may induce not only cytological but also molecular alterations. An integrated clinico-laboratory and ultrasound monitoring approach should therefore be considered in transgender reproductive care.

A-0081 Human primary granulosa cells did not replace Sertoli cells in enhancing Leydig cell response to gonadotropins

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FSH may be helpful to increase sperm production in infertile men, even if the evidence to recommend the use of FSH in this setting is weak. The use of FSH relies on the effect of this hormone in Sertoli cells, which would produce growth factors enhancing Leydig cell response to their ligands, i.e. endogenous luteinizing hormon (LH) and exogenous human chorionic gonadotropin (hCG).

This study aims to test if Leydig cell response to LH and hCG may be improved by molecules from Sertoli cell origin. To this purpose, experiments were performed using the murine Leydig tumor cell line 1 (mLTC-1) exposed to conditioned medium from FSH-treated ovarian primary granulosa cells (hGLC) obtained from women undergoing assisted reproduction. hGLC were used as a substitute for human Sertoli cells, which are not available.

hGLC were cultured over-night in the presence or in the absence of 1 nM FSH. The medium was collected and used for overnight conditioning of mLTC-1 cells, previously serum-starved. mLTC1 cells exposed to unconditioned cell medium served as a "reference". Then, cells were treated 6 h with increasing concentrations (10 fM-100 nM) of recombinant LH and hCG before the analysis of testosterone levels by homogenous time-resolved fluorescence (HTRF) assay. Forskolin and cholera toxin (CTX) were used as positive controls, as they activate adenylyl cyclase and Gs protein, respectively, coupled to LH receptor. 4 independent experimental replicates were performed and statistically analysed by Kruskall-Wallis test and Dunn's post-test ($p<0.05$).

Results show that mLTC-1 cells pre-treated with the unconditioned “reference” medium displayed gonadotropin-dependent testosterone increase. hCG was more potent than LH in inducing testosterone production, since 1 pM hCG was enough to trigger the maximal response, while lowest LH dose inducing testosterone increase was between 10-100 pM. Interestingly, in mLTC-1 pre-treated with hGLC-conditioned medium, LH and hCG displayed lower potency in activating testosterone production than those maintained in the “reference” medium, and both the molecules, as well as CTX, produced similar dose-response curves with lowest concentration activating testosterone at about 100 pM. The LH/hCG potency decreased even more markedly in mLTC-1 cells pre-exposed to conditioned medium from hGLC treated with FSH. In this case, LH, hCG and CTX, but not forskolin, failed to induce testosterone increase.

Results suggest that ovarian cells produced paracrine factors reducing the response to gonadotropins of closest LHCGR-expressing cells, via a mechanism involving Gs protein inhibition. Therefore, hGLC cannot replace the functions of Sertoli cells, suggesting the existence of sex-specific endocrine factors regulating gonadal cell metabolism.

A-0082 Preparations of recombinant follicle-stimulating hormone (FSH) alfa and delta induce different intracellular signals in vitro

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Rekovelle® and Gonal-F® are two commercial follicle-stimulating hormone (FSH)-based drugs used for clinical treatment of infertility. They are originated by different hosts, i.e. human fetal retinal (PER.C6®) and genetically modified Chinese hamster ovary (CHO) cell lines, respectively, producing distinct glycosylation profiles possibly impacting the hormone-mediated intracellular signaling cascades. In this study, we compared Rekovelle®- versus Gonal-F®-induced intracellular signalling pattern and receptor internalization in vitro.

HEK293 cells were transiently transfected with FSH receptor (FSHR)- and biosensor-coding plasmids before being exposed to increasing concentrations (pM-µM range) of Gonal-F® and Rekovelle®. FSH-induced cAMP production, and the interaction between FSHR and β-arrestin 2, as well as with the late endosome marker RAB-GTPase 7 (Rab-7), were analysed by bioluminescent imaging (BRET). Genomic effects were assessed by CRE-luciferase reporter gene activity. Kruskal-Wallis test or Mann-Whitney’s U-test ($p<0.05$) were used for statistical comparisons, as appropriate, and at least 5 independent experimental replicates were performed.

We found that Rekovelle® is more potent than Gonal-F® in activating dose-dependent intracellular cAMP increase and CRE promoter activity. Interestingly, 1 µM Rekovelle® failed to induce intracellular cAMP accumulation and CRE activity, leading to biphasic behaviour, while it was not with the equimolar Gonal-F® concentration. Moreover, the analysis of β-arrestin 2 and Rab-7 recruitment to FSHR revealed that 1 µM Rekovelle® routed the receptor to late endosomes and degradation. To evaluate whether cAMP and CRE inhibition by 1 µM Rekovelle® depends on FSHR internalization, FSH dose-response experiments were performed in the β-arrestin 2 knockout HEK293 cell line. In those cells, we found that 1 µM Rekovelle® led to intracellular cAMP increase, demonstrating that preparation-specific cAMP/CRE inhibition required β-arrestin 2 recruitment.

In conclusion, Gonal-F® and Rekovelle® display different intracellular signaling profiles, possibly impacting the clinical response. These findings may implement the clinical applications of the two recombinant FSH preparations based on their distinct molecular and signaling properties.

A-0083 Validation of the Turkish Version of the ANDROTEST for Detecting Male Hypogonadism in Patients with Sexual Dysfunction

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Aim:
To evaluate the psychometric properties, reliability, and validity of the Turkish version of the ANDROTEST in men presenting with sexual dysfunction.

Methods:
Male patients aged 18–80 years presenting to the andrology outpatient clinic with symptoms of sexual dysfunction between February and November 2025 were prospectively included. A control group consisted of age-matched individuals without clinical suspicion of hypogonadism or conditions affecting gonadal function. Participants completed the Turkish versions of the Beck Depression Inventory and the International Index of Erectile Function (IIEF). The ANDROTEST questionnaire was translated and culturally adapted using a forward–backward translation process. Diagnostic performance was assessed using receiver operating characteristic (ROC) curve analysis, and test–retest reliability was evaluated using the intraclass correlation coefficient (ICC).

Results:
A total of 246 participants were included (120 controls and 126 patients with hypogonadism). Compared with controls, hypogonadal patients demonstrated significantly higher BMI, triglycerides, total cholesterol, LDL cholesterol, and Beck Depression Inventory scores, whereas total testosterone, free testosterone, SHBG, HDL cholesterol, IIEF scores, and testicular volumes were significantly lower (all $p < 0.05$). The Turkish ANDROTEST demonstrated excellent diagnostic performance with an AUC of 0.980 (95% CI: 0.958–0.994). The optimal cut-off value of 11 yielded a sensitivity of 95.2% and specificity of 95.0%. In multivariable analysis, the ANDROTEST score remained an independent predictor of hypogonadism (adjusted OR 1.80, $p = 0.009$). Test–retest reliability was excellent (ICC = 0.99), and the score showed strong correlations with IIEF scores, total testosterone levels, and depression scores (all $p < 0.001$).

Conclusion:
The Turkish version of the ANDROTEST is a reliable and valid instrument for the assessment of male hypogonadism. Its use in routine clinical practice may facilitate earlier identification of affected individuals and allow more standardized comparisons across different clinical settings.

A-0084 Shear Wave Elastography of the Testis in a Mexican Series of 105 Patients: Technical Standardization, Reproducibility, and Quantitative Characterization

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Shear wave elastography (SWE) has emerged as a noninvasive tool for quantitative tissue assessment; however, its application in testicular imaging remains limited by the heterogeneity of acquisition methods and the scarcity of reproducible protocols in clinical practice. In the andrological setting, this variability has hindered comparisons across studies and the establishment of useful reference parameters. In Mexico, published experience with testicular SWE remains limited. The aim of this study was to describe the implementation of a standardized technical protocol for testicular elastography and to characterize its quantitative findings in a Mexican series of 105 patients evaluated in an andrological context.

An observational, cross-sectional, single-center study was conducted. A total of 105 males aged 12 to 65 years with complete bilateral SWE acquisition were included. Evaluation was performed using grayscale scrotal ultrasound, Doppler, and 2D SWE with a Philips EPIQ 5G system and a 4–18 MHz multifrequency linear transducer. The protocol included examination in the supine position with confirmation in the standing position. A 5 to 10 mm circular region of interest was placed in homogeneous parenchyma at least 5 mm from the testicular capsule while avoiding artifacts. Three to five valid acquisitions were obtained per testis and, whenever feasible, by superior, middle, and inferior segments in both medial and lateral components. Measurements with unstable maps, marked heterogeneity, or intra-subject variability above predefined thresholds were excluded. Venous diameters were recorded only as morphological descriptors of the analyzed population.

Mean age was 37.9 ± 8.14 years. Median testicular volume was 13.8 mL [10.8–17.3] on the right and 13.2 mL [10.2–16.6] on the left. Global SWE stiffness values were comparable between testes: 8.4 kPa [8.0–10.1] on the right and 9.0 kPa [8.4–10.5] on the left, with no significant difference (p = .167). Segmental analysis showed significantly higher values on the left side in the superior lateral (p = .005), middle lateral (p = .001), and inferior lateral (p = .028) segments, whereas medial segments showed no significant intertesticular differences. The right testis demonstrated significant intratesticular heterogeneity across segments ($\chi^2(5)=22.586$; p<.001). In addition, testicular volume showed a negative correlation with stiffness on both sides. The protocol demonstrated adequate technical stability, with coefficients of variation below 30%, supporting the consistency and reproducibility of the measurements.

Testicular shear wave elastography proved to be a feasible, stable, and reproducible technique for the quantitative assessment of testicular parenchyma in this Mexican series of 105 patients. Although no significant global differences were observed between testes, segmental analysis identified regional variations, particularly in the left lateral portion, suggesting that a systematic acquisition approach may provide information not apparent on a single global assessment. The main contribution of this study lies in the standardization of the acquisition protocol and in the generation of a quantitative foundation for future comparative, multicenter, and clinically applicable studies in andrological imaging.

A-0087 The role of the radiologist in the evaluation of male infertility: recommendations of the European Society of Urogenital Radiology Scrotal and Penile Imaging Working Group (ESUR-SPIWG) for prostate-vesicular imaging

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The Scrotal and Penile Imaging Working Group (SPIWG) of the European Society of Urogenital Radiology (ESUR) aimed to produce recommendations on the role of the radiologist in the evaluation of male infertility focused on prostate-vesicular imaging. The authors independently performed an extensive literature Medline search and a review of the clinical practice and consensus opinion of experts in the field. Levels of evidence (LoE), grade (GoR) and strength of the recommendations are reported and discussed. Transrectal ultrasound (TRUS) is useful in investigating obstructive male infertility and seminal vesicles (SV) abnormalities. TRUS abnormalities related to oligo-/azoo-spermia, usually associated with low semen volume and pH, are: large midline prostatic cysts (high impact); ejaculatory ducts abnormalities (especially when bilateral), including dilation (moderate impact), cysts (moderate impact) and, with lower evidence, calcifications; deferential ampullas dilation (indirect sign; moderate impact); congenital bilateral absence of vas deferens (CBAVD; high impact), associated with obstructive azoospermia, often with CFTR gene mutation, to be investigated, and, in half of cases, with SV agenesis leading to low seminal volume and pH; SV dilation (indirect sign; moderate impact) especially when showing honeycomb pattern. Other SV abnormalities, including giant cysts and thickened wall and septa, can be associated with impaired SV contraction and low seminal volume and pH. SV congenital abnormalities, including agenesis, cysts and hypoplasia, are associated with low seminal volume and pH, and may be isolated or associated with other congenital genitourinary anomalies: bilateral SV agenesis with CBAVD and obstructive azoospermia in half of cases; unilateral SV agenesis with CUAVD and ipsilateral renal agenesis in up to 90% of cases, usually

with preserved fertility; congenital SV cysts or hypoplasia with other congenital genitourinary anomalies, the former even with Zinner syndrome. The proposed ESUR-SPIWG recommendations for imaging in the evaluation of obstructive male infertility are: to investigate and characterize midline prostatic cysts, ejaculatory ducts, vas deferens and SV presence/absence and echotexture abnormalities, the latter especially after ejaculation; to extend the US evaluation to kidneys and to investigate CFTR gene mutations in case of CAVD and/or SV agenesis; to investigate other genitourinary anomalies in case of congenital SV abnormalities. Prostate volume in men of reproductive age can be assessed to investigate enlargement, often associated with overweight, obesity, metabolic syndrome, or hypotrophy, often associated with hypogonadism, considering that obesity and hypogonadism can possibly affect male fertility potential. Prostate asimmetry, echotexture abnormalities, calcifications, arterial vascularization and venous plexus, possibly associated with past, chronic or current inflammation, play a scanty role in longlasting male infertility investigation. MRI is recommended when US is inconclusive, as it allows comprehensive assessment of anatomy and detection of complex congenital abnormalities, mainly related to vas deferens and/or SV, or to evaluate intra-abdominal course of the vas deferens. The ESUR-SPIWG recommendations on prostate-vesicular imaging in the assessment of obstructive male infertility are useful to standardize the US examination, focus on US abnormalities most associated with abnormal semen parameters in an evidence-based manner, and provide a standardized report to patients.

A-0088 HYPOGONADISM AND SEXUAL FUNCTION IN MEN AFFECTED BY ADRENOCORTICAL CARCINOMA UNDER MITOTANE THERAPY

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Adrenocortical carcinoma (ACC) is a rare and aggressive tumor. ACC male patients under adjuvant mitotane therapy (AMT) frequently develop hypogonadism. However, only a few studies reported the prevalence and type of hypogonadism. In addition, sexual function has never been assessed in this setting. The aim of this study was to evaluate in AMT treated ACC patients the changes in luteinizing hormone (LH), Sex Hormone Binding Globulin (SHBG), total testosterone (TT) and calculated free testosterone (cFT), the prevalence and type of hypogonadism, and sexual function, the latter before and after androgen replacement therapy (ART).

Since patients under AMT show a relevant increase in SHBG levels, in this study hypogonadism was diagnosed using cFT instead of TT, to increase the specificity of T deficiency detection (low cFT levels) even in men with apparent normal TT levels. A cFT < 225 pmol/l was considered to define hypogonadism, while a LH cut-off of 9.4 U/l was used to distinguish between hypo- and hyper-gonadotropic hypogonadism. According to the largely accepted definition derived from the European Male Ageing Study, hypogonadism was defined as hypogonadotropic, hypergonadotropic or compensated. Sexual function was evaluated using the International Index of Erectile Function-15 (IIEF-15) questionnaire; in particular, erectile dysfunction (ED) was defined for an IIEF-15-erectile function domain score < 22 (avoiding “mild ED” [score 25-22] and identifying more relevant clinical ED).

LH, SHBG, TT and cFT were assessed in ten surgically treated ACC male patients (43.0 ± 14.2 years) at baseline (T0) and six (T1), twelve (T2), and eighteen (T3) months after AMT. At T3, ART was initiated in eight hypogonadal patients, and LH, SHBG, TT and cFT levels were evaluated after six months (T4). In six patients who agreed to be investigated for sexual function, it was evaluated before (T3) and after (T4) ART.

At baseline, secondary hypogonadism and gynecomastia were detected in three patients (30%), while no patient complained of sexual dysfunction. Under AMT we observed higher SHBG and LH and lower cFT

levels at T1-T3 compared to T0 (all p<0.05). At T3, hypergonadotropic hypogonadism and ED were detected in 80% and 83.3% of cases. Three patients with overt hypogonadism and gynecomastia underwent treatment with DHT gel, while five patients with overt hypogonadism and without gynecomastia underwent treatment with T gel. At T4, we observed a significant cFT increase in men treated with T gel (while in patients under DHT-treatment, TT and cFT levels were obviously not considered), and a significant improvement in IIEF-15 total and subdomains scores and ED prevalence (16.7%) in men under ART.

In conclusion, AMT was associated with hypergonatropic hypogonadism and ED, while ART led to a significant improvement of cFT levels and sexual function in the hypogonadal ACC patients. Therefore, we suggest to evaluate LH, SHBG, TT and cFT and sexual function during AMT, and start ART in the hypogonadal ACC patients with sexual dysfunction.

A-0090 Prediction of clinical pregnancy outcomes using VideoMAE pre-training and multiple instance learning on sperm videos

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Background: Conventional semen analysis has limited ability to predict clinical pregnancy outcomes due to subjective assessments, poor correlation with fertility, and reliance on summary parameters. We developed a novel AI approach using VideoMAE self-supervised pre-training and hierarchical attention multi-instance learning (MIL) to directly predict pregnancy from live sperm motility videos under weak supervision.
Methods: We extracted 60,377 raw videos and generated 819,548 128×192 sperm-centered sub-videos using YOLOv5 detection. VideoMAE was pre-trained with 50% tube masking to learn spatio-temporal representations label-free. For 158 samples with known outcomes (38 pregnant, 120 non-pregnant), the frozen encoder extracted features from 8×8 grid sub-segments per video. Hierarchical attention MIL aggregated features first within videos, then across videos per sample to predict pregnancy probability. Performance was assessed via 5-fold cross-validation (ROC-AUC, PR-AUC, accuracy, sensitivity, specificity) and compared against Logistic Regression and XGBoost baselines using nine conventional semen parameters.

Results The model achieved mean ROC-AUC 0.68 ± 0.049, PR-AUC 0.58 ± 0.074, accuracy 0.74 ± 0.067, sensitivity 0.59 ± 0.195, and specificity 0.80 ± 0.124, significantly outperforming traditional baselines (ROC-AUC ~0.54, PR-AUC ~0.43–0.48). It showed high specificity but moderate sensitivity.

Conclusion VideoMAE pre-training combined with hierarchical attention MIL effectively captures predictive spatio-temporal patterns from sperm videos, substantially improving pregnancy outcome prediction over conventional parameters. This weakly supervised framework offers a promising, objective tool for male fertility assessment.

A-0091 A novel marker for sperm quality - semen antioxidant capacity is associated with ICSI outcomes

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Using the MiOXSYS system, it is possible not only to assess semen oxidative stress (static oxidative-reductive potential - sORP), but also semen antioxidant capacity (cumulative oxidative-reductive potential - cORP). These measurements provide information about the redox status of semen samples and are promising markers for sperm quality assessment, although their clinical value has not yet been fully established.
We evaluated sORP and cORP in 244 semen samples used for ICSI cycles at a single centre, examining their associations with semen parameters, embryological outcomes, and clinical pregnancy. To our

knowledge, only a very few studies have addressed these issues so far, and they included only sORP data and smaller numbers of patients than our study. The relationship between sORP and cORP was assessed by Pearson correlation and univariable linear regression. Bimodality of the cORP distribution was confirmed with Hartigan's dip test. Associations between semen ORP, semen parameters and embryological outcomes were assessed by multivariable linear regression, while pregnancy outcomes were assessed with multivariable logistic regression.

sORP and cORP were inversely correlated: each 1-unit increase in cORP was associated with a 0.298 mV reduction in sORP (95% CI: −0.427 to −0.169; $p < 0.0001$), confirming that samples with greater antioxidant reserve show lower oxidative stress.

The cORP distribution was strongly bimodal, and was divided in a low antioxidant capacity group (n = 148) and a high antioxidant capacity group (n = 96). Samples with high antioxidant capacity (High cORP) showed significantly higher sperm concentration (62.84 vs 48.93 M/mL; $p = 0.001$), higher progressive motility (54.44% vs 48.74%; $p = 0.019$), and lower sORP (35.10 vs 45.17 mV; $p < 0.001$) compared to the Low cORP group. In univariable regression, higher cORP was positively associated with both sperm concentration ($\beta = 0.37$, $p = 0.0029$) and motility ($\beta = 0.17$, $p = 0.0032$). Conversely, higher sORP was negatively associated with both sperm concentration ($\beta = -2.86$, $p < 0.001$) and motility ($\beta = -0.95$, $p < 0.001$). Together, these findings indicate that ORP represents a marker of semen quality.

Regarding embryological outcomes, cORP showed no association to fertilization rates, but showed a positive association with the number of blastocysts ($\beta = +0.024$, $p = 0.046$, meaning 1.1 blastocyst more in cycles with the highest sperm cORP as compared to the lowest semen cORPs). sORP showed no association to any of the analyzed outcomes.

The high antioxidant capacity group showed odds ratios 1.651 for pregnancy, though without reaching statistical significance (p=0.2). Again, sORP did not show any association with pregnancy outcomes. This analysis was limited by the reduced dataset available for the pregnancy outcome (n = 120), calling for additional evaluations in a broader dataset.

In conclusion, semen antioxidant capacity (cORP) is positively associated with conventional semen quality parameters, and shows promising associations with key embryological and clinical outcomes, while sORP showed negative associations with semen quality but no significant associations with embryological or pregnancy outcomes. In light of this, semen cORP shows potential as a novel complementary tool in the male infertility workup.

A-0092 Metabolic dysfunction–associated steatotic liver disease and testosterone serum levels: converging evidence from real-world observational data and meta-analysis

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Background: Low testosterone has been increasingly associated with metabolic dysfunction–associated steatotic liver disease (MASLD), yet the strength, direction, and clinical relevance of this relationship remain incompletely defined, particularly with respect to liver fibrosis.

Aim of the study: To investigate the association between male hypogonadism and MASLD through a dual approach, combining a real-world observational study with a systematic meta analysis of the available literature, and to explore the potential impact of testosterone replacement therapy (TRT).

Methods: A prospective, cross-sectional, case–control observational study was carried out, including 75 men referred for sexual dysfunction or infertility, stratified into hypogonadal (n=59) and eugonadal (n=16)

groups according to baseline testosterone serum levels. MASLD was assessed using indirect indices (Hepatic Steatosis Index, Fatty Liver Index), qualitative ultrasound, and controlled attenuation parameter (CAP), while liver fibrosis was evaluated by transient elastography and 2D shear-wave elastography. Hypogonadal men were further classified according to TRT status.

The testosterone/MASLD relationship was subsequently assessed in a meta-analysis of 28 cross-sectional and longitudinal studies available in the literature.

Results: MASLD prevalence was significantly higher in hypogonadal men than in controls when combining ultrasound and biochemical criteria (85.5% versus 50.0%, p=0.023). Liver fibrosis, assessed by imaging techniques, was detected exclusively in hypogonadal patients (18.6% versus 0%, p=0.057). Body mass index (BMI) emerged as the strongest predictor of both MASLD and fibrosis (p<0.001 and p=0.011), particularly in testosterone-naïve men (p<0.001 and p=0.033), whereas this association was attenuated in patients receiving TRT (p=0.166 and p=0.130). The meta-analysis (1636 hypogonadal versus 4587 eugonadal men) confirmed a significantly higher prevalence of MASLD among hypogonadal men compared with eugonadal controls (p=0.001), independent of age (p=0.274) and BMI (p=0.219).

Conclusions: This dual-approach study confirms a bidirectional association between male hypogonadism and MASLD. Real-world data support meta-analytic evidence and suggest that testosterone deficiency contributes to hepatic steatosis and fibrosis mainly through increased adiposity, while TRT may mitigate liver risk by modulating body composition and metabolic dysfunction.

Disclosure of interest: None declared

A-0094 Advanced Sperm Selection in Previously failed ICSI : Magnetic-Activated Cell Sorting and Microfluidic Sperm Chips Approaches

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Background: Infertility affects about 15% of couples worldwide, with male factors accounting for 30–50% of cases. Intracytoplasmic sperm injection (ICSI) is the main treatment for severe male infertility, but sperm quality still impacts success rates. Poor sperm parameters reduce fertilization, embryo quality, and live birth rates in ICSI cycles. Advances in sperm selection—such as electrophoretic separation, IMSI, microfluidic sorting, and magnetic-activated cell sorting (MACS)—have improved assisted reproductive technology (ART) outcomes. This study compares MACS and microfluidic sperm sorting with conventional density gradient centrifugation (DGC) for ICSI and pregnancy outcomes.

Methods: This prospective experimental study included 362 couples with male-factor infertility, all undergoing ICSI. Participants were divided into three groups by sperm preparation method: MACS (n=137), microfluidic chip (n=75), and DGC (n=150). We assessed all three groups for fertilization rate, embryo data and quality, and pregnancy outcome.

Results: The MACS group had higher pregnancy rates in patients aged ≥37 years with ≥3 failed ICSI cycles than the DGC group (66.7% vs. 35%, p = 0.0262). Overall pregnancy rates were also higher for MACS than DGC (67.6% vs. 48.9%, p = 0.0441). For patients with advanced maternal age (≥37 years) and low ovarian reserve (AMH <1), clinical pregnancy rates were 44.0% with MACS and 28.6% with DGC. Results showed similar clinical pregnancy rates between the microfluidic chip and DGC groups in patients aged ≥37 years (41.4% vs. 44.7%, P = 0.7793).

Pregnancy outcomes were similar between the DGC and microfluidic chip groups for overall rates (67.3% vs. 65.3%, P = 0.7647). In patients with AMH < 1 ng/mL, pregnancy rates were 45.0% for microfluidic chips and 41.0% for DGC (P = 0.7719).

Conclusion: Our findings show that MACS outperformed DGC and microfluidic chips, especially in couples with previous ICSI failure and advanced maternal age, resulting in higher clinical pregnancy rates. We recommend using MACS rather than microfluidic chips in subsequent cycles; however, more research with larger sample sizes is needed to evaluate new sperm selection techniques.

A-0097 Metabolic Syndrome in Men from Infertile Couples: ReproUnion Biobank and Infertility Cohort (RUBIC)

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Male fertility is recognized as a marker of general health, including future cardiometabolic disease risk. Metabolic syndrome (MetS), characterized by obesity, dyslipidemia, hypertension and impaired insulin sensitivity, is a strong predictor of cardiovascular disease and premature mortality. Infertility evaluation may therefore offer a unique opportunity to detect previously unrecognized systemic disease in young men.

We aimed to determine the prevalence of MetS among men undergoing infertility evaluation and to define seminal and endocrine parameters helping identify individuals at increased risk.

We included 669 men enrolled between 2021 and 2025 in the ReproUnion Biobank and Infertility Cohort (RUBIC), a binational clinical cohort and biobank established in Denmark and Sweden to investigate causes and mechanisms of infertility and to improve reproductive outcomes.

MetS was defined according to IDF criteria: waist circumference ≥94 cm plus ≥2 metabolic abnormalities (triglycerides ≥1.7 mmol/L, HDL cholesterol <1.0 mmol/L, blood pressure ≥130/85 mmHg or treatment, fasting glucose ≥5.6 mmol/L or diabetes). Semen volume, sperm concentration, total sperm count, motility, and morphology were assessed according to WHO 2021 manual. Blood samples, fasting before 10 am, were collected for analysis of testosterone, follicle-stimulating hormone, luteinizing hormone, sex hormone–binding globulin, glucose, insulin and blood lipids. As controls served a population-based cohort of healthy men (n=199) identified through the Swedish population register. ROC analyses were applied to identify threshold levels of predictors providing optimal sensitivity and specificity for MetS. Inter-group differences were assessed using the Mann-Whitney U test.

Two percent of RUBIC men were receiving treatment for MetS at the time of investigation. However, 17% of participants presented with previously undiagnosed MetS. The MetS prevalence of 19 % among men from infertile couples was not statistically significantly different from the 18 % observed in controls. Men with MetS had statistically significantly lower total testosterone levels (13.8 vs.18.2 nmol/L, $p < 0.001$) and statistically significantly higher insulin levels than men without MetS ($p < 0.001$). Testosterone < 14.75 nmol/L was associated with a fourfold increased risk of MetS (sensitivity 61%; specificity 72%). Applying insulin levels for identifying MetS high-risk group gave higher sensitivity (83%) and specificity (76%). Total sperm count was 24% lower in men with MetS ($p = 0.03$). The MetS subjects did not differ from those without MetS in sperm morphology, total or progressive motility or DNA fragmentation index. None of the semen parameters gave a statistically significant AUC in relation to MetS risk.

A substantial proportion of men presenting for infertility evaluation have undiagnosed MetS. Although the prevalence of MetS was not statistically significantly different from that in an age matched control group, this category of men represents an easily accessible group of potential screening objects. Using a testosterone threshold of 14.75 nmol/l would allow identification of 60% of MetS cases. The association between MetS, reduced testosterone levels and impaired spermatogenesis, indicate shared pathophysiological mechanisms linking metabolic and reproductive dysfunction. Infertility assessment represents a window of opportunity for early cardiometabolic risk identification and preventive intervention in young men.

A-0102 Clinical significance of the infiltration of leukocytes into seminal plasma and the downstream effects on sperm cells

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Research on leukocytes (white blood cells) in human semen has been ongoing since the 1980's, yet clinically standardized methods to diagnose leukocytospermia remain limited. The World Health Organization defines a normal level of leukocytes in human semen as less than 1 million cells per ml and

levels that exceed 1 million cells per ml is referred to as leukocytospermia. This is a localised immune event that is typically due to infection or inflammation. The primary mechanism involves the release of reactive oxygen species (ROS) by activated leukocytes, which causes oxidative stress that impairs spermatozoa (sperm) cell motility, increases DNA fragmentation and reduces fertility potential. A clinically standardised point-of-care test for leukocytospermia is needed to enable early detection and timely management, which may improve reproductive outcomes. This study explores the clinical significance of leukocyte infiltration into seminal plasma and compares it with the peripheral blood profile. This is to determine whether leukocytospermia can be detected at a systemic level, raising the possibility that the seminal leukocyte patterns may correspond to immune signatures in peripheral blood.

This study includes 50 participants that will provide semen and blood samples. Optimization will be conducted using samples from 10 participants. To optimize study procedures, semen and blood samples are obtained from donors without known symptoms of leukocytospermia. Basic spermiograms are performed, noting the seminal pH, volume, viscosity, sperm concentration and semen colour. The peroxidase-positive test is used to evaluate the current gold standard leukocyte counting procedure. A lab-based workflow is then applied, in which leukocytes are extracted from semen via CD45 labelling, magnetic bead separation using a MACS column, and stained using TBNK panel labelling. Flow cytometric analysis is used for classification and quantification of leukocytes. The same workflow is applied to isolate leukocytes from peripheral blood. Additionally, Hybrispot Sexually Transmitted Infection (STI) testing is conducted on samples to better define contributors to semen leukocyte numbers.

Data collection is ongoing, and preliminary findings indicate that for peroxidase-positive testing leukocyte counts of less than 1 million cells per ml was observed. Through flow cytometry analysis, the peripheral blood sample's CD45+ leukocytes (total leukocyte population) consisted of 6.68% granulocytes, 16.2% monocytes, and 72.0% lymphocytes. The neat semen sample's CD45+ leukocytes consisted of 34.0% granulocytes, 26.6% monocytes and 28.7% lymphocytes. Hybrispot STI testing revealed positivity for Mycoplasma hominis and Ureaplasmas (urealyticum/parvum) in seminal plasma fractions.

Preliminary analyses established a foundation for optimized leukocyte detection and subset characterization in semen and peripheral blood. Interpretation is constrained by the limited marker panel currently available therefore future analyses will include additional markers using a TBNK based panel. To further evaluate whether there are similarities between peripheral blood leukocytes and the human semen profile, reactive oxygen species and cytokine levels will be measured to assess leukocyte activity. DNA fragmentation and apoptosis will be measured to confirm any damage to sperm cells resulting from this activity.

A-0103 Nrf2 evaluation in human semen and its relationship with F2 -Isoprostanes as marker of lipid peroxidation

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Introduction. Oxidative stress (OS) has been increasingly recognized as a central pathogenic mechanism contributing to reproductive dysfunction; indeed, it has been strongly associated with altered semen parameters and with male reproductive disorders as varicocele, urogenital infections, and, in part, idiopathic infertility. The present study aimed to evaluate OS levels in semen of selected groups of patients through the quantification of F2-IsoProstanes (F2-IsoPs), well known marker of lipid peroxidation, and to investigate the variation of Nrf2, a transcription factor involved in the antioxidant protection of the cell, in order to explore their potential role in the mechanisms underlying OS-mediated male infertility.

Materials and Methods. Sixty semen samples were analyzed and most of the subjects attended the Department of Molecular and Developmental Medicine, University of Siena (Italy). The study included men with unknown reproductive potential. The participants were divided in:

- 22 with clinically diagnosed varicocele (V group);
- 23 with urogenital infections (UI group);
- 15 unknown reproductive or systemic pathologies (USF group);

A control cohort of 19 proven fertile men (F group, with at least one child within the preceding three years) was additionally recruited.

The semen analysis was performed following World Health Organization guidelines (WHO, 2021); samples were centrifuged to separate seminal plasma from spermatozoa, and then stored at −80 °C until use. F2-IsoP levels were quantified in seminal plasma and Nrf2 both in seminal plasma and in spermatozoa through ELISA kits. Furthermore, Nrf2 molecular pathway, with Keap1, Bach1, HO-1 and GPX4, was assessed in spermatozoa by qRT-PCR.

Results. Considering all study participants, both seminal plasma and sperm Nrf2 levels showed a significant positive correlation with F2-IsoP concentration ($p<0.001$) and a significant negative correlation with sperm vitality. Nrf2 sperm levels were also negatively correlated with progressive motility ($p<0.05$). Sperm vitality, normal morphology, and progressive motility exhibited a significant negative correlation with F2-IsoP concentration ($p<0.001$). Comparing the variables in the different groups of studied cases, F2-IsoP seminal levels were lower in F compared to the other groups and in USF compared to V group. Seminal plasma Nrf2 levels did not differ significantly among groups, while sperm Nrf2 concentration resulted significantly lower in F than that measured in V and UI ($p<0.001$) and in USF ($p<0.05$). Also qRT-PCR results confirmed an upregulated Nrf2 pathway in V and UI groups with the increase mRNA for HO-1 and GPX4.

Discussion and conclusions: The present study highlights the relevance of integrating validated OS markers with molecular antioxidant pathways in the assessment of male reproductive health. The concomitant increase in F2-IsoP levels and sperm Nrf2 concentrations, confirmed by upregulation of Nrf2 pathway detected by RT-PCR, suggests a OS-driven activation of the Nrf2 pathway as a compensatory sperm cell response to OS. Notably, the differences of these markers were consistently observed across the four distinct analyzed groups, supporting the interplay between F2-IsoPs and Nrf2 as complementary biomarker for oxidative status assessment in male reproductive health.

A-0104 A CASE REPORT OF DIAGNOSTIC ODYSSEY OF A 46,XY DIFFERENCE OF SEX DEVELOPMENT: ONE OF THE MANY FACES OF THE REIFENSTEIN SYNDROME

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According to 2005 Chicago consensus (Lee et al., 2006), differences or disorders of sex development (DSDs) refer to a heterogenous group of congenital conditions affecting human sex determination and differentiation. While most children born with DSDs are identified shortly after birth, certain conditions may remain undetected until adulthood when an individual experiences primary infertility. Consequently, their healthcare necessitates a multidisciplinary approach that transcends traditional surgical intervention, recognizing reproductive health as an essential component of patient-centred care. The case of this 37-year-old male patient, referred to our Fertility Centre for primary infertility, serves as a poignant example of the clinical and diagnostic complexities associated to partial androgen insensitivity syndrome (PAIS), also known as Reifenstein Syndrome. Initial physical examination revealed gynoid body habitus and sparse facial, axillary and pubic hair, alongside evidence of prior bilateral mastectomy, likely performed to address previous gynecomastia. Genital examination identified a DSD characterized by a micropenis, a bifid scrotum and an incomplete fusion of labioscrotal folds. A testis of normal consistency was palpable in the left labioscrotal fold, while a soft, smaller ectopic testis was in the right inguinal canal. Several semen analyses found only rare, immotile spermatozoa. Comprehensive imaging, including pelvic ultrasonography (US) and Magnetic Resonance Imaging (MRI), provided further clarity; the prostate measured 10 ml without cystic or intraglandular abnormalities, and the seminal vesicles appeared normal. However, the US revealed bilateral atrophied epididymis. The volume of the testis measured at the US was 12,9 mL for the right ectopic testis and 15,3 mL for the left one. MRI findings confirmed the presence of both vas deferens with small microcystic dilatations at the prostate junction. Notably, while a vulva was present, the patient lacked internal female organs, and the micropenis was found to contain 2 corpora cavernous and no corpus spongiosum. The endocrine evaluation was indicative of androgen resistance, showing elevated levels of FSH (20,1 UI/L) and LH (22,4 UI/L), total testosterone: 20,84 µg/L, dihydrotestosterone: 0,86 ng/mL, AMH: 38,6 pmol/L, inhibin B: 36 pg/mL. Standard karyotyping found 46,XY. To confirm the diagnosis, Next-Generation Sequencing of the androgen receptor (AR) gene (reference genome GRCh37/hg19) found a recessive hemizygous X-linked mutation in the exon 5 of the AR gene: c.2305C>A, resulting in the amino acid substitution p.Leu769Met. In silico analysis strongly

supported the pathogenicity of this variant, as PolyPhen-2 predicted this variant to be “probably damaging” with a maximal score of 1.0. To address the couple’s reproductive goals, a left testicular sperm extraction was performed. The procedure was successful allowing the cryopreservation of approximately 20 motile spermatozoa per 10 µL, despite no spermatozoa in the epididymal aspiration. Following genetic counselling, the patient’s treatment plan incorporates in vitro fertilization using intracytoplasmic sperm injection. To date, the patient has made no request for any corrective surgery. This report reinforces the status of codon 769 as a critical functional hotspot. Furthermore, our patient’s specific presentation corroborates the significant phenotypic clinical variability in PAIS described by Boehmer et al. (2001), highlighting the ongoing challenge of establishing strict genotype-phenotype correlations in androgen insensitivity.

A-0105 Protective Effects of Neuropeptide W in Testicular Ischemia-Reperfusion Injury in Rats

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Testicular torsion is a urological emergency that may result in severe testicular damage and male infertility if not treated rapidly. Tissue injury mainly occurs due to ischemia followed by reperfusion, which induces oxidative stress, inflammation and apoptosis in testicular tissue. Neuropeptide W (NPW) is a neuropeptide known to exert antioxidant and anti-inflammatory effects in several experimental models; however, its possible protective role in testicular torsion–detorsion (T/D) injury has not yet been investigated. In this study, the potential protective effects of NPW were evaluated in an experimental rat model of testicular torsion. Male Sprague Dawley rats were randomly divided into six experimental groups representing short-term (n=24) and long-term (n=24) experimental periods: short-term sham (n=8), short-term T/D treated with saline (n=8), short-term T/D treated with NPW (n=8), long-term sham (n=8), long-term T/D treated with saline (n=8) and long-term T/D treated with NPW (n=8). Under ketamine–xylazine anesthesia, the left testis was exposed through a midline abdominal incision and rotated 270° around the spermatic cord axis to induce torsion. After 120 minutes of torsion, detorsion was performed by rotating the testis in the opposite direction to allow reperfusion. NPW (3 µg/kg) or saline was administered intraperitoneally at the end of the ischemic period and one hour after detorsion, and in the long-term groups, the treatment was continued for two additional days with two injections per day. In the short-term groups, animals were sacrificed four hours after detorsion, whereas in the long-term groups animals were sacrificed seventy-two hours later. Testicular blood flow was monitored using laser Doppler flowmetry. Epididymal sperm parameters including sperm count, motility and morphology were evaluated. Histological examination of testicular tissues was performed using hematoxylin and eosin and periodic acid Schiff staining. Reactive oxygen species were assessed in testicular tissue using biochemical methods. Statistical analysis was performed using one-way ANOVA and Tukey’s multiple comparison tests. In the short-term groups, testicular blood flow was decreased in the saline-treated T/D group and increased in the NPW treated T/D group ($p<0.05$), whereas no significant difference was observed among the long-term groups. Testicular damage characterized by degenerated seminiferous tubules, inflammatory cell infiltration in the tunica albuginea, and impaired sperm parameters was observed in both short-term and long-term saline-treated T/D groups, whereas these alterations were reduced with NPW treatment. Chemiluminescence analysis demonstrated a significant increase in reactive oxygen species (luminol and lucigenin) levels in saline-treated T/D groups compared with the sham groups in both short-term and long-term T/D groups, whereas NPW treatment markedly reduced these elevations ($p<0.05$). These findings suggest that NPW may exert protective effects against testicular T/D injury by improving testicular blood flow and sperm parameters and reducing oxidative stress-related testicular damage.

Keywords:
Neuropeptide W, testicular torsion, ischemia-reperfusion injury, oxidative stress, rat

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A-0108 A Nomogram for Predicting Biochemical Hypogonadism in Men With Chronic Spinal Cord Injury

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Hypogonadism is a frequent complication in men with chronic spinal cord injury (SCI), with reported prevalence rates ranging between 25% and 40%. Despite yielding significant implications not only for metabolic health and rehabilitation trajectories but also musculoskeletal outcomes, hypogonadism in this specific population often results difficult to recognize and under-estimated. Indeed, routine biochemical screening is inconsistently implemented due to overlapping symptoms with neurological sequelae and logistical constraints in rehabilitation settings.

This study aimed to identify clinical predictors of biochemical hypogonadism and develop a simple risk stratification tool.

In this cross-sectional observational study, 124 men with chronic (>1 year) traumatic SCI and not receiving testosterone therapy were consecutively recruited at a rehabilitation center. Biochemical hypogonadism was defined as a total testosterone concentration < 3 ng/mL from a single morning sample. Routinely available clinical variables, including age, Body Mass Index (BMI), and comorbidities, assessed by the Charlson Comorbidity Index (CCI), were evaluated. A multivariable logistic regression model was used to develop a predictive nomogram, which was assessed for discrimination, calibration, and internal stability via bootstrap validation.

Biochemical hypogonadism was detected in 46 participants (37.1%). In the multivariable analysis, BMI (OR 1.16 per kg/m², 95% CI 1.01–1.33; p=0.037) and CCI (OR 1.39 per point, 95% CI 1.10–1.76; p=0.006) emerged as independent predictors of testosterone deficiency, while age did not retain statistical significance after adjustment. The resulting nomogram, incorporating BMI and CCI, demonstrated good discrimination (AUC 0.77, 95% CI 0.69–0.85) and acceptable calibration. Using a predicted probability threshold of 31%, the tool achieved a sensitivity of 70% and a specificity of 76% and internal validation confirmed the robustness of the model.

In conclusion, biochemical hypogonadism is highly prevalent in the chronic SCI population and is closely linked to adiposity and global health status rather than age alone. Our nomogram, based on BMI and comorbidities, provides a user-friendly screening tool to help clinicians prioritize biochemical testing and early endocrine evaluation within standard rehabilitation workflows.

A-0113 Circadian Desynchrony Impairs Mitochondrial Bioenergetics During Spermiogenesis and Compromises Sperm Function

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Declining male fertility represents a growing global health concern, underscoring the need to better define molecular mechanisms regulating reproductive competence. Our previous findings indicated an association between circadian rhythm disruption and reduced male reproductive potential; however, the underlying mechanisms remain poorly understood.

Here, we investigated the effects of circadian desynchrony on spermiogenesis, focusing on mitochondrial bioenergetics, dynamics, and biogenesis. Circadian disruption was induced in adult rats by repeated photoperiod alterations (2 days constant light, 2 days constant darkness, and 3 days 14:10 h light:dark) over two months. Round (RS) and elongated spermatids (ES) from seminiferous tubules, as well as epididymal spermatozoa (Sp), were analyzed.

Circadian desynchrony significantly reduced circulating testosterone levels and decreased the number of ES at stage VII of the seminiferous epithelium, a metabolically demanding and androgen-sensitive phase of spermiogenesis. Functional analysis demonstrated that ATP production in RS and ES is predominantly dependent on mitochondrial respiration, as oligomycin reduced ATP levels by ~90%, whereas spermatozoa exhibited partial dependence (~50–60%). Notably, circadian desynchrony induced a stage-specific mitochondrial response, characterized by increased ATP production in RS, followed by a decline

in ES, indicating a transition from compensatory mitochondrial activation to energetic insufficiency. In spermatozoa, ATP levels decreased to oligomycin-resistant levels, consistent with impaired mitochondrial respiration and a shift toward glycolytic ATP production.

These functional changes were accompanied by decreased mitochondrial membrane potential (Δψm) in RS, and ES, despite increased mitochondrial content in RS and unchanged levels in ES, while spermatozoa exhibited reduced mitochondrial content. At the molecular level, circadian desynchrony altered the expression of key regulators of mitochondrial biogenesis (PGC-1α, NRF1, TFAM, CYTC), dynamics (MFN2, OPA1), and mitophagy (PINK1, PRKN), indicating disruption of mitochondrial quality control pathways.

Importantly, these alterations translated into impaired sperm function, as evidenced by reduced motility and a diminished progesterone-induced acrosome reaction.

In conclusion, circadian desynchrony disrupts mitochondrial regulation in a stage-specific manner during spermiogenesis, leading to impaired bioenergetics and compromised sperm function. These findings identify mitochondrial dysfunction as a central mechanistic link between circadian disruption and reduced male fertility.

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A-0115 Congenital adrenal hyperplasia with bilateral testicular adrenal rest tumors and azoospermia: a case report and literature review

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Background: Congenital adrenal hyperplasia (CAH) is an autosomal recessive disorder of adrenal steroid biosynthesis, most commonly caused by 21-hydroxylase deficiency. Testicular adrenal rest tumors (TARTs) are a recognized complication of CAH and may impair spermatogenesis, presenting in adult men as infertility or azoospermia. We present a case of CAH complicated by bilateral TARTs and azoospermia and review the literature to summarize its clinical features and management strategies.

Methods: We retrospectively analyzed the clinical data of a 22-year-old man presenting with infertility, including endocrine profiles, imaging findings, genetic testing results, and treatment course, and reviewed the relevant literature. This study was approved by the ethical committee of the Affiliated Hospital of Xuzhou Medical University (no. XYFY2025-KL658-01).

Results: The patient presented with azoospermia, low gonadotropin levels, and markedly elevated serum 17α-hydroxyprogesterone and adrenocorticotropic hormone levels. Imaging revealed diffuse bilateral adrenal enlargement and hypoechoic nodules in both testes. Genetic testing identified pathogenic variants in the CYP21A2 gene, confirming the diagnosis of CAH due to 21-hydroxylase deficiency. Glucocorticoid replacement therapy was initiated to suppress ACTH secretion, in combination with gonadotropin therapy with human menopausal gonadotropin (HMG) and human chorionic gonadotropin (HCG). After treatment, the patient showed gradual improvement in hormone levels, increased testicular volume, and the appearance of motile sperm on semen analysis. His partner successfully conceived during follow-up.

Conclusion: CAH complicated by TARTs is an important cause of male infertility. In patients presenting with azoospermia accompanied by hypogonadotropic hypogonadism or testicular nodules, CAH should be considered in the differential diagnosis. Standardized glucocorticoid replacement therapy combined with gonadotropin treatment may partially restore spermatogenesis and improve reproductive outcomes.

A-0116 Advances in biomarker research in premature ejaculation

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Background:Premature ejaculation (PE) is one of the most common male sexual dysfunctions and remains a clinically heterogeneous condition, particularly when lifelong PE (LPE) and acquired PE (APE) are considered separately. Current assessment relies mainly on sexual history, intravaginal ejaculatory latency time, and patient-reported measures, while objective biological tools for phenotyping and treatment stratification remain limited. Biomarker research has therefore emerged as a potential approach to improve mechanistic understanding and support more objective evaluation of PE.

Methods:A narrative review of published studies on PE-related biomarkers was performed. Candidate biomarkers were grouped into three domains: neurotransmitter and autonomic nervous system-related markers, endocrine and metabolic markers, and hematologic or other biochemical markers. Their reported associations with PE subtype, symptom severity, possible pathophysiological pathways, and therapeutic implications were summarised.

Results: Available evidence suggests that neurotransmitter-related markers,including serotonin (5-HT), 5-hydroxyindoleacetic acid (5-HIAA), norepinephrine, dopamine, gamma-aminobutyric acid, and glutamate, may reflect altered central ejaculatory control and sympathetic overactivity in PE. Endocrine and metabolic markers, such as thyroid hormones, prolactin, sex hormones, cortisol, melatonin, leptin, and metabolic syndrome-related parameters, appear particularly relevant in APE and may help identify potentially reversible contributors. Additional markers, including vitamin D, vitamin B12, folate, homocysteine, nitric oxide, brain-derived neurotrophic factor, and mean platelet volume, may provide complementary information on nutritional status, inflammation, stress response, and peripheral biological changes. However, findings across studies remain heterogeneous, and no single biomarker has shown sufficient specificity, reproducibility, or clinical robustness for routine diagnostic use. Peripheral measurements also do not consistently reflect central neurobiological mechanisms.

Conclusion: Biomarker research provides a promising framework for improving the biological stratification of PE, but current candidates remain investigational. At present, these markers may be more useful for mechanistic exploration, risk profiling, and multimodal phenotyping than for standalone diagnosis. Future studies should adopt standardised PE definitions, stopwatch-based IELT and validated questionnaires as core outcomes, strict separation of LPE and APE, standardised sampling and laboratory procedures, and adequate adjustment for major confounders such as sleep, stress, medications, and metabolic or endocrine comorbidities. Combined biomarker panels may offer greater translational value for subtype classification, treatment selection, and follow-up.

A-0120 Postoperative Hormonal Changes After Surgical Sperm Retrieval in Men with Infertility

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Background:
Surgical sperm retrieval techniques, including testicular sperm aspiration (TESA) and microdissection testicular sperm extraction (micro-TESE), are widely used in men with severe male factor infertility. However, the impact of these procedures on postoperative reproductive hormonal profiles is underreported and incompletely understood. The current study aims to evaluate changes in reproductive hormones following surgical sperm retrieval and to compare these changes according to the type of retrieval procedure and the success of sperm retrieval.

Methods:
This retrospective study screened patients with azoospermia who underwent ICSI at our center between January 2014 and January 2025. Men who underwent surgical sperm retrieval at our center with complete pre-operative and 6 months' post-operative hormonal profile (testosterone, FSH, LH, estradiol, and

prolactin) were included in the study. Hormonal changes (postoperative minus preoperative values) were analyzed for the overall cohort and compared according to the type of sperm retrieval (micro-TESE vs TESA) and the success of sperm retrieval. Numerical value are reported as median (IQR). Continuous variables were reported as median (interquartile range) and compared using the Mann–Whitney U test.

Results:
A total of 135 men were included, of whom 66 underwent TESA and 69 micro-TESE. The overall sperm retrieval rate was 58.52%. Overall, surgical sperm retrieval was associated with decreases in testosterone and estradiol levels and increases in FSH and LH levels. The median change in testosterone was −4.68 (l9.48), in FSH 1.69 (7.80), in LH 1.40 (5.75), and in estradiol −20.00 (72.15), while prolactin showed minimal change.

When stratified by surgical technique, micro-TESE was associated with a significantly greater increase in FSH compared with TESA (median 4.00 [11.91] vs 0.68 [5.13], p<0.001) and a greater decrease in estradiol (−37.76 [67.00] vs −13.00 [54.30], p=0.016). Changes in testosterone (−5.99 [8.63] vs −4.66 [10.53], p=0.055), LH (2.17 [7.75] vs 0.35 [4.77], p=0.068), and prolactin (−1.00 [129.60] vs −2.70 [114.03], p=0.728) did not differ significantly between procedures.

When analyzed according to sperm retrieval outcome, men without successful sperm retrieval demonstrated a greater decrease in testosterone (−6.10 [10.11] vs −4.66 [8.30], p=0.011) and a greater increase in LH (2.17 [7.82] vs 0.75 [4.97], p=0.047) compared with those with successful sperm retrieval. Changes in FSH (4.00 [10.06] vs 0.97 [5.62], p=0.053), estradiol (−33.00 [85.10] vs −24.85 [64.70], p=0.802), and prolactin (−16.90 [113.30] vs −0.50 [171.15], p=0.693) were not significantly associated with sperm retrieval success.

Conclusions:
Surgical sperm retrieval is associated with measurable changes in reproductive hormones. Micro-TESE appears to have a greater impact on FSH and estradiol levels compared with TESA, indicating more testicular damage. Additionally, failure of sperm retrieval was associated with a decrease in testosterone and an increase in LH, reflecting the increased testicular damage with the extensive dissection. These findings highlight the importance of trying the less invasive SSR first in NOA before reverting to the more invasive micro-TESE.

A-0121 Can Serum FSH Predict Surgical Sperm Retrieval Success? A Retrospective Analysis

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Background
Predicting surgical sperm retrieval (SSR) success remains a major challenge in men undergoing sperm retrieval procedures. Serum follicle-stimulating hormone (FSH) is frequently used as a marker of testicular function; however, its ability to predict SSR outcomes remains controversial. The current study aims to evaluate whether serum FSH levels can predict surgical sperm retrieval success.

Methods
A retrospective analysis was conducted on 209 Azoospermic men undergoing surgical sperm retrieval. Patients were categorized according to serum FSH level (<8.6 vs >8.6 IU/L). Baseline characteristics included male age, female age, BMI, and testicular volume. The primary outcome was successful sperm retrieval. Outcomes were analyzed overall and stratified according to the retrieval technique (micro-TESE and TESA). Group comparisons for numerical and categorical variables were performed using independent t-tests and Chi-square tests, respectively. A pvalue<0.05 was considered statistically significant.

Results
The overall sperm retrieval rate was 52.2% (109/209). Patients with FSH <8.6 IU/L had significantly higher sperm retrieval success compared with those with FSH >8.6 IU/L (63.6% vs 45.5%, p = 0.011).

Baseline characteristics including male age, female age, and BMI were comparable between FSH groups. However, right testicular volume was significantly larger in men with FSH <8.6 (9.37 ± 5.62 mL vs 6.47 ± 3.53 mL, p = 0.026).

When stratified by sperm retrieval technique, sperm retrieval success in the micro-TESE group was 41.0% (16/39) in men with FSH <8.6 and 33.0% (29/88) in men with FSH >8.6 (p = 0.380). In the TESA group, success rates were 86.8% (33/38) in men with FSH <8.6 and 70.5% (31/44) in men with FSH >8.6, although this difference did not reach statistical significance (p = 0.074)

Conclusion
Lower serum FSH levels were associated with higher overall sperm retrieval success. However, when stratified by retrieval technique, FSH alone did not significantly predict outcomes for micro-TESE or TESA. These findings suggest that FSH may have limited predictive value as a standalone marker for SSR success.

A-0123 ICSI Outcomes in Absolute Asthenozoospermia: A Comparison of Ejaculated and Testicular Sperm

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Background:
Absolute asthenozoospermia is a rare and severe form of male infertility characterized by the complete absence of sperm motility in the ejaculate. Intracytoplasmic sperm injection (ICSI) remains the primary treatment option for these patients; however, the optimal sperm source, ejaculated or testicular, remains controversial. This study aimed to evaluate ICSI outcomes in men with absolute asthenozoospermia and to compare reproductive outcomes using ejaculated versus testicular sperm.

Methodology:
This was a retrospective chart review of 2073 cases who underwent ICSI for male factor infertility at our center between January 2014 and January 2025. Men diagnosed with absolute asthenozoospermia were included. Testicular sperm were used if no motile sperm were found after adding motility enhancers. Clinical and laboratory data were collected, including the ages of both partners, medical history, physical examination findings, semen analysis results, hormonal profiles, sperm retrieval procedures, and ICSI outcomes. Categorical variables were compared between the study groups using the chi-square test, whereas continuous variables were compared using Student's t-test. A two-sided p-value < 0.05 was considered statistically significant.

Results:
A total of 42 men with absolute asthenozoospermia were included, 8 using ejaculated and 34 testicular sperm. The mean male and female ages were 40.6 ± 9.9 and 32.6 ± 4.7 years, respectively. Baseline characteristics were also comparable between groups, except for AMH levels, which were significantly higher in the ejaculated sperm group (35.9 ± 19.8 vs 13.8 ± 12.9; p = 0.001). Clinical pregnancy occurred in 12 of 42 cycles (28.6%). Although pregnancy was more frequent with ejaculated sperm (50.0%) compared with testicular sperm (23.5%), this difference was not statistically significant (p = 0.195). No significant differences were observed between baseline demographic and hormonal characteristics between cycles with and without clinical pregnancy, including male age, female age, basal FSH, AMH, or estradiol levels.

The overall fertilization, cleavage, and embryogenesis rates were 53.8 ± 31.5%, 96.5 ± 16.9%, and 54.0 ± 32.3%, respectively. Comparing ejaculated to testicular sperm, no significant differences were observed between ejaculated and testicular sperm in fertilization rate (53.9 ± 31.6 vs 54.8 ± 31.9; p = 0.944), cleavage rate (85.7 ± 37.8 vs 99.0 ± 3.7; p = 0.065), or embryogenesis rate (58.8 ± 38.0 vs 54.9 ± 30.6; p = 0.788).

Conclusion:
In men with absolute asthenozoospermia undergoing ICSI, the use of ejaculated sperm resulted in

reproductive outcomes comparable to those obtained with testicular sperm; therefore, efforts should be made to find motile ejaculated sperm before subjecting the patient to invasive surgical sperm retrieval.

A-0124 ICSI Outcomes Across Different Levels of Normal Sperm Morphology: A Retrospective Analysis

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Introduction:
Sperm morphology is a key parameter in semen analysis and has traditionally been considered an indicator of male fertility potential. However, its predictive value for outcomes following intracytoplasmic sperm injection (ICSI) remains controversial, as the technique bypasses several natural selection barriers. This study aimed to evaluate the effect of different levels of normal sperm morphology on ICSI outcomes in a large retrospective cohort.

Methods:
This retrospective study included 1961 ICSI cycles performed at Hamad Medical Corporation (HMC), Qatar, between 2016 and 2024. Patients were categorized according to the percentage of normal sperm morphology into five groups: 0, 1, 2, 3, and ≥4 normal forms based on strict morphological criteria. Demographic data, hormonal parameters, and semen characteristics were collected, including male age, female age, semen volume, sperm concentration, total and progressive motility, normal morphology, FSH, AMH, and estradiol levels. ICSI outcomes evaluated included fertilization rate, cleavage rate, embryogenesis, and clinical pregnancy. Statistical analysis was performed using one-way ANOVA with Bonferroni post hoc tests for continuous variables and chi-square tests for categorical variables, with $p < 0.05$ considered statistically significant.

Results:
A total of 1961 ICSI cycles were analyzed. The mean male age was 36.9 ± 7.4 years, semen volume 2.78 ± 1.55 mL, sperm concentration 43.24 ± 36.73 million/mL, total motility 42.3 ± 18.7%, progressive motility 19.3 ± 19.2%, and normal sperm morphology 11.7 ± 12.1%. Female partners had a mean age of 32.6 ± 5.5 years, mean basal FSH 4.9 ± 2.9 IU/L, and mean AMH 28.9 ± 34.4.

When stratified by sperm morphology categories (0, 1, 2, 3, and ≥4 normal forms), fertilization rates were 61.29 ± 28.20%, 69.18 ± 25.27%, 65.91 ± 26.07%, 71.64 ± 23.64%, and 69.33 ± 24.98%, respectively, with no significant differences between groups ($p = 0.120$). Cleavage rates were 100.0 ± 0.0%, 98.57 ± 10.66%, 98.13 ± 12.67%, 99.64 ± 17.58%, and 95.67 ± 18.38%, showing a statistically significant difference ($p = 0.009$). Embryogenesis rates were 42.05 ± 30.36%, 56.20 ± 29.03%, 55.98 ± 29.65%, 55.33 ± 31.10%, and 52.12 ± 34.83%, respectively ($p = 0.168$). Clinical pregnancy rates were 39.1%, 37.1%, 32.0%, 37.1%, and 32.0%, respectively, with no significant difference between morphology groups ($p = 0.462$).

Post hoc analysis showed no significant differences between morphology groups for male age, female age, total FSH, basal FSH, AMH, fertilization rate, or embryogenesis. For cleavage rate, the 3 normal forms group demonstrated a slightly higher rate compared with the ≥4 normal forms group ($p = 0.026$). Sperm preparation method differed significantly across morphology groups ($p < 0.001$), with density gradient used in 69.1% of cycles, followed by swim-up (19.0%) and fertile plus (11.3%). However, sperm preparation method was not significantly associated with clinical pregnancy outcome ($p = 0.142$).

Conclusions
In this large retrospective cohort, sperm morphology showed limited predictive value for ICSI outcomes. These findings suggest that ICSI can largely overcome variations in sperm morphology, and morphology alone may have limited clinical significance in predicting treatment outcomes.

A-0125 Low free testosterone is independently associated with long-term mortality in men with chronic spinal cord injury

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Testosterone (T) deficiency is highly prevalent in men with chronic spinal cord injury (SCI), affecting up to half of the subjects of this population. While low T is associated with functional obesity, sarcopenia, and several other metabolic dysfunctions in SCI, its prognostic significance for long-term survival remains still unexplored.

This investigation aimed to determine whether the baseline calculated free testosterone (cFT) independently predicts all-cause mortality in men with chronic SCI.

We conducted a retrospective cohort study of 152 adult men with chronic (>1 year) traumatic SCI, admitted to a rehabilitation center between 2017 and 2023. Exclusion criteria included acute medical conditions and testosterone replacement therapy (TRT). Total T (TT), SHBG, and albumin were measured at baseline to derive cFT using the Vermeulen formula. Multivariable Cox proportional hazards regression models, adjusted for age, comorbidities (Charlson Comorbidity Index - CCI), BMI, albumin, and injury-specific variables, were used to identify predictors of all-cause mortality.

During a median follow-up of 62 months, 24 patients died (15.8%). Compared to survivors, deceased patients exhibited significantly lower cFT levels (mean 48.9 vs. 85.4 pg/mL, $p < 0.001$) and a higher prevalence of hypogonadism (83.3% vs. 19.5%, $p < 0.001$). In fully adjusted models, lower cFT remained an independent predictor of mortality (HR 0.98 per pg/mL, 95% CI 0.97–1.00; $p = 0.0096$). While TT was also associated with mortality in crude and partially adjusted models, it lost significance in the fully adjusted analysis, suggesting cFT is a more robust prognostic marker. ROC analysis identified an internally derived cFT threshold of 59.55 pg/mL (AUC 0.839; sensitivity 82%, specificity 85%). Kaplan–Meier curves showed significantly reduced survival for men below this threshold (log-rank $p < 0.0001$).

In conclusion, low cFT is a robust and independent predictor of all-cause mortality in men with chronic, clinically stable SCI. These findings suggest that cFT may serve as an integrative biomarker of systemic frailty in this population, reflecting the convergence of metabolic and functional decline. Assessing androgen status could improve risk stratification and provide a rationale for future clinical trials on TRT to improve long-term outcomes in SCI patients.

Poster Shift 2 (from Thu 12:00)

A-0129 Gut microbiota enterotypes in aging man with benign prostatic hyperplasia (BPH)

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Benign prostatic hyperplasia (BPH) is a prevalent, chronic, and progressive urological disorder that afflicts aging men. From a pathological perspective, BPH is marked by the uncontrolled proliferation of prostate stromal cells, resulting in the dilatation of the prostate gland in its transitional zone. This pathological process is clinically evident through the manifestation of lower urinary tract symptoms (LUTS).The prevalence of benign prostatic hyperplasia (BPH) is escalating at an accelerated rate on a global scale. A systematic analysis of data collected from 2000 to 2019 in 204 countries provides evidence of a significant increase in the global prevalence of BPH. The analysis, which was conducted using data from men aged ≥40 years, revealed 94 million confirmed cases of BPH worldwide in 2019, marking a substantial increase from the 51.1 million cases documented in 2000. According to the most recent statistical data, the

prevalence of benign prostatic hyperplasia (BPH) is expected to increase in the near future, primarily due to the rising life expectancy of the male population.

Furthermore, BPH has been associated with a number of complications, including, acute or chronic urinary retention, urinary tract infection, bladder stone formation, bladder wall damage, hematuria, kidney damage, and erectile dysfunction. Therefore, it is imperative to implement measures aimed at mitigating the risk of developing BPH.

Recent research has focused on the composition and function of the gut microbiota and its impact on the development of prostate disease. This observation has led to the proposal of a novel concept termed the "gut-prostate axis." The precise mechanism by which the gut microbiome exerts its influence on the prostate remains to be elucidated.

The primary objective of the present investigation was to ascertain the profile and discrepancies in the composition of the gut microbiota. This study constitutes the first endeavour to identify enterotypes in men with and without BPH.

The study group included 76 men diagnosed with BPH. The control group included 81 healthy with a prostate size ≤ 30 ml and PSA less than 2.5 ng/ml.

16 S sequencing of the V3-V4 region was performed and then evaluated alpha (α) and beta (β) diversity of the faeces microbiota of BPH and control group patients.

No statistically significant difference in α diversity between the groups. Nevertheless, a significant difference was detected in the β diversity (Permanova test: F-value = 5.56, p-value < 0.001).

The identification of enterotypes revealed significant differences between the two groups (p = 0.035). In the cohort of men diagnosed with BPH, the most prevalent enterotype was identified as enterotype 3, which was characterized by a predominance of Blautia, Bacteroides, and Streptococcus. The occurrence of enterotype 3 was associated with an increased likelihood of BPH, exceeding threefold that of enterotype 1 (odds ratio [OR] = 3.24). The present findings indicate that alterations in the gut microbiota, specifically the presence of enterotype 3, may serve as a microbiological pattern associated with BPH.

A-0133 Seminal Plasma Oxylipins and NMR Metabolites in Normal and Impaired Spermatogenesis

Artem Pustovid [1], Vitalik Bezuglov [1,2], Dmitry Chistyakov [2,3], Sabina Khayat [4], Marina Andreeva [4], Maria Shtaut [4], Olga Solovova [4], Sergey Goriainov [3], Vasily Ivlev [3], Vasiliy Vasilev [3], Vyacheslav Chernykh [4], Oleg Sergeyev [1,2,4]

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Background and Objectives.
Seminal plasma (SP) is a complex fluid that reflects both testicular and accessory gland function. Current knowledge is limited regarding oxylipins and small metabolites in SP from men with normal and severe pathological sperm counts. This study aimed to identify metabolite and oxylipin-derived predictors that discriminate between normozoospermic (Normozoo), severe oligozoospermic (sOligozoo), and azoospermic (Azoo) subjects using an integrated metabolomics approach.

Methods.
Forty-one subjects with median (25th-75th percentiles) 30.8 (25.8-35.3) years old were stratified by semen parameters into three groups: Normozoo (WHO 2010 criteria, n=10); sOligozoo (sperm count <10 million/ejaculate, n=15); and Azoo (n=16). Diagnosed genetic abnormalities in the Azoo group included Klinefelter syndrome (47,XXY, n=2); Y chromosome disomy (47,XYY); X/XY mosaicism (45,X/46,X,del(Y)(q11.2), and 45,X/46,X,idic(Y)(q11.2)) with AZFb+c deletions; 46,XX male syndrome, and a reciprocal translocation 46,XY,t(1;16)(q21;p13).

Following standard ejaculate collection and centrifugation, SP samples were processed. Oxylipins analysis was performed on 0.3 mL SP using a Shimadzu 8040 UPLC-MS/MS system. Lipids were identified and quantified against standards using the Lipid Mediator Version 2 package. NMR analysis was performed on 0.2-0.3 mL SP using a Bruker AVANCE Neo 700 spectrometer and the Chenomx v9 software package.

Metabolite ratios and sums were also calculated. Group differences were evaluated using Kruskal-Wallis (KW) and Mann-Whitney tests, and discriminatory performance was assessed by ROC analysis.

Results.
We profiled 58 oxylipins and 37 NMR metabolites, including their concentrations, ratios, and sums, resulting in 532 total parameters. For the arachidonic acid (AA) metabolite anandamide (AEA), median concentrations (25th-75th percentiles) were 0.05 (0.03–0.14), 0.01 (0.01–0.03), and 0.003 (0.001–0.007) ng/mL in Normozoo, sOligozoo, and Azoo groups, respectively (KW; p=0.00003). For the AEA metabolite, prostaglandin-ethanolamide (prostamide, PGE2-EA), median concentrations were 0.94 (0.67–1.30), 0.35 (0.15–0.54), and 0.12 (0.03–0.22) ng/mL across the three groups (p=0.0005). ROC analysis revealed that the AEA/AA ratio was the strongest overall predictor, yielding AUCs of 0.96 for Normozoo vs. sOligozoo, 0.99 for Normozoo vs. Azoo, and 0.90 for sOligozoo vs. Azoo, with corresponding sensitivities/specificities of 1.0/0.89, 0.94/1.0, and 0.88/0.87, respectively (p=0.000001).

Lactate level was significantly decreased in infertile men, with median concentrations of 6.8 (6.7–8.4), 3.2 (2.3–4.3), and 2.8 (2.7–3.7) mM in Normozoo, sOligozoo, and Azoo groups, respectively (p=0.007). The logarithmic ratio of lactate to the ratio of omega-3 polyunsaturated fatty acids (eicosapentaenoic/docosahexaenoic, EPA/DHA) also decreased progressively (median (IQR): 5.8 (0.3), 4.4 (1.0), 4.0 (1.1) in Normozoo, sOligozoo, and Azoo, respectively; p=0.00009) and was highly discriminative, with AUCs of 0.98 for Normozoo vs. sOligozoo and 0.99 for Normozoo vs. Azoo. The ratio of aspartate to asparagine was a strong predictor for Normozoo vs. Azoo with AUC of 0.98 and sensitivities/specificities of 0.89/1.0.

Conclusions.

For the first time, comprehensive oxylipin and NMR metabolite profiles, including their ratios and sums, have been described in SP of normozoospermic men and patients with azoo-/severe oligozoospermia. The most robust SP markers were associated with disruptions in endocannabinoid signaling, oxylipin metabolism, energy and nitrogen balance. These findings suggest a metabolic disruption in male infertility and support the biomarker potential of combined NMR and oxylipin profiling.

A-0135 VOLUMETRIC RESPONSE OF HYPOECHOIC TESTICULAR MICRONODULES IN MEN WITH PRIMARY GONADAL FAILURE TREATED WITH TESTOSTERONE THERAPY

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Introduction. Men with primary gonadal failure often exhibit small, multiple, non-palpable, benign testicular micronodules at ultrasound examination (US), generally attributed to areas of Leydig cell hyperplasia or Leydig tumors. Elevated LH, in response to testicular damage, has been claimed to stimulate Leydig cells to generate such a 'focal lesion' appearance. Whether testosterone therapy (TRT) can reduce the micronodule volume by lowering LH has never been investigated.

Methods. Patients displaying testicular micronodules with signs of primary testicular damage and naive to androgen therapy were prospectively enrolled to undergo US, clinical and hormonal assessment before and after a minimum of 6-month TRT. Patients with congenital or acquired hypogonadotropic hypogonadism were excluded. Micronodule volume was estimated using the sphere formula (1/6 * π * D3). In the case of multiple nodules, only the two largest lesions in each testicle were analyzed.

Results. A total of 94 patients were included in the analysis (median age 34.1 years, IQR 28.1–43.6). Among them, 40 patients received testosterone replacement therapy (TRT) while 54 did not receive treatment. The karyotype distribution included 43 46,XY patients (45.7%), 48 47,XXY patients (51.1%), and three patients with other karyotypes (XXXY, XX male, XXYY).

At baseline, patients receiving TRT showed lower testicular volume compared with untreated subjects [median 4.9 (IQR 4.2–8.4) vs 10.2 (5.7–20.9) mL]. Untreated patients showed a slight increase in areolar volume over time, with a median Δ of +0.002 mm³ (IQR 0.000–0.009), corresponding to +14.3% (−0.3 to 39.1). Conversely, TRT-treated patients exhibited a marked reduction in areolar volume, with a median Δ of −0.021 mm³ (−0.046 to −0.007), corresponding to a −57.9% reduction (−89.7 to −37.4).

When the post-treatment areolar volume was analyzed using analysis of covariance adjusting for baseline volume, TRT was independently associated with a significant reduction in areolar volume ($F=32.9$, $p<0.001$; partial $\eta^2=0.286$). Baseline areolar volume was the strongest predictor of follow-up volume ($p<0.001$).
The association between TRT and reduced areolar volume remained significant after additional adjustment for age and karyotype ($F=30.3$, $p<0.001$; partial $\eta^2=0.275$), while neither age ($p=0.784$) nor karyotype ($p=0.110$) significantly influenced the outcome. Among TRT-treated patients, treatment duration was not associated with volumetric response ($p=0.864$). Conversely, TRT formulation showed a modest effect on follow-up areolar volume ($p=0.044$). Regarding hormonal parameters, TRT induced the expected endocrine changes, with testosterone increasing from 10.4±5.7 to 21.1±8.7 nmol/L and LH decreasing from 17.1±8.2 to 8.3±6.6 mUI/mL
Conclusion. This is the first demonstration that TRT can affect the size of testicular, non-palpable hypoechoic micronodules in men affected by primary hypogonadism, likely by improving LH and TTe/LH ratio.

A-0137 Effect of age on seminal parameters – Findings from a large sample of Greek men

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Introduction
Sperm quality is evaluated according to WHO guidelines and reference limits. Those limits, however, do not take into consideration the age though it is generally accepted that it affects the spermiogram results.

Purpose of the study
To investigate the effect of age on basic sperm parameters in a large dataset of semen samples provided by men within the Greek population.

Materials and methods
2,497 samples of men who underwent conventional semen analysis at Cryogonia Cryopreservation bank from 2017 to 2024 were included. The parameters were assessed according to the WHO guidelines (2010, 2021). The samples were categorized according to WHO (2021) reference limits and divided into 5 age groups: <20y, 20-29y, 30-39y, 40-49y, 50-59y. Violin graphs were drawn for each parameter per age group. Statistical analysis included correlation analysis between age and sperm parameters as well as comparisons between the age groups regarding the sperm parameters. Furthermore, the entire sample was divided into two subsets: normozoospermic samples (ARL) and non-normozoospermic samples (BRL).

Results

In the entire data set, volume showed statistically significant differences among the age groups with the highest values in the age group 20-29. Total sperm count showed the highest mean and median at the age <20 years. Progressive motility and morphology showed a steady decline with age having the highest median in the age group 20-29 and then decreased, although there were high outliers. Only 10% of samples were normozoospermic.

In the ARL subset, the volume showed statistically significant differences among the age groups with the highest median in the 30-39 age group. The mean and median of total sperm count showed a peak in the first age group. Progressive motility had highest median in the age group <20 and then decreased. In morphology, there were individual samples with extremely high values in all age groups.

In the BRL subset, the median volume increased from the age group <20 to the group 20-29 and then decreased, having the lowest value in the age group 50-59. The median of total sperm count remained constant in the two first age groups and then decreased. The median of progressive motility had the highest value in the group 20-29 and then decreased. The morphology did not show any clear trend.

Conclusions

Our data confirm that age does affect sperm parameters in different ways according to the specific sperm parameter and the existence or not of an underlined pathology. In normozoospermic samples, the most clear trends appear in volume and progressive motility; volume increases up to the fourth decade and then decreases whereas progressive motility declines after the third decade.

The present findings could act as an alarming indication to induce informed decisions of the general public and professionals in the field of andrology in the direction to promote andrological evaluation, as well as precautionary autologous sperm cryopreservation for men wishing to postpone parenthood.

A-0138 Effect of Tamoxifen on Semen Parameters in Patients with Severe Oligoasthenozoospermia and Primary Testiculopathy with Hypospermatogenesis

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Introduction Selective estrogen receptor modulators (SERMs) have been proposed as an empirical treatment for male infertility because of their ability to increase endogenous gonadotropin secretion and intratesticular testosterone levels, potentially enhancing spermatogenesis. Several studies have reported improvements in semen parameters following SERM therapy; however, available evidence is limited by small sample sizes and substantial heterogeneity among study populations. Consequently, current clinical guidelines recommend considering SERMs only as an off-label therapeutic option in selected patients with idiopathic oligozoospermia and/or asthenozoospermia. Whether these agents may also benefit patients with primary testicular impairment remains poorly investigated.

Aim To evaluate the effect of tamoxifen on semen parameters in a selected population of infertile men with severe oligoasthenozoospermia associated with primary testiculopathy and cytological evidence of hypospermatogenesis.

Materials and Methods Thirty infertile men from couples seeking natural conception or intrauterine insemination (IUI) were enrolled. All couples had explicitly declined second-level assisted reproductive techniques (ART). Inclusion criteria were: female partner age <35 years, severe oligoasthenozoospermia (<1 million progressively motile spermatozoa), FSH levels >8 IU/L (therefore not eligible for FSH treatment according to AIFA Note 74), and normal LH, total testosterone, and calculated free testosterone levels. Transrectal ultrasound excluded obstructive or sub-obstructive abnormalities of the seminal tract. Karyotype analysis and screening for Y-chromosome microdeletions were negative in all patients. All subjects underwent diagnostic testicular fine-needle aspiration, demonstrating a cytological pattern consistent with hypospermatogenesis. After informed consent was obtained, patients were treated with tamoxifen 20 mg/day for 3 months. Semen analysis was repeated at the end of treatment, and changes in semen parameters and natural pregnancy rate were evaluated.

Results Spontaneous conception occurred in 3 cases (10%). The number of progressively motile spermatozoa significantly increased after treatment (0.17 ± 0.16 vs $2.1 \pm 3.3 \times 10^6$; $p < 0.05$). In 50% of patients, the number of progressively motile spermatozoa exceeded 1×10^6 following treatment. Tamoxifen administration was associated with a significant increase in serum FSH levels (13.4 ± 3.6 vs 24.1 ± 12.9 IU/L; $p < 0.05$), suggesting activation of the hypothalamic–pituitary–testicular axis.

Conclusions In this carefully characterized population of infertile men with severe oligoasthenozoospermia and primary testicular impairment, tamoxifen therapy was associated with a significant improvement in semen parameters and a clinically relevant rate of spontaneous pregnancies. These findings suggest that pharmacological stimulation of the hypothalamic–pituitary–testicular axis may enhance residual spermatogenic activity even in patients with elevated baseline FSH levels and cytological evidence of hypospermatogenesis. From a clinical perspective, tamoxifen may represent a therapeutic option in selected patients who are not candidates for gonadotropin therapy and who wish to attempt natural conception or less invasive assisted reproductive techniques before proceeding to advanced ART. Larger controlled studies are warranted to confirm these findings and to better define the role of SERMs within the therapeutic algorithm of male infertility.

A-0140 Residual Testicular Volume Determines Early Semen Parameter Changes After Orchiectomy in Testicular Cancer

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Background: The impact of orchiectomy on semen parameters in men with testicular cancer (TC) is not fully understood, particularly regarding inter-individual variability and determinants of postoperative spermatogenic resilience. While previous studies reported overall declines in semen quality after orchiectomy, the potential role of residual testicular reserve and endocrine–genetic factors remains poorly characterized.

Methods: We performed a retrospective monocentric cohort study including 85 men with TC undergoing semen cryopreservation both before orchiectomy (T0) and within one month after surgery (T1), prior to any gonadotoxic treatment. Longitudinal changes in sperm concentration (SC) were analysed using linear mixed-effects models. Determinants of postoperative change (ΔSC) were assessed through multivariable regression and baseline-adjusted ANCOVA models. Inter-individual variability was explored using clustering of longitudinal trajectories and a composite semen quality index integrating multiple semen parameters. Exploratory analyses also evaluated potential genetic modulation.

mixed-effects modelling confirmed a significant longitudinal effect of orchiectomy on SC. Residual testicular volume (RTV) was significantly associated with baseline SC and emerged as the only independent predictor of postoperative change ($\beta = -1.79$, $p = 0.008$). ANCOVA models confirmed that RTV independently predicted postoperative SC beyond baseline values. Clustering analysis identified three distinct spermatogenic trajectories: stable profile, moderate decline, and pronounced decline. Composite index analysis confirmed marked inter-individual variability in semen quality dynamics. Exploratory analysis suggested a potential role of FSHB polymorphism in modulating baseline spermatogenic capacity.

Conclusions: Orchiectomy is associated with heterogeneous spermatogenic responses rather than uniform deterioration. Residual testicular volume appears to be a key determinant of postoperative spermatogenic dynamics, supporting a reserve-dependent model of response. These findings highlight the importance of individualized fertility counselling and reinforce the role of early semen cryopreservation in men with TC.

A-0145 Unmasking Primary Hypogonadism After Significant Weight Loss Induced by Tirzepatide in a Male Survivor of Hodgkin and Non-Hodgkin Lymphoma

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Background:
Male survivors of haematological malignancies face a heightened risk of long-term gonadal dysfunction due to both the underlying disease and gonadotoxic therapies. Differentiating between central and primary hypogonadism in these patients can be challenging, as treatment-related damage may affect both the gonads and the pituitary. Furthermore obesity, more prevalent in this population than in the general one, can obscure the clinical picture. We report a distinctive case in which significant tirzepatide-induced weight loss unmasked previously misclassified primary testicular failure.

Case Presentation:
A man with a history of Hodgkin lymphoma diagnosed at the age of 10 (treated with ABVD ×6 and BEACOPP ×6) and relapsed as non-Hodgkin lymphoma at the age of 22 (treated with R-CODOX-M/IVAC-R), was first referred to our clinic at the age of 23. He reported erectile dysfunction, low libido, bilateral testicular hypotrophy (bilateral testicular volume [BTV] 14 mL) and obesity (BMI 32.0 Kg/m²). Laboratory testing revealed total testosterone (TT) levels of 8.2 nmol/L (reduced levels confirmed on repeated samples), LH 3.2 mIU/mL, FSH 7.6 mIU/mL, Inhibin B 38.1 pg/mL, SHBG 20.3 nmol/L and cfT 204 pmol/L, consistent with secondary hypogonadism. After organic causes had been excluded, hypogonadism was

deemed likely obesity-related. Azoospermia was confirmed on two semen analyses, according to WHO operating procedures. A 9-month course of gonadotropin therapy (hCG and uFSH) led to testicular volume increase (BTV 19 mL) and serum TT increase (14.3 nmol/L); bilateral micro-TESE was persued, and revealed a Sertoli-cell-only syndrome. Testosterone replacement therapy (TRT) was then initiated.

Despite continuous lifestyle intervention, his BMI progressively increased to 36.0 Kg/m² (absolute weight 114 Kg), in association with incident diagnoses of impaired glucose intolerance (IGT) and mixed dyslipidaemia. In November 2024, tirzepatide was initiated (titrated up to 10 mg/week), and TRT was discontinued. After 12 months, he achieved a 21% weight loss (absolute weight lost 24 Kg), with significant improvement in metabolic parameters. Remarkably, TT levels increased spontaneously to 18.0 nmol/L, alongside a marked rise in LH (9.3 mIU/mL) and FSH (14.8 mIU/mL) and hypogonadal symptoms vanished.

Discussion and Conclusion:
This case illustrates how obesity-related suppression of the hypothalamic-pituitary-gonadal axis can mask underlying primary hypogonadism. Significant weight loss revealed the true aetiology of gonadal failure and highlighted the intricate relationship between metabolic and testicular function. Crucially, even mild to moderate obesity can profoundly alter hormonal profiles and spermatogenesis, acting as a powerful confounder. Therefore, diagnostic and therapeutic approaches used in normal-weight individuals should not be uncritically applied in obese patients when assessing gonadal health.

A-0146 Post-vasectomy pain syndrome and vasectomy complications

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Introduction
Vasectomy is an increasingly utilized method of permanent male contraception. No-scalpel vasectomy (NSV) is the preferred technique due to its favorable safety profile and low complication rates. However, complications such as infection, hematoma, and chronic post-vasectomy pain syndrome (PVPS) can occur. The true incidence and risk factors for PVPS remain uncertain. This study aims to evaluate the rates of PVPS and other complications following vasectomy, as well as to identify associated risk factors.

Materials and Methods
This retrospective study evaluated 1,774 sexually active men undergoing vasectomy between 2016 and 2024 at a single tertiary-referral center. Baseline data, including age, number of children, and BMI, were collected preoperatively. Postoperative follow-up assessed complications, need for repeat surgery, and results of post-vasectomy semen analysis (PVSA) performed three months after the procedure. Statistical analysis utilized nonparametric tests and multivariate logistic regression to identify independent risk factors for complications (significance: $p < 0.05$).

Results
Patients had a median age of 42 years (IQR 7.0), median BMI 26.95 (IQR 4.96), and median follow-up of 101 months (IQR 97–104). Most (90%) completed PVSA; 94.9% achieved azoospermia at first analysis, with 2% requiring re-vasectomy, and 0.2% undergoing vasovasostomy or TESE for regret. Postoperative recovery was uneventful in 97.3% of cases; infection and hematoma rates were 1.5% and 1.1%, respectively. PVPS occurred in 0.7% ($n = 14$); most were managed with NSAIDs, a minority ($n = 3$) required escalated treatment, including one surgical re-intervention. Multivariate analysis identified postoperative complications ($p < 0.001$) and higher BMI ($p = 0.012$) as independent risk factors for PVPS; age was not significant.

Conclusions
No-scalpel vasectomy offers high efficacy and safety for male sterilization, with rare complications and a low PVPS incidence. Postoperative complications and elevated BMI independently increase PVPS risk and may merit consideration during preoperative counseling and risk stratification.

A-0149 WOULD A PDE5-INHIBITOR NON-RESPONDERS WITH ERECTYLE DYSFUNCTION AND COMPENSATED DIABETES MELLITUS BENEFIT FROM DAPAGLIFLOZIN ASSOCIATION?

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The first-line therapy for erectile dysfunction (ED) in patients with diabetes mellitus (DM) consists of phosphodiesterase type 5 inhibitors (PDE5i). However, the rate of PDE5i non-responders is significantly higher in diabetic patients compared with the general population, and therapeutic options remain limited in patients with compensated diabetes who do not respond to standard PDE5i therapy.

The aim of this study was to evaluate whether the addition of dapagliflozin could improve erectile function in patients with compensated diabetes mellitus treated with metformin who were refractory to PDE5 inhibitor therapy.

The study included 23 men aged 48–64 years with type 2 diabetes mellitus (duration 3–20 years) controlled with metformin monotherapy (fasting glucose 5.9–7.0 mmol/L, HbA1c <7%) and erectile dysfunction with inadequate response to PDE5 inhibitor therapy for a period ranging from 2 months to 6 years. All patients underwent baseline evaluation according to the clinic protocol, including body mass index, lipid profile, and hormonal assessment. Patients received dapagliflozin 10 mg daily with adjustment of the metformin dose while continuing PDE5 inhibitor therapy at the previously prescribed regimen.

Patients were evaluated after 3 months using the International Index of Erectile Function (IIEF-5). Improvement in erectile function was observed in 10 patients (43%), with the mean IIEF-5 score increasing from 12.5 to 18.6 (±3) ($p < 0.05$). No improvement was observed in 6 patients (26%), with IIEF-5 scores of 14 at baseline and 14.3 at follow-up ($p > 0.05$). A decrease in erectile function was observed in 7 patients (30%), with IIEF-5 scores changing from 14.1 at baseline to 12.8 at follow-up. Adverse effects leading to treatment discontinuation occurred in 5 patients (22%), mainly urinary tract infections and pollakiuria.

The results suggest that the addition of dapagliflozin may improve erectile function in approximately 40% of diabetic patients who are refractory to PDE5 inhibitor therapy, potentially enhancing the response to PDE5 inhibitors. However, the relatively high rate of non-responders and treatment discontinuation, together with the limited sample size, indicate the need for further studies to identify the subgroup of patients who may benefit most from this therapeutic approach.

A-0153 TESA AND M-TESE IN MEN WITH AZOOSPERMIA AFTER ONCOLOGICAL TREATMENT

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Introduction: Oncological diseases, surgical treatment, chemotherapy, radiotherapy have a negative impact on spermatogenesis, up to azoospermia. The progress in the effectiveness of oncological treatment makes it possible to include an increasing group of azoospermic men with a history of anticancer therapy in the IVF-ICSI protocol (in-vitro fertilization, intracytoplasmic sperm injection) thanks to the obtaining sperm from testicular tissue. Objectives: The effectiveness of testicular biopsies were analyzed: percutaneous using 16-gauge needle (TESA, testicular sperm aspiration) and open procedure using operating microscope (m-TESE, microdissection testicular sperm extraction). Material and methods: During the period 2018-2025, in the nOvum Fertility Center, 896 testicular biopsies (TESA - 456, m-TESE - 440) were done, including 54 patients from the non-homogeneous group of azoospermic men after oncological treatment: TESA 16/456 (3,5%), m-TESE 38/440 (8.63%); age 17-45 years (avg. 32.6). Anamnesis: lymphoma(5), Hodgkin's lymphoma(7), non-Hodgkin lymphoma(2), anaplastic lymphoma(2), acute myeloid leukemia(1), chronic myeloid leukemia(1), neuroblastoma(2), mesenchymoma malignum(2), RMS(1), osteosarcoma of the lower extremity(2), seminoma testis(13), non-seminoma testis(3), choriocarcinoma testis(1), GCNIS(3), extragonadal germline tumor(2), hypothalamic astrocytoma(2), ventricular astrocytoma(2), ependymoma(1), liposarcoma(1), fibrosarcoma(1). After confirmation acceptance of IVF-ICSI all patients were diagnosed with azoospermia according to EAU (European

Association of Urology) guidelines. TESA were performed as an outpatient procedure, under short intravenous anesthesia (procedure time - 15-30 minutes); m-TESE – as a one-day surgery, under general anesthesia, laryngeal mask (procedure time - 60-90 minutes). Prevention of infections: cephalosporin 1-dose (TESA), 5-day therapy (m-TESE). Techniques: TESA – percutaneous puncture of the testis with a Menghini 16 G biopsy needle; m-TESE – surgical removal of promising tubules in which sperm may be found under Leica M8602x2 microscope, 25 x magnification. Parts of the collected samples were fixed in Bouin's solution for histopathological examination and were sent to a cooperating pathologist. The evaluation of each fragment of testicular tissue was performed according to the Testicular Biopsy Score Count by Johnsen (Hormones, 1970). The other part of the tissue was cryopreserved in liquid nitrogen (minus 196 degrees Celsius) as a deposit for the planned IVF-ICSI in the future. Results: Generally, both types of biopsies were accepted. The Clavien-Dindo classification of surgical complications II° - 1 after TESA - sheath hematoma, conservative management was applied. Spermatozoa were found in 17/54 (31,5%): TESA 5/16 (31,3%): seminoma (1), nonseminoma (1), choriocarcinoma testis (1), chronic myeloid leukemia (1), ependymoma (1); m-TESE 12/38 (31.6%): seminoma (3), GCNIS (2), nonseminoma(2), Hodgkin lymphoma (2), anaplastic lymphoma (1), non-Hodgkin lymphoma (1), liposarcoma (1). Conclusions: In the group of patients with azoospermia after oncological treatment it is possible to perform a testicular biopsy TESA and m-TESE. In the case of finding sperm in testicular tissue, it is possible to include them in reproduction in the IVF-ICSI protocol.

A-0157 Effects of glucagon-like peptide 1 receptor agonists on testicular dysfunction: A systematic review and meta-analysis

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Background: Hypogonadism and infertility are two conditions that are heavily affected by overweight and obesity in the male patient. Glucagon-like peptide 1 agonists (GLP-1RAs) are a recently introduced class of antidiabetic drugs with powerful weight-loss effect; this may induce an indirect positive effect on the testicular function. Nevertheless, recent evidence also suggests a potential direct influence of these molecules on the gonadal function.

Objectives: Our study aims at evaluating the effects of GLP-1RAs on hormone secretion in male patients and comparing their impact on the testicular function with other antidiabetic agents or weight-lowering drugs.

Materials and methods: A literature search was conducted using PubMed, EMBASE, and Scopus database to assess the effects of GLP-1RAs on hormone levels, sperm parameters, and erectile function in overweight and obese men. Before-after analysis and comparison between therapy with GLP-1RAs and other treatment regimens were performed.

Results: Seven studies (n = 680) were included in the quantitative analysis. Treatment with GLP-1RAs produced a significant increase in total serum testosterone (TT), with a standardized mean difference of 1.39 ng/mL (95% confidence interval: 0.70, 2.09; $p < 0.0001$). Free serum testosterone (FT), sex hormone-binding globulin (SHBG), luteinizing hormone (LH), and follicle-stimulating hormone (FSH) showed similar increase, while weight, body mass index (BMI), waist circumference (WC), and glycated hemoglobin (HbA1c) decreased. Meta-regression showed a significant negative correlation between standardized mean difference in TT levels before-after treatment and percentage change in weight and BMI. When compared with other treatment options, GLP-1RAs showed a comparable effect on serum androgens, but greater BMI reduction and increase in serum gonadotropins and indexes of the erectile function.

Conclusion: Our systematic review and meta-analysis suggest a possible role for GLP-1RAs in the therapy of functional hypogonadism related to overweight and obesity, while also promoting weight loss. The limitations of the current literature do not allow to demonstrate a direct action of GLP-1RAs on the testicular function.

A-0158 The effects of stress on the mammalian blood-testis barrier

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The blood-testis barrier (BTB) is a highly specialized structure formed by junctional complexes between Sertoli cells. It is essential for maintaining the microenvironment required for spermatogenesis, protecting developing germ cells from harmful influences. The BTB is composed of tight junctions, ectoplasmic specializations, gap junctions, and desmosomes. Any disruption to its integrity can impair fertility. Stress is known to alter protein expression within the BTB, potentially affecting its permeability, but the precise effects remain poorly understood.

Methods: To investigate this, social isolation as a stress model was employed in male Wistar rats (n=18). Animals were divided into two groups: stressed (isolated for 7 days) and control (group-housed). At 18 weeks, rats were sacrificed, and blood plasma was collected for corticosterone and testosterone analysis. Testes were harvested to examine BTB-associated proteins. Western blotting and immunohistochemistry were used to assess focal adhesion kinase (FAK), phosphorylated FAK (Tyr397, Tyr407), occludin, zonula occludens-1 (ZO-1), and total/phosphorylated p38 MAPK. FAK–occludin interactions were analyzed via co-immunoprecipitation, while transmission electron microscopy (TEM) was used to evaluate BTB ultrastructure.
Results: Corticosterone levels were significantly elevated in stressed rats ($P < 0.0001$), confirming stress induction, while testosterone remained unchanged. Protein analysis revealed trends toward reduced expression of FAK, FAK-Tyr397, ZO-1, and p38, alongside increased phosphorylation of p38. Occludin levels were stable, and results for FAK-Tyr407 were inconclusive. Immunohistochemistry suggested non-significant decreases in occludin, ZO-1, and p38, while FAK localization remained unclear. Co-immunoprecipitation results were similarly inconclusive.

Conclusion: No visible, significant compromise to the integrity of BTB was observed during stress. Nonetheless, we observed graphical reductions in the expression of tight junction proteins, which is an indication of a potential loss of BTB integrity. These findings therefore suggest that BTB is at risk of being compromised during stress.

A-0159 Distal Penile Gangrene following Winter's shunt For Ischeamic Priapism

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BACKGROUND: Ischemic priapism is a painful and persistent penile erection lasting more than four hours. It disproportionately affects patients with the SS Genotype. Surgical management could be with a distal winters glanulocavernosal shunt with the rare complication of penile gangrene.

CASE SUMMARY: A 40-year-old man; known HBSS gentleman presented with a 4-day history of priapism. He subsequently had a winter shunt procedure, resolution of tumescence but developed discoloration of the mid-portion of the phallus that evolved over the next few days to wet gangrene, sepsis and a need to have a debridement by partial penectomy. He recovered well and the wound has granulated well as he awaits phalloplasty and penile implant.

DISCUSSION: Penile gangrene is a devastating complication of priapism.in this patient he had megalophallus and had had two sessions of shunt procedures inserted on the right cavernosa in the distal third of the penile shaft giving a bulbous and firm form to the shape of the phallus. Doppler ultrasound confirmed the absence of blood flow in the mid-section of the phallus. The progressive nature of the wet gangrene causing sepsis necessitated a debridement with preservation of the proximal two-fifths of the organ.
CONCLUSION: The Penile gangrene is a rare and irreversible complication that must be recognized early to prevent further tissue loss. A high index of suspicion, prompt and decisive action whilst having full disclosure with the patient could preserve penile tissue and length.

KEY WORDS: Ischemic priapism, Penile gangrene, Partial penectomy

A-0207 Androgen sensitivity and hypogonadotropic hypogonadism correlate differently and independently with cardiovascular risk in severe obesity

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Background: Androgen activity has been variably associated with cardiovascular disease (CVD) in the literature. Obesity is known to be linked to both hypogonadism and an increased risk of CVD. However, evidence regarding the relationship between increased androgen sensitivity and CVD remains heterogeneous and inconclusive. Objective: To evaluate, in a large cohort of patients with severe obesity: (I) the length of CAG repeats in the androgen receptor (AR) gene, as a marker of androgen sensitivity, as compared with the general population; and (II) the potential association between gonadal function and androgen sensitivity with CVD. Methods: A total of 163 adult male patients (age >18 years) with severe obesity (BMI >35 kg/m²) were consecutively enrolled in a cross-sectional observational study. Clinical history was collected for each participant, and gonadal function was assessed by calculating free testosterone (cFT) and measuring gonadotropin levels. Hypogonadism was defined as persistent cFT <225 pmol/L and classified as hypogonadotropic (HH) or hypergonadotropic based on LH levels (cut-off ≤9.4 IU/L). Androgen receptor sensitivity was evaluated by analyzing the length of CAG repeats in the AR gene, using PCR amplification followed by DNA fragment analysis. Androgen sensitivity was categorized as reduced (>24 CAG repeats), intermediate (21–24 repeats), or increased (<21 repeats). Results: Chi-square analysis assessing androgen sensitivity revealed a significantly higher frequency of AR gene CAG repeats <21 ($p < 0.0001$) in our cohort, indicating greater androgen sensitivity than the general population. Logistic regression analysis investigating the association between CVD with gonadal function and androgen sensitivity demonstrated an independent association with both a lower number of CAG repeats ($p = 0.037$) and the presence of HH ($p = 0.041$), after adjustment for age, BMI, arterial hypertension, type 2 diabetes mellitus, obstructive sleep apnea (OSA), and smoking history (pack-years). Conclusions: We report, for the first time, a higher prevalence of increased androgen sensitivity in patients with severe obesity compared with the general population. Moreover, both increased androgen sensitivity and, conversely, the presence of HH are independently associated with CVD, suggesting the involvement of distinct pathogenic mechanisms: on the one hand, higher androgens are a known risk factor for CVD; on the other, HH may reflect an underlying systemic inflammatory state capable of exerting deleterious effects on both the cardiovascular system and the central regulation of the hypothalamic–pituitary–gonadal axis.

A-0208 Impact of Chemotherapy on Sperm Functional Characteristics in Patients With Testicular Germ Cell Tumours

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Testicular germ cell tumours (TGCT) are the most common malignancies among young men and represent a major threat to reproductive health. Both the disease itself and oncological treatments, particularly chemotherapy, can compromise spermatogenesis and semen quality. To evaluate sperm qualitative parameters in patients diagnosed with TGCT who underwent orchiectomy, assessed before chemotherapy and at 3 and 6 months post-treatment, to monitor their reproductive health with respect to the type of oncological therapy. Semen samples from seminoma and non-seminoma patients were analysed before and after chemotherapy. Standard spermiogram, flow cytometry (viability, apoptosis, DNA damage, acrosome integrity), sperm motility clustering, and western blot detection of histone modifications (H4K12ac, H3K9ac, H3K36me3) were used to assess chemotherapy-related sperm alterations. Before chemotherapy, non-seminoma patients showed higher sperm count and volume than seminoma patients. Chemotherapy, particularly BEP, caused severe impairment, with azoospermia and necrozoospermia observed. Normozoospermia declined rapidly in non-seminoma patients, while seminoma showed milder effects. DNA fragmentation, apoptosis, and acrosome integrity remained unchanged. Notably, histone H4K12ac abundance correlated positively with motility, sperm count, morphology, and DNA integrity. Non-seminoma patients had initially better semen quality, which declined rapidly during chemotherapy. Our findings highlight the need for extended sperm analysis in cancer and pathological conditions. Importantly, sperm quality correlated positively with histone H4K12ac abundance, underscoring its clinical relevance in chromatin regulation and paternal contributions to embryonic development. Sperm quality in TGCT patients showed marked variability. Non-seminoma diagnosis appeared less detrimental than seminoma, yet BEP chemotherapy severely impaired sperm parameters, especially in non-seminoma cases. These results highlight the importance of individualized post-chemotherapy monitoring using standard semen analysis, cytometry-based qualitative assessments, and epigenetic profiling of histone modifications to optimize fertility preservation and guide optimal treatment strategies by andrologists.

A-0212 Steatosis Indices and Penile Vascular Health: Rethinking the Liver–Erection Axis

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Context: Vasculogenic erectile dysfunction (VED) is the most common form of erectile dysfunction (ED) and is closely linked to cardiometabolic disease. Hepatic steatosis indices have been associated with cardiovascular risk, yet no study has evaluated their relationship with objective penile vascular measures assessed by penile color Doppler ultrasonography (PCDU).

Objective: To examine the association between non-invasive steatosis indices—Fatty Liver Index (FLI), Hepatic Steatosis Index (HSI), and Dallas Steatosis Index (DSI)—and PCDU-derived parameters of VED, including peak systolic velocity (PSV) and intima–media thickness (IMT).

Methods: In this retrospective study, 96 men evaluated for ED underwent dynamic PCDU after intracavernosal alprostadil injection. Clinical, biochemical, and metabolic data were collected. Associations between steatosis indices and PCDU parameters were analysed. Multiple regression identified independent predictors of PSV, and ROC analysis assessed the performance of FLI in detecting reduced PSV (<35 cm/s).

Results: Reduced PSV was observed in 24% of participants and was associated with older age, higher FSH and LH levels, lower total and free testosterone, and higher FLI values, while HSI and DSI did not differ between groups. FLI correlated inversely with PSV ($R = –0.305$; $p = 0.003$). In multivariable analysis, only PSV and age independently predicted FLI ($p = 0.009$). ROC curve analysis indicated that an FLI threshold ≥67.6 identified reduced PSV with 56% sensitivity and 82% specificity.

Conclusions: FLI is independently associated with impaired penile arterial inflow and may represent a practical, non-invasive marker of vascular andrological risk. These findings suggest that FLI could assist early risk stratification in men with ED.

A-0214 Efficacy and safety of transurethral seminal vesiculoscopy for oligo-astheno-teratozoospermia: A systematic review and meta-analysis

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Introduction: Oligo-astheno-teratozoospermia (OAT) is a significant cause of male infertility, often associated with ejaculatory duct obstruction (EDO) or seminal vesicle pathology. Traditional diagnostic and therapeutic approaches for EDO can be invasive or lack precision. Transurethral seminal vesiculoscopy (TUSV) has emerged as a minimally invasive technique for direct visualization, diagnosis, and treatment of seminal vesicle and ejaculatory duct abnormalities. However, its overall efficacy and safety profile in improving semen parameters and fertility outcomes for OAT patients remain to be systematically evaluated. This systematic review and meta-analysis aims to synthesize the current evidence on TUSV for OAT.

Methods: A comprehensive systematic review was conducted following PRISMA guidelines. Electronic databases including PubMed, Embase, Web of Science, and Cochrane Library were searched from inception to April 2024 for studies reporting on TUSV in OAT patients. Keywords included 'transurethral seminal vesiculoscopy', 'ejaculatory duct obstruction', 'oligoasthenoteratozoospermia', 'male infertility', and 'semen parameters'. Studies reporting on semen parameters (sperm concentration, motility, morphology) and clinical pregnancy rates post-TUSV were included. Two independent reviewers screened titles, abstracts, and full texts, extracted data, and assessed study quality using the Newcastle-Ottawa Scale for observational studies. A meta-analysis was performed using random-effects models to pool outcomes where appropriate.

Results: Twenty-two studies, comprising 1,587 OAT patients undergoing TUSV, met the inclusion criteria. The pooled analysis demonstrated significant improvements in semen parameters post-TUSV. Mean sperm concentration increased from 6.8 ± 4.2 million/mL pre-TUSV to 18.5 ± 7.1 million/mL post-TUSV (mean difference 11.7 million/mL, 95% CI 9.5-13.9, $p < 0.001$). Progressive motility improved from 12.1 ± 6.5% to 34.8 ± 9.3% (mean difference 22.7%, 95% CI 19.8-25.6, $p < 0.001$). Normal morphology also showed a modest but significant increase from 1.8 ± 1.1% to 3.5 ± 1.9% (mean difference 1.7%, 95% CI 1.2-2.2, $p < 0.001$). The overall spontaneous clinical pregnancy rate following TUSV was 28.3% (95% CI 23.5-33.5). Complications were generally mild and transient, with epididymitis (3.2%) and hematospermia (2.1%) being the most common.

Conclusions: Transurethral seminal vesiculoscopy is an effective and relatively safe procedure for improving semen parameters and achieving spontaneous pregnancies in selected OAT patients, particularly those with suspected ejaculatory duct obstruction or seminal vesicle pathology. This systematic review provides strong evidence supporting its role in the diagnostic and therapeutic algorithm for male infertility. Further prospective, randomized controlled trials are warranted to confirm these findings and establish optimal patient selection criteria.

A-0216 Driving diagnostic excellence: 25 Years of External Quality Assessment (EQA) in Y Chromosome microdeletion testing

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Introduction: Molecular diagnosis of Y-chromosome microdeletions is a routine genetic test in the evaluation of infertile male patients. Given its diagnostic and prognostic relevance, it is essential that this analysis is performed according to the highest quality standards. For the past 25 years, the European Academy of Andrology (EAA) and the European Molecular Genetics Quality Network, Community Interest Company (EMQN CIC), have supported the advancement of Y-chromosome microdeletion testing through an External Quality Assessment (EQA) program accredited under ISO 17043 - the international standard that defines competence requirements for proficiency testing providers.

Aim: To quantify the impact of the EAA/EMQN EQA program on the molecular diagnosis of Y-chromosome microdeletions.

Methods: A 25-year analysis was conducted to assess the effectiveness of the EAA/EMQN EQA program in reducing genotyping errors and improving reporting practices.

Results: The EAA/EMQN EQA program has had a substantial impact on reducing diagnostic errors. At the start of the program, the overall genotyping error rate was nearly 8%, whereas it has now stabilized below 2% of all reported cases. In addition, the program has markedly improved reporting quality: the proportion of analyses receiving a full interpretation score has steadily increased and currently reaches approximately 80%. Despite these advances, further progress is still needed. Several laboratories have yet to adopt the currently recommended best-practice multiplex PCR setup or to implement the mandatory deletion extension analysis.

Conclusion: Participation in the EAA/EMQN EQA program has led to major improvements in the molecular diagnosis of Y-chromosome microdeletions and provides clear benefits to the roughly 140 laboratories worldwide that take part each year. Therefore, enrolment in this EQA program is strongly recommended for all diagnostic laboratories performing Y-chromosome microdeletion analysis.

A-0218 TESTOSTERONE PROTECTS FROM METABOLIC SYNDROME-ASSOCIATED LUNG DYSFUNCTION IN A HIGH-FAT DIET RABBIT MODEL

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Background and Objective: Metabolic syndrome (MetS), a cluster of physiopathological conditions including obesity, dyslipidemia, hypertension, and insulin resistance, is increasingly recognized as a driver of impaired lung function. In the male population, MetS is frequently characterized by the onset of hypogonadotropic hypogonadism, marked by low levels of testosterone (T) and gonadotropins. While clinical observations, reinforced by the respiratory vulnerabilities seen during the COVID-19 pandemic, suggest a correlation between MetS and decreased pulmonary compliance, the underlying mechanisms remain largely unknown. This study aimed to investigate the mechanisms determining the onset of lung dysfunction and evaluate the potential protective, anti-inflammatory, and antifibrotic effects of T therapy using a non-genomic animal model.

Methods: Male New Zealand White rabbits were used to recapitulate the human MetS phenotype through exposure to a high-fat diet (HFD; standard diet supplemented with cholesterol and peanut oil). Animals were assigned to several experimental groups: regular diet control (RD, 12 weeks), HFD for 6 weeks (HFD6W), HFD for 12 weeks (HFD12W), and HFD groups supplemented with T either for the full 12 weeks (HFD+T12W) or for the final 6 weeks (HFD12W+T6W). Lung function was assessed via spirometry to measure the pressure of the airway opening (PAO), a functional parameter of airway resistance and reduced compliance. Histomorphological and biomolecular analyses were performed on isolated lung samples using Picrosirius Red staining for collagen deposition, RAM11 immunohistochemistry for macrophage activation, and semi-quantitative RT-PCR for gene expression of inflammatory and fibrotic markers.
Results: HFD successfully induced MetS features, hypogonadism, and progressive lung impairment. By 6 weeks, early macrophage remodeling and elevated fatty acids were observed. By 12 weeks, HFD rabbits exhibited a significant increase in PAO compared to RD controls, demonstrating clear functional impairment. Molecular analysis confirmed a prominent pro-inflammatory and pro-fibrotic status in the lungs of HFD animals, characterized by the significant upregulation of markers including IL1β, LOX1, RORγt, TLR2, COL1A1, COL3A1, and TGFβ1. Histological findings showed marked peribronchiolar fibrosis and macrophage clustering. Conversely, T administration (both 6-week and 12-week protocols) significantly improved metabolic parameters and lung ventilation while counteracting macrophage dysregulation. T therapy reversed the mRNA expression of inflammatory and fibrotic markers and reduced collagen deposition. Notably, a strong inverse correlation was found between plasma T and PAO; T levels below a threshold of 3.76 nM predicted abnormal pulmonary function with >80% sensitivity and specificity.

Conclusions: This study demonstrates that MetS causes progressive lung injury mediated by systemic inflammation, macrophage dysregulation, and peribronchiolar fibrosis. Testosterone deficiency appears central to this pathology, as T administration effectively mediates anti-inflammatory and antifibrotic effects, potentially involving cAMP-signaling and the modulation of Th2/Th17 cytokines and EMT markers. These results highlight the validity of the HFD rabbit model and suggest that clinical assessment of T and metabolic status is crucial for mitigating the respiratory consequences of MetS.

A-0219 Enzyme-mediated generation of DNA double-strand breaks reveal accessible sperm chromatin regions

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Sperm chromatin represents the most highly compacted and specialized DNA organization in nature. Packaging by protamines into toroidal and rod-like structures results in extreme DNA condensation, rendering most of the genome transcriptionally silent and largely inaccessible. Nevertheless, protamine-bound toroids are interconnected by linker regions of reduced compaction, and a minor fraction of histone-retained domains also persists, both of which remain potentially accessible to enzymatic activity. The aim of our ongoing project is to map and sequence these accessible regions of the sperm genome. To this end, we induce double-strand breaks (DSBs) specifically within accessible chromatin regions, enabling their subsequent tagging and sequencing. Here, we aim to compare the patterns of DSBs generated in accessible sperm chromatin using two different endonucleases: Deoxyribonuclease I (DNase I) and Fragmentase.

For each condition tested, we used 30 million sperm from four different porcine ejaculates. Each sample was first permeabilized through incubation in 0,25% Triton-X100, then washed twice in PBS, and after incubated at 37ºC with 10 units of DNase I for 0, 5, 10 or 30 min; or with 2µL of NEBNext dsDNA Fragmentase for 0, 15, 30, 60, 120, 180 or 240 min. Reactions were stopped by adding 50mM EDTA. The incidence of DSBs was evaluated using the neutral Comet assay, following the protocol adapted to boar sperm.

DNase I incubation caused a dose-dependent increase in double-stranded DNA damage, as measured by the Olive Tail Moment (OTM). OTM was 1.1±0.2, 120.0±34.9, 122.0±29.6, and 143.0±32.5 after 0, 5, 10, and 30 min of incubation, respectively, with statistically significant differences compared to controls observed up to 30 min ($P<0.05$). Fragmentase incubation also induced a dose-dependent increase in DSBs. OTM values were 1.4±0.5, 11.4±7.8, 14.6±4.8, 24.1±3.8, 29.6±5.7, 37.6±9.2, and 42.8±15.3 after 0, 15, 30, 60, 120, 180, and 240 min of incubation, respectively. Statistically significant differences compared to controls were observed up to 240 min of incubation ($P<0.05$). No statistically significant differences were found among 120, 180, and 240 min ($P>0.05$). Comparison of the highest levels of damage for each condition revealed that DNase I generated 3.3-fold more DSBs compared to Fragmentase ($P=0.028$).

This study demonstrates that both strategies are able to induce DNA damage in accessible regions of the genome. Nevertheless, DNase I generates more extensive damage than Fragmentase, which can be attributed to their distinct mechanisms of action. DNase I is a classical endonuclease in which DSBs arise stochastically from independent cleavage events on each DNA strand. Conversely, Fragmentase is a two-step enzymatic system in which DNA is nicked prior to DSBs formation by a nick-dependent endonuclease, resulting in more coordinated cleavage events. These differences in DNA damage patterns are likely driven by differences in enzymatic architecture and catalytic requirements. This becomes particularly relevant in the context of sperm chromatin, where accessibility is extremely limited and restricted to specific linker and histone-retained domains. Future experiments will aim at mapping such induced DSBs across the genome, enabling the identification of accessible regions in sperm chromatin.
Funding: Consolidación Investigadora scheme (CNS2024-154471;MICIU/AEI/10.13039/501100011033)

A-0221 Changes in IIEF, PEDT and Orgasmometer Scores According to Attachment Styles as Measured by the Relationship Questionnaire

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Introduction & Objective.

Attachment styles - secure, fearful, preoccupied, and dismissing - are based on a person's model of the self and other, and can be measured with the Relationship Questionnaire. It is largely believed that attachment styles can influence an individual's behaviour, including sexual function, but data in these regards is poor. To bridge this gap, we aimed to investigate a population of sexually active, heterosexual men in a stable relationship, using different validated questionnaires to measure different aspects of sexual function across different attachment styles.

Methods.
All questionnaires were administered through an online, anonymous survey; participants were recruited through social media. The Relationship Questionnaire was used for assessment of the attachment style. Erectile function, orgasmic dysfunction and perceived orgasmic intensity were measured with the International Index of Erectile Function (IIEF), the Premature Ejaculation Diagnostic Tool (PEDT) and the Orgasmometer, respectively. The Generalized Anxiety Disorder scale (GAD-7) and the Patient Health Questionnaire (PHQ-9) were also used to measure anxiety and depression, respectively, to adjust statistical analysis for confounding variables. Analysis of covariance was performed using the statistical software JASP (Version 0.18.3).

Results.
Among 362 male study participants, 208 were included in subsequent analyses (31 non-heterosexual, 97 not in a relationship, 26 both). The mean age of study participants was 27.6±7.49 years. The secure attachment style was the most prevalent (avoidant, n = 48; fearful, n = 38; preoccupied, n = 40; secure, n = 61). In the ANCOVA model adjusted for GAD-7, PHQ-9 and age, a significant difference was found across attachment styles ($p = 0.016$); post-hoc analysis showed a significant difference only between secure and fearful attachment styles (27.0±2.14 vs 25.42, $p = 0.026$). No significant effect was found for attachment styles in regards to PEDT ($p = 0.552$) or Orgasmometer scores ($p = 0.825$).

Conclusion
The present study aims to fill a gap in regards to how attachment styles might influence sexual behaviours by using several validated questionnaires in a small, but highly selected population. Our results show that the attachment styles influence sexual function only in a limited way, with only erectile function being slightly affected. However, it should be pointed out that these data come from a survey on social media, and thus suffer from the same limitations of other online studies.

A-0223 Age disparity in couples and the sexual and general health of the male partner

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Introduction: Several robust epidemiological studies suggest that men are often engaged in sexual relationships with younger women with a variable, age-dependent age difference. However, the ageing process determines a significant worsening of the andrological status, which favors the onset of erectile dysfunction and hypogonadism.

Objectives: To analyze the effects of differences in age between the partners [delta (Δ) age (M - F)] on patients referring to the Andrology Unit of Careggi University Hospital for male sexual dysfunction.

Materials and methods: A monocentre cohort of 4055 male subjects was evaluated by SIEDY structured interview. The cross-sectional analysis assessed the psychobiological and relational correlates. The rate of

forthcoming major cardiovascular events (MACE) was investigated in the longitudinal analysis. All the models have been adjusted for age, education, lifestyle, and chronic disease score.

Results: Δ age (M-F) shows a stepwise increase, according to the increasing age bands of the male partner. Δ age (M-F) was associated with a greater number of children, at the cost of more conflictual relationships within the family. The phenotype of these relationships is characterized by the report of a partner with a higher sexual desire and a higher ability to reach climax. Men seeking a younger partner show more often a histrionic personality (p = 0.023) and higher testosterone levels (p = 0.032). However, having a younger partner doesn't improve the ability to obtain a full erection. Kaplan-Maier analysis of a longitudinal subgroup of patients followed longitudinally (N = 1402) for 4.3 ± 2,59 years, showed that patients in the fourth quartile had a higher rate of forthcoming MACE versus those in the first quartile (p = 0.005).

Discussion and conclusion: In subjects with sexual dysfunctions (as in the general population) age-different relationships increase as a function of male ageing. A greater Δ age (M-F) is associated with specific men and relationship features and a higher risk of MACE.

A-0227 Phenotyping of male factor infertility and development of a new pathophysiology-based diagnostic classification: evidence from a 700-patient clinical cohort

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Background
Male factor infertility (MFI) is frequently labelled idiopathic when evaluation relies primarily on semen analysis, potentially overlooking endocrine and pathophysiological mechanisms that may guide targeted therapies.

Objective
To clearly phenotypically define MFI and develop a new classification using pathophysiology-based diagnostic-therapeutic criteria following comprehensive clinical, biochemical, genetic and imaging assessment.

Design and Setting
Retrospective monocentric cohort study conducted at a tertiary referral academic centre.

Patients
Seven hundred male partners of infertile couples evaluated between January 2023 and October 2025 after exclusion of isolated female factor infertility.

Main Outcome Measures

Prevalence of the different MFI categories, hormonal patterns across phenotypes, and proportion of idiopathic infertility after deep diagnostic work-up.

Methods
All patients underwent clinical/medical, familial and personal history and physical examination, hormonal evaluation (total testosterone, FSH, LH), testicular ultrasound, semen evaluation. Semen microbiological evaluation, transrectal ultrasound, genetic evaluation (karyotype, Y chromosome microdeletions, CFTR gene mutations, androgen receptor gene mutations, and gene panel for hypogonadotropic hypogonadism) were performed following Italian guidelines.

Results
We propose a new classification of MFI with the relative prevalence: I – infection and/or inflammation (22.1%); II – obstructive azoospermia and retrograde ejaculation (4.6%); III - primary spermatogenic failure (high FSH 38.8%/normal FSH 17.5%); IV – organic and functional hypogonadotropic hypogonadism (8.6%); V - idiopathic semen alterations (1.9%); VI - unexplained couple infertility (3.3%); VII - significant varicocele (2.6%); VIII - immunity-related semen conditions (0.6%).

Conclusions
Deep phenotyping substantially reduces the proportion of idiopathic infertility and identifies clinically meaningful subgroups with immediate therapeutic impact. A pathophysiology-driven classification may support management strategies aimed at restoring natural fertility and optimizing reproductive outcomes.

A-0238 Sexual Health Interventions in People Living with HIV (PLWH): A Systematic Review

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Introduction: Over 40 million people live with HIV (PLWH) worldwide. Although antiretroviral therapy (ART) has transformed survival, sexual dysfunctions (SDs) remain highly prevalent and clinically relevant. Beyond impairing intimacy, SDs are associated with ART nonadherence, viro-immunological decline, and sexual risk behaviors. Hypogonadism and SDs also share mechanisms with major comorbidities, contributing to cardiovascular and metabolic risk. Despite their established impact on HIV outcomes, SDs are often overlooked in care.

Objectives: This systematic review aims at synthesize evidence on the clinical management of SDs in PLWH.
Methods: We systematically searched MEDLINE, Embase, and ClinicalTrials.gov up to March 2025 for peer-reviewed studies on SD management in people living with HIV. Animal studies, reviews, abstracts, and letters were excluded. Two reviewers independently screened records, extracted data on population, interventions, and outcomes, and assessed risk of bias using ROB 2.0 for trials and Newcastle–Ottawa Scale for observational studies.

Results: From 1,206 records, 13 studies met inclusion criteria, comprising randomized trials, cohort studies, and case series published between 1995 and 2024 across the USA, UK, Nigeria, Portugal, and Kenya. In women with HIV, counselling and transdermal testosterone significantly improved sexual function and reduced depressive symptoms. In men, testosterone replacement therapy, including topical and intramuscular formulations, appeared to improve libido, erectile function, and sexual satisfaction, even in eugonadal individuals. Phosphodiesterase-5 (PDE5) Inhibitors showed variable efficacy. Risk of bias was moderate to high in most observational studies, whereas randomized trials displayed mixed quality.

Conclusion: Evidence on the management of sexual dysfunctions in PLWH remains limited and heterogeneous. The available literature is methodologically weak, and largely excludes transgender and gender-diverse individuals. Pharmacological interventions, including testosterone replacement therapy (TRT) have shown some improvements in sexual function in small studies, but the quality of evidence is low and long-term safety, dosing, and clinical benefit remain uncertain, while PDE5 inhibitors remain first-line for erectile dysfunction. Psychological and counselling interventions demonstrate benefit, particularly in women and individuals with complex psychosocial needs. Critically, sexual dysfunctions in PLWH are underrecognized due to limited provider training, scarce dedicated sexual health services, and stigma, highlighting the need for multidisciplinary care. High-quality, targeted studies are urgently required to guide evidence-based management and integrate sexual health into routine HIV care.

A-0243 Revisiting male infertility: insights into sexual dysfunction from a meta-analysis

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Background: Infertility affects approximately 10–15% of couples worldwide, with male factors contributing to nearly half of cases. Beyond reproductive impairment, infertility is associated with significant psychological distress and may negatively impact male sexual health. Sexual dysfunction, including erectile dysfunction (ED), premature ejaculation (PE), hypoactive sexual desire, and orgasmic disorders, is increasingly recognized among men undergoing infertility evaluation. However, the overall burden and spectrum of sexual dysfunctions in this population remain incompletely characterized.

Aim of the study: This meta-analysis aimed to estimate the pooled prevalence of male sexual dysfunctions in partners of couples evaluated for infertility and to explore potential determinants of heterogeneity.

Materials and Methods: This meta-analysis was registered in PROSPERO and a comprehensive literature search was performed in MEDLINE, EMBASE, Cochrane Library, and Web of Science from inception to March 2026. Eligible studies included cross-sectional, cohort, or case-control designs reporting prevalence of male sexual dysfunction in infertile populations using validated instruments, such as the International Index of Erectile Function (IIEF)-15 questionnaire. Pooled prevalence estimates and effect sizes were calculated using random-effects models with Freeman–Tukey transformation. Subgroup analyses and meta-regression were performed to investigate potential sources of heterogeneity, including age, infertility duration, semen parameters and hormonal levels.

Results: Ten studies (13 comparisons) including 4,319 infertile men and 2,718 fertile controls were analysed. Age and body mass index (BMI) were comparable between groups. Infertile men demonstrated significantly worse erectile function scores (IIEF-15 domain mean difference −0.34, 95% CI −0.5 to −0.1; $p<0.001$) and a higher prevalence of ED (OR 2.1, 95% CI 1.3–3.4; $p=0.002$). PE was also significantly more frequent among infertile men (OR 2.8, 95% CI 2.0–3.8; $p<0.001$). Orgasmic function and intercourse satisfaction were significantly reduced ($p<0.001$ and $p=0.030$, respectively), while sexual desire and overall satisfaction did not differ significantly between groups ($p=0.130$ and $p=0.960$, respectively). Meta-regression analyses showed that erectile function differences were associated with total testosterone serum levels ($p=0.006$), but not with semen parameters or gonadotropins levels. No significant associations were found between PE and hormonal or semen variables.

Conclusion: This meta-analysis demonstrates that male partners of infertile couples have a significantly higher burden of sexual dysfunction, particularly ED and PE, independent of semen quality. These findings support the concept of a complex, bidirectional relationship between infertility and sexual health, likely mediated by psychological, relational, and hormonal factors. The association between erectile function and testosterone suggests a potential biological contribution, whereas the lack of correlation with semen parameters underscores the multifactorial nature of sexual dysfunction in this population. Routine assessment of sexual health should be integrated into infertility evaluation and management.

A-0244 Infertility-Related Stress and Hypothalamic–Pituitary–Adrenal Axis Function in Patients Undergoing Assisted Reproductive Technologies: From Psychometric Assessment to Endocrine Biomarkers

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Background:
Several studies suggest that stress may directly affect assisted reproductive technologies (ART) outcomes; however, no validated instruments are available to accurately measure stress. Chronic stress is known to modulate the hypothalamic–pituitary–adrenal (HPA) axis, potentially leading to relative hypocortisolism. Thus, evaluation of the HPA axis should be considered for its potential interference with reproductive function.

To specifically evaluate stress related to infertility, we conducted a pilot study on male partners of infertile couples to develop an ad hoc questionnaire, called Trait Relationship between Infertility and stress (TRIS) questionnaire. We then designed a multicenter prospective pilot study aimed at quantitively assessing chronic stress in couples undergoing infertility treatment through integrated psychometric and endocrine evaluation.

Aim of the study:
To identify quantitative biomarkers of chronic stress related to infertility, integrating psychometric evaluation of infertility-related stress, developing an ad hoc questionnaire (TRIS questionnaire) together with validated instruments, with assessment of HPA axis activity in patients undergoing ART.

Materials and methods:
A two-steps clinical trial was designed. Step 1: a longitudinal prospective cohort pilot study on male partners of infertile couples (January to August 2025). During routine andrological evaluation, patients completed the TRIS questionnaire, which consists of two domains: one exploring the role of infertility on general anxiety and another investigating its role in the sexual function/dysfunction. Step 2: a prospective multicenter clinical trial was started, enrolling six groups of participants: (i) 40 females with primary infertility undergoing ART, (ii) 20 age-matched female fertile controls, (iv) 20 males attending ART for male factor couple infertility, (iii) 40 males, partner of infertile couples attending ART, (iv) 20 age-matched male fertile subjects. All participants underwent clinical evaluation, validated psychometric testing (SF-36, PSS, GAD-7, PHQ-9, STAI-Y1/Y2) together with the TRIS questionnaire. Basal 8:00 AM serum cortisol and ACTH were measured in all subjects; a 250-µg ACTH (Synacthen®) stimulation test is performed when morning cortisol is <14 µg/dL.

Results:
Step 1: Twenty-three male partners of infertile couples were enrolled and completed the TRIS questionnaire. The sexual function/dysfunction domain of the TRIS questionnaire showed significant correlation with sperm concentration (Rho's Spearman 0.486, p=0.019), total sperm number (Rho's Spearman 0.597, p=0.003), and progressive motility (Rho's Spearman 0.575, p=0.004). No correlation was found between the general anxiety domain and fertility parameters.

Step 2: To date, eight males have been enrolled at Modena Center (seven in group V and one in group VI). At the Belgrade center, thirty-one female patients have been enrolled, with twenty-six allocated to groups I and five to group II.

Conclusions:
The preliminary results of our ongoing two-step trial confirm that infertility-related stress is associated with semen quality in men evaluated for couple infertility. The TRIS questionnaire may represent a useful tool for capturing infertility-specific psychological burden that may be clinically relevant for male reproductive function. The ongoing prospective study integrating psychometric assessment with evaluation of HPA axis aims to further clarify the biological correlates of chronic stress in infertility and to explore potential endocrine biomarkers in patients undergoing ART.

A-0245 Fresh versus cryopreserved Microtese sperm in NOA: ICSI outcomes and the impact of severe testicular histology

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Introduction: MicroTESE is currently regarded as the gold standard technique for sperm retrieval (SR) in non obstructive azoospermia (NOA), offering the possibility of biological paternity to patients. Because successful SR (SSR) cannot be reliably predicted before surgery, sperm retrieval is performed before oocyte pickup and eventually retrieved sperm are cryopreserved for further use in ICSI. However, an average low post thaw sperm survival rate (33%) is usually reported, therefore in recent years the use of fresh testicular sperm has been proposed, coupling surgical SR with oocyte pick up on the same or on next day to achieve better ICSI outcomes, particularly in cases of historical or predicted poor sperm yield. Unfortunately, studies have not found difference in ICSI outcome with the use of fresh and frozen sperm. The present study aimed to compare the ICSI outcome using fresh versus frozen/thawed testicular sperm.

Materials and Methods: Data were retrospectively drawn from a cohort of 222 patients with NOA undergoing microTESE: SSR was achieved in 127 cases (57.2%). Patients were divided in two groups: group A was comprised of 127 couples undergoing 145 fresh ICSI cycles, group B had 64 patients with a prior failed fresh ICSI cycle who underwent 101 further ICSI cycles using supernumerary frozen/thawed sperm. Sperm yield was classified as poor, moderate or excellent based on the average number of sperm retrieved. Testis histology was performed and specimens were classified in 5 categories: hyalinosis (Hya), Sertoli cell only (SCO), maturation arrest (MA), hypospermatogenesis (Hypo), or mixed patterns (Mix).

Results: In group A, 1298 metaphase II oocytes were injected, of which 548 fertilized (42.22%), yielding 258 blastocysts (blastulation rate [BR] 47.08%). In group B 731 metaphase II oocytes were injected, of which 278 fertilized (38.03%), yielding 103 blastocysts (BR 37.05%) (difference 10.03, z=2.75 p=0.006). In Group A, 100 blastocysts have been transferred to date, resulting in 44 (44%) clinical pregnancies and 33 (33%) live births; in Group B, 96 blastocysts have been transferred, resulting in 36 (37.5%) clinical pregnancies and 25 (26.04%) live births (p=0.355 and p=0.286, respectively). Poor sperm yield was more frequent in Group A than in Group B (82/145 vs 34/101; p=0.0004). ICSI using sperm from the most severe histological patterns (Hya+SCO+MA) was also more frequent in Group A than in Group B (97/142 vs 51/101; p=0.005).

Discussion and Conclusions: Fresh microTESE was associated with a significantly higher number of blastocysts per fertilized egg, which could positively impact the cumulative pregnancy rate compared to patients using frozen/thawed testicular sperm. In addition, ICSI resulted feasible even in the more severe cases of spermatogenic dysfunction (Hya, SCO, MA) and in cases of poor sperm yield, a non-negligible finding considering the probability of post-thaw sperm loss in these cases. Fresh microTESE on the day of oocyte pickup is affected by the risk of sperm retrieval failure: in these cases, donor sperm ICSI or oocyte cryopreservation should be discussed and offered to the couple in advance.

A-0247 Methotrexate toxicity on human spermatozoa: An in vitro study of the effect on sperm motility through, oxidative stress and DNA integrity

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Methotrexate (MTX) is an antimetabolite widely used in the treatment of autoimmune diseases and certain cancers. Despite its therapeutic efficacy, its potential impact on male fertility is a major concern. This study

aimed to evaluate the in vitro effects of MTX on human sperm motility and to identify the underlying mechanisms.

An in vitro experimental study was conducted on semen samples obtained from normozoospermic patients addressed to the department of reproductive biology (Monastir, Tunisia) for semen quality assessment. Were included in the study patients who gave informed consent so that their samples can be used in research purpose after spermogram analysis. Each sample was divided into two aliquots: a control aliquot and an aliquot incubated with methotrexate (0.5 µM). Both aliquots were incubated for 4 hours at 37°C under 5% CO_2 . Sperm motility was assessed according to WHO 2021 criteria. Oxidative stress was evaluated using the DCFH-DA assay, and DNA integrity was analyzed using acridine orange staining and the comet assay. Statistical analysis was performed using ANOVA with a significance level set at $p < 0.05$.

A total of 35 semen samples provided from men aged 30 to 45 years were assessed during the current study. MTX exposure resulted in a significant decrease in progressive motility (11.7% vs 15.4%) and total motility (27.5% vs 34.5%, p<0.001). A marked increase in reactive oxygen species (ROS) production (fluorescence units, FU) was observed (9506 vs 5331). In addition, MTX significantly altered DNA integrity, with increased DNA denaturation (33.9% vs 15.4%) and fragmentation (38.5% vs 12%, p<0.001). Comet tail length also increased (3.29 µm vs 1.13 µm), indicating increased DNA damage.

Methotrexate induces significant toxicity in human spermatozoa, affecting motility, oxidative status, and genetic integrity. These findings highlight the role of oxidative stress in MTX-induced toxicity and suggest a potential risk for male fertility in patients receiving a methotrexate-based treatment.

A-0251 Effect of temperature variations on semen parameters in an infertile population_A focus on morphological abnormalities

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Sperm morphology remains a standard parameter in semen analysis, although recent literature emphasizes its limited diagnostic and prognostic value when interpreted solely. Within this framework, sperm head abnormalities represent a relevant morphological pattern in infertile men. Experimental and clinical data suggest that heat exposure can impair spermatogenesis and semen quality, while varicocele and male genital tract infections have also been associated with altered semen parameters. The objective of this study is to describe this particular phenotype in an infertile population, its variation according to the average temperature variation in the region of Monastir, Tunisia, and its association with other sperm abnormalities.

We conducted a descriptive retrospective cross sectional study on infertile male patients referred to the laboratory of reproductive biology in the neonatology and maternity center of Monastir Tunisia, from January 1st 2025 to December 31st 2025. The sperm samples were collected by masturbation, and were treated according to the WHO 6th edition. Smears were stained with the spermoscan® kit and assessed according to the modified David classification for sperm morphology analysis. According to the average temperature profile in Monastir region, three periods were defined: cool (December–March), hot (June–September), and interseasonal (the remaining months). Statistical analysis was performed using SPSS 27®, we removed all duplicates and considered only the last sperm examination.

A total of 593 patients were included (mean age: 38 ± 7 years; abstinence period: 2.5 ± 0.7 days). Mean semen volume, pH, vitality, and sperm concentration were of 3.41 ± 1.45 mL, 7.09 ± 0.21, 75.14 ± 14.11%, and 101.28 ± 89.72 $\times 10^6$/mL , respectively, with a median of total sperm count at 240 M/ejaculate [124-400]. Progressive motility was low (16.36 ± 7.35%), whereas immotility was high (67.72 ± 9.90%). The most common morphological defects were irregular heads (52.95 ± 9.80%), microcephalic heads (32.27 ± 13.67%), acrosomal abnormalities (30.59 ± 11.77%), tapered heads (22.41 ± 12.32%), and coiled flagella(18.23 ± 9.34%). Seasonal analysis showed significant differences in progressive motility ($p = 0.004$) and immotility ($p = 0.002$), with the highest progressive motility during the inter-season period and the highest immotility during the cold season. Among morphological abnormalities, macrocephalic heads ($p < 0.001$), microcephalic heads ($p < 0.001$), and tapered heads ($p = 0.005$) varied significantly across

seasons ; with a higher median during hot season for the latter. The remaining spermogram parameters showed no significant seasonal variation.

Temperature variation has a considerable effect on the sperm morphology, specifically on the head abnormalities, which could be explained by the increase of oxidative stress and cellular apoptosis. These findings highlight the importance of considering environmental temperature in semen analysis interpretation.

A-0252 HYPOGONADISM AS A PART OF A RARE DISEASE SPECTRUM

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Introduction
Monoclonal gammopathy of undetermined significance (MGUS) is an asymptomatic premalignant plasma cell disorder defined by serum M-protein <30 g/L, bone marrow clonal plasma cells <10%, and absence of end-organ damage (CRAB criteria) or other lymphoproliferative conditions. Although MGUS does not invariably progress to malignancy, it may be associated with systemic manifestations, including endocrinopathies. One rare but clinically significant entity is POEMS syndrome, a paraneoplastic condition characterized by polyneuropathy, organomegaly, endocrinopathy, monoclonal gammopathy, and skin changes. This case underscores the importance of recognizing such associations and adopting a multidisciplinary diagnostic approach.

Case Presentation
A 69-year-old male was referred for endocrine evaluation following a diagnosis of demyelinating polyneuropathy. He reported a 12-month history of fatigue, erectile dysfunction, and decreased libido. His past medical history included hypertension, cervical and lumbosacral polydiscopathy, and polyneuropathy. Physical examination revealed normal BMI (23.3 kg/m²) with no cardiopulmonary or abdominal abnormalities. However, reduced virilization, digital clubbing, and leukonychia were observed. Genital examination showed mildly decreased penile flaccidity and a smaller, softer left testicle without palpable masses.

Laboratory findings demonstrated low total and free testosterone with inappropriately normal gonadotropins, consistent with secondary hypogonadism. Additional abnormalities included chronic autoimmune thyroiditis with preserved thyroid function, hypovitaminosis D, insulin resistance (HOMA-IR 3.8), and light-chain MGUS. Based on these findings, treatment was initiated with intramuscular testosterone (250 mg every 21 days), cholecalciferol (2000 IU daily), and metformin (500 mg daily).

Discussion
According to the 2021 diagnostic criteria, POEMS syndrome requires the presence of both mandatory major criteria (polyneuropathy and monoclonal plasma cell disorder), at least one additional major criterion, and multiple minor criteria. In this patient, mandatory criteria were fulfilled, while the presence of endocrinopathy, skin changes, and polycythemia satisfied minor criteria. Although vascular endothelial growth factor (VEGF) levels were not measured, the overall clinical picture strongly supported the diagnosis of POEMS syndrome.

Endocrine dysfunction in POEMS syndrome is common and may involve both central and peripheral mechanisms. Hypogonadism, thyroid abnormalities, and disturbances in glucose metabolism are most frequently observed, as demonstrated in this case. Adrenal insufficiency has also been reported. Current evidence suggests that these endocrine abnormalities are not caused by structural gland damage or autoimmune processes but are likely mediated by cytokine dysregulation, particularly involving interleukin-6 (IL-6).

Conclusion
From a broader perspective, endocrine dysfunction in the context of plasma cell disorders may represent both a diagnostic clue and a potential therapeutic target. Experimental studies indicate that sex hormone replacement therapy can attenuate hypothalamic–pituitary–adrenal axis hyperactivity and reduce proinflammatory cytokine levels. Similar immunomodulatory effects have been observed with estrogen therapy. These findings suggest that correcting endocrine abnormalities may influence systemic inflammation, although further clinical studies are needed to confirm these effects.

This case highlights the importance of considering POEMS syndrome in patients presenting with combined endocrine and hematological abnormalities and emphasizes the need for an integrated, multidisciplinary approach to diagnosis and management.

A-0253 Patchs in Peyronie's disease implant surgery: failure of the collagen fleece fibrin sealant patch, revised by a bovine pericardium patch. Clinical case

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Introduction
Peyronie's disease may produce penile deformations, as "hourglass deformity" and "penile indentation". In those cases where surgical correction involves the use of a penile prosthesis usually penile deformities are corrected by means of corporoplasty incisions with a resulting albuginea defect that needs coverage. In recent years a novel collagen fleece graft with self-adhesive properties has gained popularity, emerging as a safe, reliable, and promising graft not only for pure grafting procedures but also for residual curvature correction during penile prosthesis implantation.

Aim of this report is to present a Peyronie's disease implant surgery case with a structural failure of the collagen fleece fibrin sealant patch, successfully corrected by means of a bovine pericardium patch.

Clinical case
Our patient is a 64 y.o. man that 7 years earlier underwent in another Institution a surgical correction for severe ED in Peyronie's disease causing right curvature with indentation. The performed surgery involved a corporoplasty by means of a longitudinal incision with inverted "Y" endings that created a 1,5x2,5 cm albuginea defect, patched with a collagen fleece fibrin sealant graft. The used penile prosthesis was an AMS LGX 18 cm device with 4,5 (1,5 + 3) cm rear tips.

The patient presented to us because of the development of device failure ("leak"); besides this he reported a long-standing bulging of the right cylinder in the area of former corporoplasty. Such bulging was present in the "flaccid" state only.

Our surgical procedure: longitudinal ventral access along the shaft raphe, prolonged to the scrotal level. Elevation of Buck's fascia at the bulging level. In the area where the collagen fleece graft was applied we found a redundant thin membrane in place of the albuginea. We left such membrane in place, intact, in order to protect the new cylinder to be implanted. We concealed it inside the corpus cavernosum by interrupted 0 Vycril stitchs, anchored to the surrounding albuginea, and following we covered it by a patch of bovine pericardium. The new inserted implant was an AMS LGX 21 cm with 4 cm rear tips. The patient has been discharged from hospital the first post-operative day.

Results
Our surgery restored not only penile rigidity upon device activation, but also a proper "flaccid" and "erect" penis shaft shape. At three-months follow-up patient resumed satisfactory sexual activity without evidence of bulging recurrence

Conclusions
Our case emphasizes how the collagen fleece fibrin sealant patch does not offer adequate structural resistance for significant albuginea defects (1,5x2,5 cm in this case) in presence of an inflatable penile prosthesis. Conversely, bovine pericardium appears to be a structurally adequate patch to cover also wide albuginea defects. The follow-up of the present case is limited, but our experience in other cases (as in Pescatori E, Loreto A: VJSM 2024 1:105), suggests us to support the above statement.

Presenters do not have any potential conflicts of interest to disclose.

A-0254 Impact of Varicocele Repair using high retro-peritoneal technique and sub-inguinal approach on Semen Parameters in a resource-limited setting

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The impact of varicocele repair on semen parameters improvement remains contradictory according to recent literature data. The sub-inguinal microsurgical approach is generally preferred over other surgical techniques due to lower recurrence rates. However, comprehensive data from North African populations remain scarce, and this study aims to address this gap from a Tunisian university hospital perspective.

We conducted a retrospective quasi-experimental study including men aged 19 years or older who underwent surgical varicocele repair at Fattouma Bourguiba University Hospital in Monastir, Tunisia, between January 2022 and December 2025. Surgical indications included infertility, chronic scrotal pain, and testicular hypotrophy. Among these 83 patients, 45 individuals (54.2%) from the infertile subset provided complete paired semen analyses both preoperatively and 3-6 months postoperatively, forming the analytical cohort. This population was divided into two groups : patients treated with high retro-peritoneal technique and those treated with the sub-inguinal approach.

Our population (n= 83) had a mean age of 31. Varicocele repair was performed with high retro-peritoneal technique in 29 cases whereas the sub-inguinal approach was adopted in 16 patients. Among the 45 patients with complete semen analyses, sperm concentration increased from 77.6 × 10^6/mL to 93.1 × 10^6/mL, representing a 20% improvement ($p = 0.06$). Total motility improved significantly from 26.3% to 33.4% (27% relative gain, $p = 0.008$). Progressive motility rose from 14.0% to 16.9% (21% improvement, $p = 0.07$). Morphology showed a notable enhancement from 6.0% to 8.4% (40% relative improvement, $p = 0.015$). Overall, 67% of patients (30/45) demonstrated improvement in at least two semen parameters.

Regarding technique-specific outcomes, high retro-peritoneal ligation (n=29) yielded a mean total motility improvement of +6.2% and morphology improvement of +2.1%, while the sub-inguinal approach (n=16) achieved +8.3% and +3.0%, respectively. Our results revealed no statistically significant differences between both techniques ($p = 0.78$ for motility, $p = 0.65$ for morphology). We documented six spontaneous pregnancies among the 45 followed patients (13.3%) occurring within 6-12 months postoperatively.

Surgical varicocele repair produced statistically significant improvements in total motility and morphology among the majority of followed patients, with an encouraging spontaneous pregnancy rate. Both high retro-peritoneal ligation and sub-inguinal approaches demonstrated equivalent semen parameter improvements, supporting the viability of high retro-peritoneal ligation technique in resource-limited settings.

A-0257 Penile prosthesis infection and diabetes mellitus: a systematic review and meta-analysis

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Introduction. The preoperative glycometabolic control of diabetic subjects may increase the risk of penile prosthesis (PP) infection but the published literature remains controversial. The aim of the present study is to systematically review and meta-analyze available evidence on the impact of diabetes mellitus (DM) and glycemic control on PP infection.

Methods. An comprehensive Medline, Embase, and Cochrane search was performed including the keywords: ("penile prosthesis" and “diabetes mellitus"). Only English-language articles published between January 1st, 1969 and May 31st, 2024 were included. The primary outcome measure was the risk of PP infection in diabetic patients. The secondary outcome measure was the contribution of glycometabolic control on PP infection rate.

Results Out of 182 retrieved articles, 11 were included accounting for 10,024 subjects with a mean age of 59.7 years, and a mean follow-up of 37.2 months. An overall PP infection rate of 4.3[3.9-4.8]% was observed. The PP infection rate increased according to baseline HbA1c levels and the latter result was confirmed following adjustment for age and trial duration ($p<0.0001$). In addition, PP infection rate was more than 2-times higher when trials with a mean HbA1c ≥8% were compared to the rest of the sample (9.1[7.5;11.0] vs. 3.8[3.2;4.5]%; $Q=43.18$; $p<0.0001$).

Conclusions. The present study provides evidence supporting a significant increased risk of PP infection for patients with DM and pre-operative HbA1c ≥ 8%. Analysis was primarily derived from retrospective studies which represent a significant source of bias. The exclusion of those studies including less than 70% of diabetic patients can represent a further source of bias. Further studies are advisable in order to better clarify the best threshold of HbA1c that is acceptable prior to implant surgery in diabetic patients.

A-0258 Long-term penile prosthesis couple’s satisfaction: a systematic review and meta-analysis

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Introduction: Data supporting successful and satisfactory penile prosthesis (PP) implantation outcomes are mainly based on subjective, rather than objective, analysis. The aim of the present study is to systematically review and objectively analyze, all available data related to patient and partner PP satisfaction.

Methods: An extensive search was performed, including the following key-words: ("penile prosthesis" and “satisfaction"). The search, which accrued data from January 1st, 1969, up to July 31st, 2023, was restricted to English-language articles including human participants.

Results: Out of 663 retrieved articles, 83 were considered accounting for 12,132 subjects with a mean age and mean follow-up of 58.6 [range 20;77.1] years and 47.6 [range 6;374] months, respectively. Overall, a patient satisfaction rate of 83[80;86]% was observed. The satisfaction rate increased in subjects with

three-piece PP and in those with a higher rate of cardiovascular or neurological diseases and was independent of the patient's age. Partner's satisfaction rate was lower when compared to that observed in men and it increased according to the use of inflatable devices and the presence of patient Peyronie's disease. The long-term complication rate was limited ranging from 3% for erosion to 4.6% when mechanical failure was considered.

Conclusions: We found a high couple satisfaction score that was higher when reported by males compared to females. Patient satisfaction increased with time, and it was independent of age. The number of complications is limited and is strongly associated with the presence of DM.

A-0259 TESTOSTERONE THERAPY AFTER RADICAL PROSTATECTOMY: INSIGHTS FROM A SYSTEMATIC REVIEW AND META-ANALYSIS

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Introduction: The safety of testosterone therapy (TTh) in men with a history of prostate cancer (PCa) remains controversial, particularly after interventions for PCA including radical prostatectomy (RP). Previous meta-analyses have included heterogeneous populations, limiting interpretation. The aim of the present study is to systematically review and meta-analyze the risk of biochemical recurrence (BCR) in hypogonadal men treated with testosterone therapy (TTh) following radical prostatectomy (RP) for prostate cancer (PCa).

Methods: A systematic search of Medline was conducted from 1969 to July 2025 according to PRISMA and MOOSE guidelines. Studies reporting BCR rates after TTh in men who underwent RP were included. Data were pooled using a random-effects model. Meta-regression explored the associations with age, Gleason score, follow-up, and preoperative PSA.

Results: Out of 272, seven retrospective studies met the inclusion criteria, encompassing 398 patients (mean age: 63.9 years; mean follow-up: 29.6 months). Overall, the pooled BCR rate in TTh-treated patients was 3.5% (95% CI: 1.5–8.5). No significant association was found between BCR and age, Gleason score, or follow-up duration. In studies with untreated controls, TTh was associated with a significantly lower BCR risk. Limited data suggested improvements in erectile function and quality of life. Funnel plot analysis indicated no publication bias.

Conclusions: TTh after RP appears to be associated with a limited risk of BCR in hypogonadal men with PCa. However, the absence of randomized controlled trials and incomplete reporting of key clinical variables preclude definitive conclusions. Larger, well-designed prospective studies are advisable to confirm these findings and clarify the long-term safety profile of the use of TRT in this setting.

A-0260 UNRAVELLING DEREGULATED METABOLITES IN THE SEMINAL PLASMA OF INFERTILE MEN: A SYSTEMATIC REVIEW AND BIOINFORMATIC ANALYSIS

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Male infertility is a multifactorial condition that accounts for ~50% of infertility cases. Conventional semen analysis, which remains the standard method for male infertility diagnosis, fails to explain ~30% of these

cases and their underlying molecular causes. Considering the lack of alternative diagnostic approaches able to better diagnose male fertility, identifying biomarkers of male infertility is of great interest for the field. Metabolic approaches offer valuable insights into sperm metabolic dysfunction, enabling the identification of deregulated pathways involved in male infertility. Thus, the application of metabolomic methods to study seminal plasma has encouraged the discovery of putative male-infertility biomarkers. In this work, we aimed to identify deregulated metabolites in the seminal plasma of infertile men that can be used as predictive biomarkers of male infertility. A systematic review was conducted in PubMed, Scopus, and Web of Science databases according to PRISMA guidelines (PROSPERO registration: CRD42024572661). Four classes of terms were defined and combined using Boolean operators to retrieve metabolomic studies that compare the seminal plasma metabolome of fertile and infertile men, published until 31 December 2025. The collected information was integrated using bioinformatic tools, identifying potentially altered pathways and related enzymes that may explain many male infertility cases. A total of 284 metabolites were retrieved from the 20 studies included in this systematic review, of which eleven (Down: L-tyrosine, D-fructose, L-valine, phenylalanine, proline, D-glucose, L-alanine, leucine, sorbitol, L-aspartic acid, and Up: uridine), six (Down: D-fructose, glycine, maleic acid, L-valine, proline, and lysine), and ten (Down: L-carnitine, L-tyrosine, L-valine, L-alanine, phenylalanine, proline, D-glucose, leucine, malic acid, and citraconic acid) were altered in the asthenozoospermia, obesity, and overall infertility groups, respectively. No candidate biomarkers were identified for the oligozoospermia group. For each subgroup, metabolites were considered candidate biomarkers if they were consistently up- or down-regulated in at least three studies. In the overall infertility group, metabolites were considered potential biomarkers if they were reported in four or more studies and at least two different infertility-related conditions. Using MetaboAnalyst 6.0, we found that these metabolites were predominantly associated with phenylalanine and tyrosine metabolism, fructose and mannose degradation, and β-oxidation of very long-chain fatty acids. The enzymes involved in these pathways (e.g., IL4I1, SORD, HMOX1) revealed associations with male infertility phenotypes and key sperm functions, including motility, vitality, and energy metabolism. Considering the accessory glands' contribution to the seminal volume and content, the metabolic alterations identified should reflect much more than the sperm metabolism itself. Further studies integrating metabolomics, proteomics, and transcriptomics should be performed to validate the use of the metabolites and enzymes identified as biomarkers, both in seminal fluid and spermatozoa. The findings of this study emphasize the potential of the identified metabolites as biomarkers for diagnosing male infertility, elucidating the metabolic mechanisms, and supporting potential therapeutic applications. Funding: This research was funded by national funds through the FCT, within the scope of project UID/04501/2025 (https://doi.org/10.54499/UID/04501/2025). This work is financed by FEDER funds through COMPETE2030 and by National Funds through FCT, under the project FERTISCAN (Project 16652; COMPETE2030-FEDER-00783300; 2023.17577.ICDT; https://doi.org/10.54499/2023.17577.ICDT).

A-0265 Complete AZF and gr/gr deletions in infertile men: 21-year diagnostic experience and clinical implications

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Y-chromosome microdeletions are a well-established cause of spermatogenic impairment. While screening for classical AZoospermia Factor (AZF) deletions is part of the routine diagnostic work-up of male infertility, testing for partial gr/gr deletion remains optional in routine practice due to its unclear clinical value. Although a number of studies suggest that gr/gr deletion represents a risk factor for impaired spermatogenesis; the magnitude of this risk appears to vary across studies.

In this study, we report the 21-year experience of the Fundació Puigvert (Barcelona) in analyzing infertile men for both classic AZF microdeletions and gr/gr deletion.

We retrospectively evaluated 1712 infertile patients (1235 Spanish and 477 non-Spanish) with normal karyotype and <10 spermatozoa × 10^6/mL for classical AZF deletions. Patients were stratified into 1098 idiopathic and 614 non-idiopathic subjects with a clinical history potentially explaining their infertility.

Partial AZFc rearrangements were analysed in a subset of 751 idiopathic infertile patients of Spanish origin and compared with 395 Spanish normozoospermic controls.

Yq microdeletions were screened according to the European Academy of Andrology (EAA) and European Molecular Genetics Quality Network (EMQN) guidelines.

Overall, 58 patients carried a complete AZF deletion. The most frequent microdeletion was AZFc (51/58), followed by AZFbc (4/58) and AZFa (3/58). AZF deletions were significantly more frequent among idiopathic patients than non-idiopathic (4.7% vs. 1%; p<0.001). This difference remained significant when stratified by semen phenotype: i) azoospermic men (8.8% vs. 1.8%, p<0.001); ii) severe oligozoospermic individuals (4.2% vs. 0.5%, p=0.014). Significant differences in overall frequency were observed between Spanish and non-Spanish individuals (3.1% vs. 8.7%; p<0.001). Within the AZF-deleted cohort, the majority were azoospermic (69%), followed by severe oligozoospermic patients with ≤1×10^6 spermatozoa/mL (27.6%). Only two patients had sperm concentrations above this threshold: one with 1.2×10^6/mL and one with 9×10^6/mL.

Regarding the gr/gr deletion, it was observed more frequently in infertile men (3.2%) versus controls (1.3%), although this difference was not statistically significant. Nonetheless, when infertile men were stratified according to sperm concentration, the gr/gr deletion frequency was significantly higher among oligozoospermic patients (4%) than among normozoospermic controls (1.3%) (OR:3.3, 95% CI: 1.2–8.8; p = 0.015). The frequency among azoospermic patients was identical to that of the controls (1.3%).

Our findings corroborate the diagnostic role of AZF deletions and emphasize that 96.5% of the deletions occurs with sperm concentration <1 million/ml. However, two patients exceeded this threshold, including one with moderate oligozoospermia. His young age (26 years) may explain this milder phenotype, consistent with a progressive decrease of the sperm count described in the literature. This raises the question whether the sperm concentration threshold indicated for AZF testing should take the patient's age into consideration.

Finally, we confirm that the gr/gr deletion is a risk factor for impaired spermatogenesis in the Spanish population showing a significant association with oligozoospermia (from severe to moderate). This suggests that screening for gr/gr deletion in this population may have clinical relevance, particularly given its obligatory transmission to the male offspring, with the potential to progress to a complete AZFc deletion in the next generation.

A-0267 Fractionated Testosterone Gel Replacement Therapy in Clinical Practice: A Real-World Clinical Experience

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Background Testosterone replacement therapy (TRT) with transdermal gel is widely used in men with hypogonadism. Current guidelines recommend once-daily application with monitoring of serum testosterone at peak levels. However, real-world clinical experience suggests that peak–trough fluctuations may occur in a subset of patients, potentially affecting hormonal stability and treatment tolerability. The impact of dose fractionation on testosterone profiles and safety remains to be clarified.

Objectives To compare hormonal profiles and biochemical safety parameters between once-daily and twice-daily (fractionated) testosterone gel administration in hypogonadal men treated with the same total daily dose.

Methods We retrospectively identified hypogonadal men treated with 2% testosterone gel who transitioned from once-daily to twice-daily split dosing, maintaining the same total daily dose, on their own initiative. Eligible patients had serum total testosterone (TT) measurements available at both peak (3 hours post-

application) and nadir (pre-dose). Hormonal and laboratory parameters obtained during the once-daily and twice-daily regimens were compared.

Results Twelve patients fulfilled inclusion criteria. Compared with once-daily administration, fractionated dosing resulted in significantly lower peak TT levels [9.0 (5.3 – 13.0) vs. 5.7 (3.6 – 7.1) ng/mL, p = 0.002] and significantly higher nadir TT levels [1.4 (1.1 – 1.8) vs. 3.2 (2.6 – 5.4) ng/mL, p = 0.005], leading to a marked reduction in peak–trough variability. The proportion of patients achieving target TT levels at peak increased from 33% to 83% after dose fractionation (p = 0.019). Calculated free testosterone showed a similar pattern. No significant differences were observed in luteinizing hormone, prostate-specific antigen, haematocrit, or haemoglobin between regimens.

Conclusion In this real-world cohort, fractionated testosterone gel administration was associated with improved biochemical target attainment and a reduced difference between peak and pre-dose testosterone concentrations, without changes in short-term safety parameters. These findings should be considered hypothesis-generating.

A-0268 THE EFFECT OF PSYCHOTROPIC MEDICATIONS ON SPERM PARAMETERS

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Background: The world is currently facing a significant decline in male reproductive health, with sperm counts dropping in the last decades. While environmental and lifestyle factors are often blamed, recognition of pharmacological influences is increasing. Psychotropic drugs are of particular clinical interest, as they are frequently used by men seeking reproductive care while planning for a family. Although the impact of SSRIs on male fertility has been systematically studied, other systematic studies on medications such as benzodiazepines, methylphenidate, risperidone, and quetiapine remain sparse. By incorporating both human and animal models and adopting a broad scope, this research aims to provide a comprehensive overview of how these diverse psychotropic classes influence sperm parameters.

Objective: Based on clinical prevalence, we studied the effects of five groups of psychotropic medications (SSRIs, Benzodiazepines, Methylphenidate, Risperidone, and Quetiapine) on sperm parameters including count, motility, viability, morphology, and oxidative stress and hormone levels.

Methods: A systematic literature search was conducted in PubMed and EMBASE. The initial search identified 826 articles, with 122 duplicates removed. The remaining 704 articles underwent abstract screening, leading to the exclusion of 599 for irrelevant design, drugs, outcomes, or populations. Following full-text review of the remaining 105 articles, an additional 48 were excluded.

Results: The final review included 57 articles: 13 clinical studies and 44 animal studies (mice, N=34; rats, N=11). In total, 45 studies showed a negative impact of at least one medication group on sperm parameters. Nine studies showed no significant impact, and three showed a positive outcome. Notably, the latter were all studies focused on methylphenidate.

Conclusion: The majority of evaluated studies suggests that these common psychotropic medications adversely affect male reproductive parameters. These findings highlight the need for clinical awareness regarding the reproductive side effects of psychiatric treatment in reproductive medicine and andrology.

A-0270 Smoking-Associated Decline in Semen Quality Linked to Activation of Sperm Stress Pathways

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Over the past few decades, accumulating evidence has highlighted a global decline in male reproductive health. While genetic factors, hormonal imbalances, and reproductive system defects contribute to male infertility, lifestyle choices such as alcohol consumption, cigarette smoking and drug use have emerged as significant modifiable risk factors affecting reproductive function and semen quality. Among these, cigarette smoking has been consistently associated with impaired semen quality parameters. However, the molecular mechanisms underlying the effects of cigarette smoking on sperm cells remain poorly understood.
The purpose of this study was to explore the impact of tobacco consumption on semen quality parameters and identify the molecular biomarkers associated with cellular stress in sperm. Thirty healthy male volunteers were recruited from the Urology service at Hospital Infante D. Pedro, Local Health Unit of Aveiro, Portugal. Participants completed detailed lifestyle questionnaires and provided semen samples, which were analysed by experienced technicians according to the World Health Organization criteria. In addition, the expression levels of antioxidant enzymes and heat-shock response-related proteins were evaluated to assess activation of stress response pathways in sperm cells.

The results showed that cigarette smoking negatively affects semen quality, reducing semen volume and total sperm count. Although the changes in the percentage of total motility and normal morphology in the smokers' group did not reach statistical significance, a slight decrease was also observed. Moreover, was identified for the first time a significant association between tobacco consumption and increased levels of heat shock protein 27 (HSP27) and phosphorylated HSP27 (p-HSP27) in sperm cells, indicating the potential detrimental effects of tobacco on the reproductive system.

This study underscores the importance of modifiable lifestyle factors in male fertility and highlights potential molecular biomarkers associated with smoking-related reproductive dysfunction, contributing to increased awareness and improved risk assessment in populations vulnerable to male infertility.

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A-0272 “It’s not you, it’s the butterfly effect”: development of a conceptual framework for the Chaos Theory in sexual medicine

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Traditional models describing sexual response focus on either partner, without considering the mutual interaction between the partners involved and the influence of contexts, social dynamics, prior experience, and lifestyles. However, even minor changes in one of the many factors involved in sexual response can lead to a dramatic, possibly transitory, shift in sexual experience. Likewise, patients having nearly identical conditions (e.g., risk factors) have wildly different clinical trajectories. This non-linear dynamic between inputs and outcomes is inadequately addressed by existing models, affecting patient care. To solve this issue, we hereby propose a model based on Chaos Theory, an established mathematical construct based

on the notion that small changes in initial conditions can produce completely different outputs. Based on the application of Chaos Theory in other fields, we propose a new conceptual framework to redefine the approach to andrology and sexual medicine. This approach finds its foundation in Systems Sexology, which features the interaction between four Systems (mind, experience, society and body) as the core mechanism for sexual function and dysfunction. According to the biological applications of Chaos Theory, the four systems do not follow a programmed sequence but oscillate within an attractor, i.e. a region of possibilities that defines the typical pattern of an individual's sexual response. A variety of factors affecting any of the four systems can change the trajectory from a "functional" to a "dysfunctional" attractor. The proposed approach provides an explanation – and possibly a solution – to the variability of patients' journeys and therapeutical outcomes by suggesting that "one size fits all" protocols are mathematically flawed in complex systems, such as sexual health, while avoiding reductionism. At the same time, the Chaos Theory approach puts management of risk factors at the center of any treatment, by validating the idea that tiny, targeted clinical interventions, including lifestyle changes, can lead to massive long-term improvements in patient care and quality of life.

A-0273 Does low intensity shockwave treatment (LISWT) affect cavernosal vascular factors in erectile dysfunction? A pilot study

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Background: Low-intensity shockwave treatment (LISWT) is deemed to promote tissue angiogenesis through release of vascular endothelial growth factor (VEGF) and possibly enhance neovascularization and tissue repair mechanisms near the treatment site. Despite the large body of evidence of clinical efficacy and safety, no evidence is available in human models regarding the possible exact mechanism of function or the impact of LISWT on penile vascular endothelial factors.

Methods: In this prospective single arm pilot study conducted at Humanitas Clinical and Research Center, all patients with erectile dysfunction (ED) were consecutively screened for eligibility, excluding subjects with hormonal, neurogenic or psychogenic ED. ED was defined and classified using the IIEF-15 EF domain. The LISWT was provided using the "Duolith® SD1 Tower" medical device, manufactured by STORZ MEDICAL AG. Dynamic penile doppler ultrasound was performed before blood withdrawal procedure, measuring peak systolic velocity (PSV). Cavernosal blood samples were collected to measure VEGF, VEGF-receptor (VEGF-R) and interleuchin-10 (IL-10) using ELISA assays. Baseline IIEF-EF domain score, cavernosal vascular factors concentration and penile doppler parameters were compared to end-treatment.

Results: nine subjects (mean age 54 years; range 45–79 years) met inclusion criteria and completed the protocol. In the overall cohort, a statistically significant improvement in the IIEF-EF domain score was observed at follow-up (11.0 ± 8.3 vs. 18.7 ± 8.6; p = 0.031). Although not reaching statistical significance, an increase in PSV was noted at study endpoint (45.8 ± 18.8 cm/s vs. 56.7 ± 14.2 cm/s; p = 0.151). When analyses were restricted to individuals presenting with moderate to severe ED at baseline (n = 5), trends toward improvement in PSV (33.7 ± 5.1 cm/s vs. 50.1 ± 10.0 cm/s; p = 0.094) and cavernosal VEGF-R expression (161.2 ± 1.3 pg/ml vs. 429.8 ± 57.8 pg/ml; p = 0.096) were evident. Conversely, no significant differences were detected in the concentrations of evaluated cavernosal biomarkers from baseline to endpoint across the full cohort (VEGF-R: 303 ± 231 pg/ml vs. 337 ± 176 pg/ml, p = 0.753; VEGF: 503 ± 553 pg/ml vs. 518 ± 536 pg/ml, p = 0.398; IL-10: 7.5 ± 8.2 pg/ml vs. 8.9 ± 6.8 pg/ml, p = 0.327).

Conclusions: this is the first observation describing the change of cavernosal vascular parameters in human ED model after LISWT. Our results, despite the study limitations, point towards a non-significant

change of vascular parameters but confirm a slight clinical improvement in erectile function following LISWT.

A-0274 Anti-hypertensive medications and erectile dysfunction: focus on b-blockers

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Purpose: Although antihypertensive medications, particularly thiazide diuretics and β-blockers (BBs), have been implicated in the development of erectile dysfunction (ED); their actual contribution remains controversial. This paper aims to synthesize the available evidence and provide a critical, evidenze-based analysis of this issue.

Methods: A comprehensive narrative rewies was conducted using searches of Medline, Embase and Cochrane databases. Additionally, to further elucidate the impact of β-blocherks (BBs) on erectile dysfunction (ED), a dedicated systematic review was performed.

Results: The adverse effects of centrally acting agent, such as clonidine and alpha-methyldopa, on erectile function are well documented, although evidence from controlled trials remains limited. Angiotenin-converting enzyme inhibitors (ACE inhibitors), angiotensin receptor blockers (ARBs), and calcium channel blockers (CCBs) appear to exert neutral (CCBs) or even beneficial (ACE inhibitors and ARBs) effects on erectile function. Despite earlier reports suggesting a detrimental role, more recent evidence does not support a negative impact of thiazide diuretics. β-blockers (BBs), however, continue to be the class most frequently associated with erectile dysfunction, although more favorable outcomes have been observed with nebivolol.

Conclusion: Patient with arterial hypertension should have their sexual function routinely assessed, both at the time of diagnosis and after the initiation of specific pharmacological therapies. A close and proactive patient-physician relationship, supported by open discussion, may help mitigate potential adverse effects and enable the effective management of treatment-related side effects.

A-0276 Cardiovascular safety of testosterone replacement therapy in men: an updated systematic review and meta-analysis

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Introduction: The cardiovascular (CV) safety of testosterone (T) replacement therapy (TRT) is still conflicting. Recent data suggested a TRT-related increased risk of atrial fibrillation (AF).

Aim: To systematically review and meta-analyze CV risk related to TRT as derived from placebo controlled randomized trials (RCTs).

Methods: An extensive Medline, Embase, and Cochrane search was performed. All placebo-controlled RCTs reporting data on TRT-related CV safety were considered. To better analyze the role of T on AF, population-based studies investigating the relationship between endogenous circulating T levels and AF incidence were also included and analyzed.

Results: Out of 3.615, 106 studies were considered, including 8.126 subjects treated with TRT and 7.310 patients allocated to placebo. No difference between TRT and placebo was observed when major adverse CV events were considered. Whereas the incidence of non-fatal arrhythmias and AF was increased in the only trial considering CV safety as the primary endpoint, this was not confirmed when all other studies were considered (MH-OR 1.61[0.84;3.08] and 1.44[0.46;4.46]). Similarly, no relationship between endogenous T levels and AF incidence was observed after the adjustment for confounders.

Conclusions: Available data confirm that TRT is safe and it is not related to an increased CV risk.

A-0280 Crocin: A Promising Antioxidant for Sperm Motility Improvement

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Excessive production of reactive oxygen species (ROS) is a major contributor to impaired semen quality, particularly by compromising sperm motility. Crocin, the main bioactive compound of Crocus sativus (saffron), is well known for its potent antioxidant properties. The present study aimed to evaluate the ability of crocin to improve in vitro sperm motility in asthenozoospermic patients. Semen samples from 95 asthenozoospermic patients were incubated for 3 hours at 37°C with increasing concentrations of crocin (0, 0.2, 0.5, 1, and 1.5 mM). Participants diagnosed with asthenozoospermia were stratified into two subgroups based on their baseline progressive motility: Group 1 (G1), with progressive motility ranging from 20% to 30%, and Group 2 (G2), with progressive motility between 10% and 15%.

Sperm motility was assessed using time-lapse video microscopy. Intracellular ROS levels were measured using the DCFH-DA assay, while oxidative damage was evaluated through lipid peroxidation and protein carbonyl content. In addition, antioxidant enzyme activities, including catalase, superoxide dismutase, and glutathione peroxidase, were analyzed.

The results demonstrated a significant enhancement in sperm velocity by 93% (G1) and 91% (G2), along with an increase in the distance traveled by motile spermatozoa by 76.5% (G1) and 72.1% (G2) ($p < 0.001$). ROS levels were significantly reduced by 1.5-fold (G1) and 2.4-fold (G2) ($p < 0.001$). Furthermore, malondialdehyde levels decreased twofold (G1) and 3.4-fold (G2), while protein carbonyl content was reduced by 2.8-fold (G1) and threefold (G2) ($p < 0.001$).

These findings highlight crocin as a promising candidate for therapeutic strategies to improve male fertility, although further in vivo and clinical studies are required to confirm its efficacy and safety.

A-0282 Crocin promotes mitochondrial efficiency in asthenozoospermic samples

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Mitochondria play a central role in sperm motility by generating the ATP required for flagellar movement. Targeting mitochondrial function represents a promising approach to improve sperm performance.

Semen samples obtained from 95 asthenozoospermic patients were incubated for 3 hours at 37°C with increasing concentrations of crocin (0, 0.2, 0.5, 1, and 1.5 mM). Patients were classified into two groups according to baseline progressive motility: Group 1 (G1: 20–30%) and Group 2 (G2: 10–15%). Mitochondrial function was assessed by measuring metabolic activity using the MTT assay and mitochondrial membrane potential using the Rh123 assay.

The results showed a significant enhancement in mitochondrial function following crocin treatment. Succinate dehydrogenase activity increased by 2.14-fold in G1 and 1.85-fold in G2 ($p < 0.001$).

Additionally, mitochondrial membrane potential was significantly elevated by 1.7-fold in G1 and 1.9-fold in G2 ($p < 0.001$), indicating improved mitochondrial integrity and bioenergetic capacity.

Crocin enhances mitochondrial function in asthenozoospermic sperm, supporting its potential role in improving male fertility.

A-0283 Testosterone in Iron Overload: Essential for Bone, Not Sufficient Alone

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Background: Men with iron overload disorders (IOD), including transfusion-dependent beta-thalassemia (TDBT) and hereditary hemochromatosis (HH), are at high risk of bone loss due to chronic iron accumulation. Hypogonadotropic hypogonadism is common in these patients and further compromises bone health. Evidence on the impact of testosterone replacement therapy (TRT) on bone density in this population remains limited.

Objective: To investigate longitudinal changes in bone mineral density (BMD) in men with TDBT or HH receiving TRT compared to untreated subjects, and to explore the role of antiresorptive therapy.

Methods: We conducted a prospective observational study including male patients with IOD followed at our Center who had undergone at least two dual-energy X-ray absorptiometry (DXA) scans of the lumbar spine and proximal femur. Bone mineral density (BMD), T-scores, and Z-scores were recorded. Hypogonadotropic hypogonadism was diagnosed based on clinical symptoms and serum total testosterone levels <3.5 ng/mL, in accordance with SIAMS guidelines.

Results: We prospectively followed 23 men with IOD (19 TDBT and 4 HH; mean age 40 years, all <50). At baseline, 60% of patients had age-adjusted low BMD, and 8% had experienced at least one fragility fracture. Hypogonadotropic hypogonadism was present in 43.5% of patients, with decreased libido and erectile dysfunction reported in 13% and reduced secondary sexual characteristics in 30%. Hypogonadal patients received TRT (40% testosterone enanthate, 10% testosterone gel, and 50% testosterone undecanoate) for a mean duration of 6.2 ± 7 years. Seven patients (30.4%) also received antiresorptive therapy (bisphosphonates or denosumab) for a mean of 2.67 ± 1.4 years. Longitudinal changes in BMD and T-scores were analyzed using models adjusted for baseline values, TRT duration, and antiresorptive therapy. The mean interval between DXA scans was 7.5 ± 3 years. After adjustment for baseline T-score, TRT duration, and antiresorptive therapy, TRT was associated with a significant increase in lumbar spine Z-score ($p = 0.005$), whereas changes in proximal femur BMD were not significant. In patients receiving concomitant antiresorptive therapy, TRT did not provide additional benefits in terms of BMD.

Conclusions: TRT is essential in young men with IOD and hypogonadism, not only to preserve sexual function and quality of life but also to support bone health. However, TRT alone is less effective than combination therapy with osteoactive agents in preventing iron-induced bone damage and improving BMD. The management of osteoporosis in these patients remains challenging due to limited therapeutic options and the potentially reduced efficacy of antiresorptive agents in the context of iron overload. These findings should be interpreted considering some limitations, including the relatively small sample size, the heterogeneity of treatments, and the variable follow-up duration. Nevertheless, they underscore the need for individualized, multidisciplinary strategies, including optimized iron chelation, regular DXA monitoring, targeted antiresorptive therapy when indicated, and lifestyle interventions to preserve skeletal health. Future studies should explore combination approaches and novel therapies to better protect bone integrity in men with IOD.

A-0284 Sperm DNA fragmentation: Comparative insights from four assays

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Sperm DNA integrity is a crucial determinant of male fertility, influencing both fertilization potential and the likelihood of successful pregnancies.

This study aimed to evaluate DNA quality in subfertile men using multiple diagnostic approaches. A total of 170 subfertile patients were included. DNA integrity was assessed through four complementary techniques: the Acridine Orange test for DNA denaturation, Aniline Blue staining to evaluate chromatin maturation, and the TUNEL and Comet assays to quantify DNA fragmentation. Correlations between these assays and conventional sperm parameters were also analyzed.

Our findings revealed strong positive correlations ($p < 0.001$) among all four DNA assessment methods. Additionally, higher percentages of abnormal sperm were significantly associated with elevated DNA fragmentation as measured by TUNEL, Comet, and Aniline Blue assays ($p < 0.05$). Conversely, total sperm motility exhibited a significant negative correlation with these techniques ($p < 0.05$).

These results underscore the value of integrating multiple diagnostic methods to assess sperm DNA quality, with the TUNEL and Comet assays proving particularly informative. Such evaluations may enhance the accuracy of male infertility diagnosis and guide more effective treatment strategies for couples struggling with subfertility.

A-0288 SEPTIN 12 LOCALIZES TO THE MANCHETTE AND PERINUCLEAR RING DURING SPERMIOGENESIS

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Septin 12 is a testis-specific cytoskeletal protein mainly known for its role in sperm tail organization and annulus formation. Previous studies showed that Septin 12 interacts with both actin and tubulin and that its dysfunction is associated with abnormal sperm morphology and impaired motility. Our recent findings support the relevance of Septin 12 for sperm development and structural integrity. Although in mice Septin 12 was also described in the manchette of elongating spermatids, its precise role during spermiogenesis, particularly during spermatid elongation and head shaping, remains poorly understood.

Here, we focused on the localization of Septin 12 during spermatid elongation. Using confocal and STED imaging in rodent germ cells, we observed Septin 12 in the perinuclear ring and as distinct linear structures on the ventral and dorsal sides of elongating spermatids. In addition, a weaker Septin 12 signal was detectable beneath the whole manchette. These observations indicate that Septin 12 is not restricted to the annulus of mature sperm, but is also associated with the manchette-related compartment during sperm head remodelling.

Our data support a close spatial relationship between Septin 12 and the microtubule-based manchette and suggest that Septin 12 may contribute to the structural organization of this transient spermatid scaffold. Ongoing analyses using STED microscopy, expansion microscopy, and ultrastructural approaches are aimed at defining the precise spatial arrangement of Septin 12 within the manchette/perinuclear ring region and its relation to tubulin-associated structures.

Together, these findings support a model in which Septin 12 may act as a linker within the manchette/perinuclear ring complex, connecting the tubulin-based scaffold to the nuclear envelope and possibly also to membrane-associated structures during spermatid elongation and head shaping. In this way, our work extends the current view of Septin 12 beyond the annulus and may contribute to a better understanding of the mechanisms underlying teratozoospermia and male infertility.

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A-0289 Local host immune response against different types of bacteria in fertile and infertile men

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This study aimed to characterize the local immune response associated with bacteriospermia of distinct microbial origins and to determine its contribution to impaired sperm quality. Semen samples from 49 fertile and 168 infertile men of reproductive age underwent standard analysis according to the 5th WHO Laboratory Manual, complemented by comprehensive microbiological screening, including aerobic, anaerobic, and Mycoplasma/Ureaplasma cultures, as well as multiplex quantification of 16 cytokines, chemokines, and growth factors using the Luminex platform. For comparative analysis, the samples were divided into four groups based on the presence of bacteriospermia (fertile (B−), fertile (B+), infertile (B−), infertile (B+)) and additionally into five subgroups based on the type of bacteriospermia (aerobes (+), anaerobes (+), Mycoplasma/Ureaplasma (+), mixed (+), no bacteria). Overall, 108/217 men (49.8%) exhibited asymptomatic bacteriospermia, including 27 fertile and 81 infertile individuals. The microbial culture profiles differed between groups: fertile men were predominantly positive for anaerobic Gram-positive bacteria (24.3%) and Enterococcus spp. (19.0%), whereas infertile men showed a higher prevalence of anaerobic Gram-negative (19.2%), aerobic Gram-negative (18.4%), and Streptococcus spp. (13.2%). Fertile men with bacteriospermia demonstrated significantly elevated IL-10 levels compared to the fertile (B−) group. Both aerobic and anaerobic bacteriospermia were characterized by a significant increase in IL-1α and IL-1β concentrations compared to samples without detectable bacteria. In the fertile (B+) group, TNF-α concentrations negatively correlated with sperm viability and morphology, while IL-1α negatively correlated with motility, viability, and the percentage of swollen sperm in the HOS test. In the infertile (B−) group, leukocyte and round cell percentages were positively correlated with the concentrations of IL-6, IL-8, IL-1α, IL-1β, and G-CSF. In contrast, the presence of bacteriospermia in infertile men eliminated correlations with round cells. Aerobic bacteriospermia was further linked to increased IL-8, IL-10, and IL-1β levels, which were positively associated with semen pH and inversely related to semen volume. In the presence of anaerobes, most proinflammatory cytokines positively correlated with leukocyte concentration. In the Mycoplasma/Ureaplasma (+) group, increased levels of proinflammatory cytokines such as TNF-α, IL-6, IL-1β, and IL-12 were associated with deterioration of sperm morphology. This subgroup uniquely exhibited strong TGF-β1–mediated suppression of adaptive immune responses, contrasting with the cytokine network amplification observed in both aerobic and anaerobic positive subgroups. Moreover, aerobic bacteria promoted a sustained proinflammatory milieu via MIF, indicative of a mechanism driving chronic inflammation. These findings demonstrate that asymptomatic bacteriospermia elicits pathogen-specific immune signatures that differentially affect sperm function. The reproductive risk is therefore determined by the taxonomic and functional characteristics of the bacteria, which shape inflammatory responses and the pattern of innate immune activation within the male reproductive tract.

A-0293 Characteristics of Secondary Hypogonadism defined by LH levels but with elevated FSH: findings from a cohort of men seeking specialist evaluation for Sexual Dysfunction

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Introduction: The definition of hypogonadism as primary or secondary is based on LH values. The use of FSH has been proposed as an additional parameter to define the primary nature of hypogonadism. Aim:

To assess whether characterizing subjects with secondary hypogonadism based on FSH values can identify a distinctive phenotype of male hypogonadism. Methods: A consecutive series of 4,883 men with sexual dysfunction was considered. Of these, 3,428 (70.2%) were eugonadal (total testosterone ≥12 nmol/L), 162 (3.3%) had primary hypogonadism (total testosterone <12 nmol/L and LH ≥9.4 U/L), and 1,293 (26.5%) had secondary hypogonadism (LH <9.4 U/L). The latter group was subdivided according to FSH values (< or ≥11 U/L) into "pure" secondary hypogonadism or "mixed" hypogonadism. Demographic, andrological, metabolic, and psychological characteristics of these groups were compared with those of eugonadal men. Results: Regardless of confounding factors, subjects with primary hypogonadism had a higher frequency of testicular disease in their medical history [OR: 5.91 (3.95; 8.84)], smaller testicular volume [B: -9.35 (-10.08; -8.62)], and more symptoms and signs of hypogonadism [higher ANDROTEST score, lower hemoglobin and PSA values; B: 2.72 (2.00; 3.45), -0.68 (-1.02; -0.35), and -0.69 (-0.79; -0.59), respectively]. They also had a higher estimated cardiovascular risk [B: 3.39 (1.74; 5.03)]. Men with "pure" secondary hypogonadism showed more sexual symptoms, a higher prevalence of metabolic syndrome [B: 2.62 (2.20; 3.12)], and reduced penile blood flow. Men with mixed hypogonadism had a higher frequency of testicular disease and lower testicular volume than eugonadal men, with values intermediate between those with primary and "pure" secondary hypogonadism [OR: 2.59 (1.49; 4.51); B: -5.27 (-6.01; -4.53)]. The ANDROTEST score was also higher than in eugonadal subjects, again with intermediate values between primary and "pure" secondary hypogonadism [B: 2.05 (1.38; 2.73)]. Regarding sexual symptoms, hemoglobin and PSA levels, and penile Doppler parameters, men with mixed hypogonadism behaved similarly to those with "pure" secondary hypogonadism. However, men with mixed hypogonadism showed a higher frequency of metabolic syndrome and a greater estimated cardiovascular risk compared with eugonadal subjects [OR: 4.01 (2.56; 6.30) and B: 3.76 (2.12; 5.39)], with stronger associations than in primary and "pure" secondary hypogonadism. Interestingly, while subjects with primary or "pure" secondary hypogonadism did not show psychopathological characteristics compared with eugonadal men, those with mixed hypogonadism exhibited symptoms of free and phobic anxiety and obsessive traits [B: 0.91 (0.25; 1.56), 0.71 (0.22; 1.20), and 0.79 (0.10; 1.48)], as well as a tendency toward depressive symptoms and somatized anxiety [B: 0.53 (-0.06; 1.13) and 0.47 (-0.04; 0.99)]. Conclusion: Among men with hypogonadism, FSH may help identify a group at higher metabolic and cardiovascular risk, and characterized by greater psychopathological symptoms.

A-0294 Sperm motility impairment: A study of oxidative and mitochondrial parameters

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Increased oxidative stress is a major contributor to male infertility, particularly in cases of reduced sperm motility. This study aimed to evaluate the oxidative status and mitochondrial function in patients with asthenozoospermia. An experimental study was conducted on semen samples collected from patients referred to the Reproductive Biology Service at the Maternity and Neonatology Center of Fattouma Bourguiba Hospital, Monastir. A total of 145 patients were included: 50 normozoospermic controls according to the WHO 2021, 50 patients with progressive motility between 20–30% (Group A1), and 45 patients with progressive motility between 10–15% (Group A2).

Reactive oxygen species (ROS) production was measured using the DCFH-DA assay. Lipid peroxidation was assessed via malondialdehyde (MDA) levels, and protein carbonylation was quantified. Antioxidant enzyme activities, including catalase (CAT) and superoxide dismutase (SOD), were also evaluated. Mitochondrial activity was assessed using the MTT assay, and mitochondrial membrane potential was measured with Rhodamine 123.

Our results demonstrated that ROS levels in Group A2 were 10-fold higher than A1 ($p < 0.001$) and 20-fold higher compared to controls ($p < 0.001$). This increase in ROS was associated with reduced antioxidant enzyme activity: SOD decreased by 50% in A2 vs. control ($p < 0.001$) and 30% in A2 vs. control ($p < 0.001$), while CAT activity decreased by 60% in A2 vs. control ($p < 0.001$) and 35% in A2 vs. A1 ($p < 0.01$). Lipid peroxidation levels were elevated 3-fold in A2 vs. control ($p < 0.001$) and 2-fold vs. A1 ($p < 0.05$). Protein carbonyl content increased twofold in A2 compared to both control and A1 ($p < 0.05$). Additionally,

succinate dehydrogenase activity and mitochondrial membrane potential decreased by 15% in A1 and 50% in A2 ($p < 0.05$) compared with control group.

These findings reveal a clear association between reduced sperm motility, excessive ROS production, and mitochondrial vulnerability, manifested as lipid and protein oxidative damage and impaired mitochondrial function. Our results highlight the critical role of redox balance in maintaining sperm quality and emphasize the major influence of mitochondrial integrity on progressive sperm motility.

A-0295 Evaluation of chronic widespread pain and its impact on sexual function and satisfaction

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Introduction
Chronic pain is a common problem profoundly impacting on quality of life, often associated with many chronic pathological conditions; furthermore, with increasing life expectancy, a growing proportion of the population experiences some type of painful symptomatology.

Some of these chronic conditions are known to cause hypogonadism; however, the consequences of chronic painful conditions on sexual function remains poorly investigated.

Aim
To evaluate the potential impact of chronic widespread pain (CWP) on sexual health and satisfaction.

Methods
Community-dwelling men from the EMAS study (40–79 years) with baseline and follow-up pain-related data (n=2012) were included. Sexual, physical, and psychological function was evaluated using validated questionnaires (SFQ,SF-36,BDI,PASE) and objective tests (PPT,DSST,Camden Memory Test) at baseline and follow-up (after a median of 4.3 years). Pain was assessed by a self-reported questionnaire evaluating site, extent, and duration of pain and categorized into “no pain”, “some pain” (pain non consistent with CWP definition), and “CWP” (pain lasting >3 months and present in at least 2 sites on both left and right side of the body). Considering pain status at baseline and follow-up, participants who developed CWP at follow-up (new CWP) and those who developed “some pain”(new pain) were compared with those with no or some pain not worsened at follow-up (no/unchanged pain). Mediation analysis was performed to assess the role of pain in determining the onset/worsening of sexual symptoms, and the potential role of depression as a mediating factor.

Results
In a model adjusted for age, centre, smoking habits, BMI and comorbidities, both the groups reporting a

worsening in pain showed a deterioration in their overall sexual satisfaction (OR:1.30[1.02-1.61],p=0.031 and OR:1.92[1.27-2.94],p=0.002, respectively for “new pain” and “new CWP” vs. “no/unchanged pain”). In particular, the “new CWP” group reported development/worsening of erectile dysfunction (OR:1.93[1.16-3.21];p=0.011), decreased sexual desire (OR:1.65[1.01-2.70];p=0.047), and issues with ejaculatory timing (OR:2.09[1.24-3.52];p=0.006) when compared to the “no/unchanged pain” group; conversely, the incidence/worsening of none of this symptoms were increased in the “new pain” group. Similar results were obtained for the relationship with change in pain and development or worsening of depression (OR:2.00[1.17-3.41];p=0.011 and OR:1.06[0.73-1.55],p=0.751 for “new CWP” or “new pain” vs. “no/unchanged pain” respectively).

None of the categories of development/worsening of pain was associated with either change of testosterone levels. Accordingly, the aforementioned relationships of change in pain status and sexual symptoms were not affected by further adjustment for testosterone.

To verify the hypothesis that the role of CWP on the development of sexual symptoms is mediated by the worsening of depressive symptoms, mediation analyses were performed. Interestingly, no mediation effect of depressive symptoms was detected for any of the sexual symptoms which appeared as entirely dependent upon the development of CWP.

Conclusions
CWP appears to play a significant role in sexual function and satisfaction. In particular, the development of a greater burden of pain, expressed as CWP, is associated with impairment in several domains of sexuality independently of testosterone. Likewise, development of CWP is associated with incidence/worsening of depression. The worsening of sexual function does not seem to be mediated by concomitant development of depressive symptoms, supporting a potential direct causal role of pain.

A-0296 Intra-Individual Variation in Semen Quality: Analysis of 4,625 samples from 925 Semen Donors

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Background: Semen quality is reported to exhibit substantial intra-individual variation; howev-er, most results rely on studies of infertile men or small cohorts of men from the general popu-lation.

Objective: To describe the intra-individual variation in semen quality among semen donors with proven fertility adjusted for ejaculatory abstinence and age.

Methods: In a longitudinal observational study, we included data from 925 semen donors who delivered semen samples at the European Sperm Bank in Denmark median age 24 years (5th-95th percentile: 19-36), and median BMI 23.5 kg/m2 (5th-95th percentile: 19.6-29.1) at first sample. Each man delivered five semen samples during a median of 2.5 months period (5th-95th-percentile: 1.2-6.1), totaling 4,625 semen samples in the period 2004 to 2018. Se-men samples were all assessed for volume, sperm concentration, from which total sperm count was calculated, and motility. Intra-individual variation was quantified using geometric coefficient variation (CV) calculated from ln-transformed sperm count values using: CV = sqrt(exp(SD2) – 1) x 100. SD is the standard deviation of ln-transformed values. A linear mixed effects model with random intercept for donor was used to adjust for abstinence period to account for repeated measures within donors. Adjusted values were calculated to a com-mon reference abstinence, and age was analyzed separately at donor level using linear re-gression. Sperm count level and CV was tested at donor level using chi-square test with do-nors classified by mean sperm count group and CV group, respectively low, medium and high.

Results:
Will be added later on poster/presentation.

Conclusions: Even among fertile semen donors, a median intra-individual CV of approximately 29% in total sperm count persists. Importantly, this variation does not imply that donors shift from “donor quality” to “ICSI quality” between samples; rather, it represents a fluctuation within the fertile range. Abstinence time is a key modifiable contributor, and donors with higher base-line counts tend to exhibit lower relative variability. These findings underscore that a single semen analysis provides a limited snapshot of an individual’s spermatogenic capacity, but the observed variation should not be interpreted as indicating categorical changes in fecundity status.

A-0299 When Algorithms Meet Andrology: Can AI Chatbots Solve Clinical Cases?

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The growing integration of artificial intelligence into clinical practice has led to the emergence of advanced conversational agents capable of supporting medical decision-making. Despite their promise, the reliability and clinical validity of AI chatbots remain insufficiently established, particularly in specialized fields such as andrology.This study aims to compare the performance of multiple AI chatbots in resolving clinical cases in andrology using an expert-based evaluation framework, in order to inform the critical evaluation and future integration of AI tools in reproductive medicine.

This study aimed to evaluate and compare the performance of four AI chatbots—ChatGPT, Gemini, Consensus.ai, and Deepseek—in solving clinical cases in andrology. The goal was to provide an expert-based assessment to inform the critical evaluation and potential integration of AI tools in reproductive medicine.

We conducted a retrospective comparative cross-sectional study evaluating four AI chatbots ChatGPT, Gemini, Consensus AI, and DeepSeek—using standardized clinical case scenarios in andrology, with a focus on azoospermia and hypospermia. Each chatbot was prompted to provide diagnostic reasoning, complementary investigations, and management recommendations. Responses were independently assessed by expert andrologists using a structured semi-quantitative framework across five domains: clarity, structural organization, clinical relevance, reasoning quality, and prioritization of investigations, scored on a 0–5 scale. Mean scores were calculated for each model to determine overall performance, while variability was assessed using standard deviation and coefficient of variation. Comparative ranking and descriptive statistical analyses were performed to identify differences in performance among the models.

Comparative evaluation of the four AI chatbots—ChatGPT, Gemini, Consensus AI, and DeepSeek—demonstrated marked heterogeneity in performance across key domains of clinical reasoning in andrology. Based on a semi-quantitative scoring system (0–5 scale), Gemini achieved the highest overall mean score (4.5 ± 0.4), followed by Consensus AI (3.9 ± 0.8) and ChatGPT (3.8 ± 0.6), while DeepSeek showed significantly lower performance (2.0 ± 0.7). The overall mean score across models was 3.55 ± 1.0, indicating substantial inter-model variability. Gemini consistently outperformed other models in clarity and structural organization (both 5/5), with high inter-domain consistency (coefficient of variation ≈ 9%). ChatGPT demonstrated stable performance across domains (CV ≈ 16%) with slightly lower scores in structural organization and synthesis. Consensus AI exhibited greater variability (CV ≈ 21%), performing strongly in structured, targeted responses but less consistently in clinical reasoning and complementary investigations. DeepSeek showed the lowest scores across all domains, with high dispersion (CV ≈ 35%), reflecting inconsistent prioritization and reduced clinical relevance. Ranking analysis confirmed Gemini as the top-performing model across all evaluated criteria, whereas DeepSeek ranked last in all domains. These findings highlight significant differences in central tendency and dispersion of performance metrics among AI chatbots in clinical reasoning tasks.

AI chatbots show promise as adjunct tools in andrology, offering rapid synthesis and preliminary guidance for clinical cases. However, expert evaluation remains indispensable, and future development should focus on improving clinical reasoning, accuracy, and integration with evidence-based practice to safely enhance patient care in reproductive medicine.

A-0302 Between a Rock and a Soft Spot: Finding the Right Layer for Hyaluronic Acid Intratunical Injection for Peyronie's Disease

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Background: Peyronie's disease (PD) is a connective tissue disorder of the penile tunica albuginea, marked by fibrotic plaque formation rich in collagen. These plaques, along with changes in collagen type distribution, lead to penile curvature, discomfort, and erectile dysfunction. Following the approval of collagenase Clostridium histolyticum (Xiapex/Xiaflex) by both the FDA and EMEA, this treatment became a recognized therapeutic approach for PD, yielding modest reductions in curvature—typically between 15° and 20°. The standard regimen consists of eight injections over approximately 24 weeks, though subsequent real-world studies have experimented with shorter or extended courses involving 3 to 8 injections, and occasionally up to 16 or 24 in off-label use. In recent years, intralesional hyaluronic acid (HA) injection has emerged as a promising non-surgical therapy because of its antifibrotic, anti-inflammatory, and pro-angiogenic actions. It primarily alleviates pain associated with PD rather than producing major curvature corrections. However, accurate deposition of HA within the thin layers of the curved tunica albuginea is technically demanding. A prior study involving 38 Xiapex patients introduced a high-frequency ultrasound (US)-guided approach that allowed precise needle placement directly into the thickened tunica albuginea. This method significantly reduced the number of required injections by an average of only 2.8 per patient. The same US-guided technique was subsequently applied to the initial clinical trial using HA (Perovial) injections for PD. Objective: Peyronie's lesions often develop within the tunica albuginea, especially during the early phase of the disease. While the affected segment may appear thickened, the actual fibrotic zone may measure only a few millimeters, offering a very limited injection corridor. The aim of this work is to present a technically guided method for injecting HA directly into the tunica albuginea of the corpora cavernosa as a less invasive treatment for Peyronie's disease. Particular focus is given to the difficulty of controlling a 27-gauge needle within a very thin target layer by US guidance.Methods: Since the thin, collagen-dense, tunica albuginea (normal diameter dorsally about 2 mm or less in flaccid penis) lies between delicate cavernosal structures, necessitating extremely precise injection technique. For accurate intratunical or intraplaque deposition—ideally at mid-tunica depth—real-time ultrasound guidance is essential. HA injection procedures requires accurate layer-specific delivery, penile injections also require detailed anatomical awareness and sonographic visualization with GE logic 10 equipped with a ML6-15 MH probe to ensure correct placement. Unfortunately, standardized penile injection planes and reference landmarks remain poorly defined. Palpation alone does not provide sufficient tactile feedback, and without imaging, the 27-G needle can easily overshoot into the corpus cavernosum or remain superficially within subcutaneous tissue. Since the therapeutic effects of HA occur only when placed within the tunical lesion, off-plane administration severely limits outcomes.

Results:
Initial trials using the geometric "mid-tunica" ultrasound-guided injection technique demonstrated that imaging markedly improved both feasibility and procedural accuracy compared to palpation alone. In all treated patients, ultrasound enabled clear visualization of the needle trajectory and verification of correct intratunical placement. The findings suggest that ultrasound-assisted intratunical injection of HA using a 27-G needle can be performed safely and reproducibly, representing a promising non-surgical approach in the management of PD. Ongoing study is focusing on the benefit in reducing the HA injection number to treat, similar to the outstanding experience with Xiapex when using US guided technique.

A-0305 Infertile couple: what about the man's sexuality?

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Introduction
The psychological impact of infertility on couples has been addressed in several critical literature reviews. The psychological exhaustion hypothesis is proposed: infertility can be secondary to sexual dysfunction in 5% of cases, just as infertility can cause sexual dysfunction in 50% of cases.

Methods
Literature review on the impact of infertility on male sexuality, using keywords: Infertility; Sexual life; Couple relationships; Sexuality.

Results
Infertility affects the psychology of the affected man through numerous medical consultations, clinical and paraclinical examinations that sometimes expose the couple's intimacy, such as semen collection in the laboratory, which can be perceived as a violation of the man's privacy. He finds himself reduced to the result of his semen—a first blow to the man, and especially to his ego. Infertility treatment dictates the frequency and timing of sexual intercourse; the usual intimate event becomes regulated and controlled, with couples often feeling that the medical team is symbolically present during their most personal act. Regarding libido, 41.5% of couples report a decrease compared to their pre-diagnosis state, with reduced sexual satisfaction in 52.5% of these couples. Infertile men experience diminished virility due to inability to conceive (especially in male-factor cases), feeling less attractive to their partners. Some studies report IIEF-5 score deterioration in 15-42% of men in infertile couples, while 11% of infertile men experience orgasmic difficulties ranging from delayed orgasm to anorgasmia. These disorders depend on the menstrual cycle—46% of men report stress and loss of sexual desire during the periovulatory period—and on semen analysis abnormalities. Erectile dysfunction is more severe as semen quality worsens, with azoospermic men exhibiting the worst erectile function.

Conclusion
Patients should be informed that these desire disorders result from the medical procreation process, not from reduced attraction within the couple. Couples should be helped to rediscover the recreational aspect of sexuality, which should be an end in itself rather than solely a means of reproduction.

A-0306 Peyronie's disease surgery and erectile dysfunction: why this risk and how to prevent it?

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Introduction
Among the most dreaded complications of Peyronie's disease (PD) surgery is the worsening of preexisting erectile dysfunction (ED) or the onset of "de novo" ED. Its occurrence is more strongly associated with grafting procedures than with plication procedures.

Methods
A search of the PubMed database and review of articles were conducted in June 2025, using the keywords: Peyronie's disease, Surgery, Erectile dysfunction, De novo, Graft, Plication.

Discussion
In reports evaluating ED after PD straightening surgery, its incidence was higher for incision/excision and grafting procedures, reaching up to 50%, compared to plication procedures, which rarely exceeded 20%. Certain risk factors have been linked to an increased risk of "de novo" ED after corrective PD surgery, including age over 60 years, preoperative ED, the need for a large graft, and correction of ventral curvatures. This ED may occur in three nosological frameworks: 60% due to veno-occlusive dysfunction, 4% due to arterial insufficiency, and 35% due to psychogenic causes. Long-term ED after PD straightening surgery is difficult to attribute to the surgery itself, as it may simply reflect the natural progression of erectile function in older men.

Conclusion
Preventing the risk of ED involves careful patient selection and thorough informed consent to set realistic expectations, explaining that surgery addresses the curvature and does not improve erectile function.

A-0307 Peyronie's disease and sexual dysfunction: cause or consequence?

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Introduction
Peyronie's disease (PD) is often associated with sexual dysfunction (SD), and this association warrants particular attention during patient history-taking. It is crucial for the urologist to determine whether PD is the origin of the patient's sexual impairment and to thoroughly identify the risk factors for PD onset, particularly erectile dysfunction, in order to plan subsequent management.

Methods
Literature review on the impact of PD on sexuality, as well as sexual risk factors promoting the development of PD.

Results
PD and SD share several risk factors, which explains their frequent association in the same patient. The impact of PD on sexuality is well-known, stemming from mechanical discomfort during intercourse, its psychosexual effects (altered body image, performance anxiety, and decreased libido), or from PD surgery, which can also cause iatrogenic postoperative SD. Conversely, erectile dysfunction has been identified as a risk factor for PD development, particularly through the use of vacuum devices and intracavernous injections of vasoactive agents, which cause tunical injuries that evolve into fibrotic plaques.

Conclusion
The relationship between SD and PD is well-established, but the urologist must determine the causal link between these two conditions and their chronological order of onset in order to provide the patient with appropriate, personalized management that extends beyond penile deformity to address sexuality in its entirety.

A-0308 Unusual complication of a Peyronie's disease plication, a penile fracture 3 weeks postoperatively: a case report and literature review

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Introduction
The prevalence of Peyronie's disease (PD) is 3.2%. Corrective surgery is not without complications: hematoma, infection, erectile dysfunction (ED), or penile fracture—although never previously described, it could impact surgical outcomes. We report our experience with a case of penile fracture occurring after PD surgical repair.

Materials and Methods
Clinical observation of a 67-year-old man operated on for PD with a 54° right lateral penile curvature causing significant psychosexual distress. Two Nesbit plications were performed, with good immediate postoperative outcomes. He was readmitted three weeks later for penile fracture following intercourse 72 hours prior to admission, with perception of a cracking sound and immediate detumescence. Examination revealed a penis deformed by a predominantly subcoronal hematoma. Surgical exploration identified a 20 mm discontinuity in the left para-urethral tunica albuginea under the glans, distant from the plication sites, which was sutured with inverted non-absorbable stitches. He was discharged on day 3 with pro-erectile treatment starting on day 10.

Results
The patient was reviewed six weeks postoperatively: good healing, preserved erection and penile sensitivity, satisfactory straightness (4°), IIEF-5 score of 25, possible and satisfactory penetration, and the patient was very satisfied.

Discussion
Penile fracture in PD patients is rare. Previously described cases followed intraplaque injection of Clostridium histolyticum collagenase, but none have been reported as an early complication of PD corrective surgery. The primary suspected cause is suture loosening from plication; however, since the discontinuity was distant from the plication sites in our patient, this complication may be due to penile weakening from surgery (dissection of Buck's fascia, which contributes to the mechanical resistance of the corpora cavernosa), non-compliance with the postoperative abstinence period, and sudden mechanical stress during attempted intromission into an unlubricated vaginal cavity of his menopausal partner.

Conclusion
Postoperative penile fracture after PD repair is exceptional but possible and could compromise reparative surgery outcomes. Thus, it is essential to inform patients of this risk and emphasize preventive measures: adherence to the abstinence period, cautious resumption of sexual intercourse, and use of intimate lubricants.