ABSTRACT

Posters

BASIC SCIENCE

P001 | Non-obstructive azoospermia as a sentinel for early diagnosis of late-onset Fanconi anemia

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In the majority of cases, the clinical manifestations of Fanconi anemia (FA) appear during childhood. Nevertheless, in about 10% of patients, the diagnosis is delayed until adulthood due to slow progressive bone marrow failure. Late diagnosis may occur especially when individuals have no symptoms or present subtle findings that may be overlooked. The reason for diagnosis in these patients used to be the appearance of FA-related cancers or cancer treatment related life-threatening toxicity. Mouse models suggest that genes belonging to the FA pathway play a role in spermatogenesis in particular in primordial germ cell proliferation and meiosis. Based on the above observations, we hypothesize that defects in any of the 22 FA genes may lead to non-obstructive azoospermia in adulthood, even in the absence of overt anemia or other signs of FA. In this study, we aimed at diagnosing late-onset FA before the appearance of severe clinical complications. In particular, our main objectives were the identification and phenotypic characterization of late-onset FA cases through the genetic analysis of a highly selected group of infertile men and to provide novel knowledge on the genetic etiology of adult-onset FA. We performed targeted next-generation sequencing of the 22 FA genes in 100 patients affected by azoospermia and showing mild/borderline hematological alterations and in 78 fertile men with normal spermatogenesis and hematological values. We have identified a likely pathogenic (LP) hemizygous splicing variant in the X-chromosome linked FANCB gene, in a patient with azoospermia due to sertoli cell only and mild hematological alterations. No recessive LP variants were identified neither in patients nor in controls. Interestingly, three patients carried two heterozygous variants in two FA genes. Both were classified as variants of uncertain significance (VUS) and predicted to be pathogenic for the majority of in silico tools. The testicular biopsies of these patients ranged from sertoli cell only to incomplete meiotic arrest. A similar digenic condition was not observed in the controls. Data on DEB-induced chromosomal breakage test in leukocytes is not yet available. In conclusion, if the DEB-induced chromosomal breakage test in the FANCB mutation carrier confirms the pathogenicity of the variant, our study will provide further evidence of an expected high frequency of FA in infertile men. In that case, the screening of mutations in the FA genes in a specific group of infertile men with mild hematological parameters may have the potential to identify undiagnosed FA before the appearance of other severe clinical manifestations of the disease.

Keywords: genetics, non-obstructive azoospermia, Fanconi anemia, NGS

P002 | Steroidogenesis and androgen/estrogen signaling in in vitro matured testicular tissues of prepubertal mice

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Chemotherapy has a recognized toxicity on germ cells that could lead to infertility. In order to preserve and restore the fertility of prepubertal patients, testicular biopsies are frozen/thawed and need to be matured to produce spermatozoa for assisted reproductive technology. In vitro maturation developed in mice has a poor yield. Since steroid hormones play an essential role in spermatogenesis, it appears necessary to ensure that their synthesis and mechanisms of action are not altered in in vitro cultured tissues. The aim of this project was therefore to study steroidogenesis and the androgen and estrogen signaling during in vitro maturation of prepubertal mouse testicular tissues. Histological, RT-qPCR, Western blot analyses and measurements of cholesterol and steroid hormones levels and of aromatase activity were performed. First of all, a similar number of Leydig cells (LCs) is found after 30 days of organotypic culture (D30) and at 36.5 days postpartum, the corresponding in vivo time point. However, LCs are...
ABSTRACT
Throughout first wave of spermatogenesis: transcriptomic analysis of in vitro maturation of fresh and frozen murine testicular tissues

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Introduction and Objective: Cancer treatment in young boys is known to decrease male fertility. Freezing of prepubertal testicular tissue followed by a culture is an experimental strategy that may be used to preserve and restore male fertility before gonadotoxic treatment. To date, this strategy is not available in humans and is still difficult in mice. The aim of this study is to evaluate the impact of freezing and culture procedures by a high-throughput next-generation sequencing of testicular explants from an animal model allowing a complete first spermatogonial wave.

Methods: Testicular tissues from 6.5 days postpartum (jpp) mice were cultured to study (i) the impact of in vitro culture on the first wave of spermatogenesis at key stages: four days (D4), D16 and D30 (compared to corresponding in vivo controls) and (ii) the impact of freezing procedure after thawing and at the end of culture. RNAs of testicular tissues were extracted, the libraries sequenced and the gene list compared with sources of functional information to detect enriched terms.

Results: Two principal component analysis highlights three main groups: (i) 6.5 dpp, 10.5 jpp, D4; (ii) D16, 22.5 dpp, D30; and (iii) 36.5 dpp. In spite of a complete functional spermatogenesis, the culture system results in numerous differentially expressed genes (DEGs) compared to in vivo physiological conditions (8,456 DEGs). Tissues differentiate similarly up to in vitro D16 and in vivo 22.5 dpp; however, explants at D30 expressed a strong under-expression of transcripts (mainly related to steroidogenesis and insulin growth factor). Fresh and frozen tissues conditions are comparable before and after culture (only 45 DEGs).

Conclusion: This study shows that culture system, although lowering complete spermatogenesis, needs to be improved. Besides, this study confirms that testicular tissue freezing has a little impact, reinforcing the idea that the preservation of germinal tissue remains a promising strategy.

P003 | Throughout first wave of spermatogenesis: transcriptomic analysis of in vitro maturation of fresh and frozen murine testicular tissues

P004 | The role of piRNA-associated proteins in testicular function

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Background: Spermatogenesis is a process of multiple mitotic and meiotic divisions where sperm are produced from spermatogonial stem cells in the testes and is regulated by the hypothalamic-pituitary-gonadal axis. Worldwide, ~15% of all couples struggle to conceive and the fertility rates continue to decline. Thus, more research on reproductive genetic and epigenetic factors that may reveal new causes of the increasing fertility crisis is needed. Our group has recently demonstrated that pathogenic mutations in certain piRNA-associated proteins, like PNLDC1, causes spermatogenic arrest and non-obstructive azoospermia due to a lack of mature pachytene piRNAs. In this project, we seek to further describe the role of piRNAs in male reproduction and endocrinology.

Materials and methods: From the biobank at the Department of Growth and Reproduction at the Copenhagen University Hospital (Rigshospitalet), we used archived testis biopsies from men representing normal (n = 1) or impaired spermatogenesis (n = 9). Immunohistochemistry was conducted essentially as previously described (Nielsen et al., 2019), to stain for the piRNA-associated proteins TDRKH (1:1000, Proteintech, 13528-1-AP) and PIWIL1 (1:250, CST, #2079S) proteins.

Results: In the tissue section with normal spermatogenesis, a prominent TDRKH expression was seen in spermatocytes and spermatogonia, whereas PIWIL1 was highly expressed in spermatocytes and round spermatids. In contrast, tissue sections from patients with reduced spermatogenesis displayed fewer tubules with TDRKH and PIWIL1 expression as well as a clear reduction in expression level in those tubules showing expression of either TDRKH or PIWIL1.

Conclusion: Preliminary data indicate that men with impaired spermatogenesis show lower levels of piRNA-associated proteins, albeit more data is needed to draw a conclusion.
Prepubertal boys subjected to oncological treatments are at a higher risk of subfertility. Currently, cryopreservation of immature testicular tissue containing spermatogonial stem cells is the only way to preserve the fertility in prepubertal boys subjected to high-gonadotoxic treatments, such as total body radiation and/or high-dose chemotherapy [1]. However, today, there is no technique available to mature these stem cells into functional sperm in humans. Hence, novel functional in vitro models are required. The testicular organoid is a novel model that resembles the in vivo development of testis. Most protocols used for testicular organoids formation used Matrigel [2]. However, Matrigel is a poorly defined xenogeneic extra cellular matrix (ECM) protein. Due to existing batch-to-batch variations as well as its xenogeneic character, it has limited use for clinical application. Therefore, artificial scaffolds and defined recombinant human ECM proteins need to be developed. This project aims to develop a defined and synthetic Matrigel-alternative for the expansion and differentiation of murine testicular organoids.

Here, we used a Noviogel, which is a synthetic biomimetic hydrogel based on polyisocyanopeptides (PIC), for the generation of testicular organoids. Testes of 11 days postpartum (dpp) old C57BL/6 mice (n = 40) were digested into testicular single-cell suspensions and applied to the three-layer gradient culture system for testicular organoids formation [3]. We compared the effect of combinations of Noviogel with three different types of laminins (LN121, LN521 or LN111), on testicular organoid formation for 7 days in vitro (n = 4). Our results demonstrated that Noviogel alone is not sufficient to support testicular organoid growth. However, Noviogel supplemented with LN111, provided a supportive environment for testicular organoid formation. The effects of LN111 were superior to effects shown for LN121 and LN521. To investigate the organoids formation, the organoids were sectioned and stained with periodic Acid-Schiff (PAS) staining. Organoids cultured in Noviogel supplemented with LN111 exhibited cord-like structures, similar to in vivo control tissue samples.

Overall, our study demonstrates that primary mouse testicular cells are capable of forming testicular organoids with a compartmentalized tubular structure in a synthetic defined hydrogel-based three-dimensional model. Moreover, our results show the importance of the ECM for testicular organoid formation. This could bring valuable knowledge for the development of human testicular organoids that may further be used to develop a novel fertility restoration option for the prepubertal boys who have undergone cancer therapies.
SOF-L (p < 0.0001) and SOF-H (p < 0.0001) groups as compared to control, with a more significant increase in SOF-H as compared to SOF-L (p < 0.0001). However, PCNA immuno-reactive germ cells showed a significant reduction in both SOF-L (p < 0.0001) and SOF-H (p < 0.0001) groups as compared to control, with a more significant reduction in SOF-H as compared to SOF-L (p < 0.0001).

Conclusions: Sofosbuvir might have negative effects on the pituitary-testicular functions of the rats. These negative effects might be dose-dependent with more negative effects with a higher dose. These testicular negative effects might be mediated through the significant increase of both germ cell apoptosis, and seminiferous tubules collagen deposition, with a reduction of germ cell proliferation.

P008  | Study of positive, negative and zwitterionic liposomes on human spermatozoa

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Liposomes are vesicles consisting of single or multiple concentric lipid bilayers encapsulating an aqueous compartment and show several potential applicative uses, including basic research on spermatozoa. Three types of rhodamine-loaded liposomes (positive and negative charged and zwitterionic) were tested in vitro on human spermatozoa to visualize their possible interaction. Fifteen semen samples were analysed following WHO guidelines (2010) and used for experiments. Empty liposomes diluted 1:10,000 were incubated for 1 hour with basal semen samples, swim-up upper and lower fractions for motility evaluation. Subsequently, basal samples and swim-up upper fractions were centrifuged and spermatozoa were used to evaluate the membrane mitochondrial potential (MMP) by JC-1, DNA status by acridine orange (AO) and acrosome integrity using Pisum Sativum Agglutinin (PSA). Semen analysis revealed normal sperm parameters ranged from 25th to 75th centiles. After incubation with rhodamine-loaded liposomes, more than 95% of sperm were stained red, indicating a high level of adhesion or fusion, mainly in the midpiece. Regarding motility evaluation, sperm from basal samples incubated with positive and negative liposomes showed a significantly higher motility percentage than that of control and samples treated with zwitterionic liposomes (p < 0.05). Since spermatozoa from swim-up upper fraction are highly motile, no differences were visible among controls and swim-up selected sperm treated with liposomes. Instead, sperm from swim-up lower fraction, incubated with positive liposomes, showed a significant increased motility compared to controls and sperm treated with zwitterionic liposomes (both p < 0.05). Spermatozoa of both basal and swim-up upper fraction incubated with positive liposomes had a significant decreased percentage of sperm with double-stranded DNA respect to the other groups (p < 0.01). Sperm treated with positive liposomes showed significantly higher MMP than controls (p < 0.05 both in basal and swim-up selected samples) and sperm treated with zwitterionic liposomes (p < 0.05 in swim-up selected samples). Spermatozoa from basal and swim-up fractions showed intact acrosomes both in controls and after liposome treatments.

In conclusion, these liposomes are promising and available devices in the study of human spermatozoa. They could be studied in different situations, functionalized modifying their surface with other kind of lipids and proteins, or loading with different compounds.

P009  | Presence of Apelin in human spermatozoa and testis

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Adipocytokines, a family of hormones and cytokines produced and secreted by adipose tissue, have shown regulative activities in various physiological and pathological conditions of the reproductive tissues. In particular, in male reproductive tissue, some adipocytokines have shown anti-inflammatory and anti-oxidative stress properties. Among these adipocytokines, apelin and its receptor APJ have been studying in different body systems, but a little is known about their role in male reproduction.

Semen samples of 53 individuals were investigated following WHO guidelines (2010) and apelin was dosed both in seminal fluid and sperm cells by ELISA assay. In addition, apelin localization was explored in ejaculated spermatozoa and human testicular tissue by immunofluorescence technique.

For the first time, ELISA experiments demonstrated the presence of apelin in human spermatozoa and its absence in human semen. Sperm apelin level was negatively correlated with sperm concentration (p < 0.01), rapid progressive motility (p < 0.001), slow progressive motility (p < 0.05), total progressive motility (p < 0.001), sperm vitality (p < 0.001) and sperm morphology (p < 0.001).

By grouping patients following the threshold value for normal morphology, defined by WHO (2010), group 1 with normal sperm morphology >4% showed a significant lower apelin level compared to group 2 with sperm morphology ≤4% (p < 0.001). In addition, immunofluorescence analysis on spermatozoa of group 1 showed a weak apelin signal in sperm tail, while sperm from group 2 expressed a strong apelin labelling in sperm tail, cytoplasmic residues and post-acrosomal sheath. In normal human testicular tissue, apelin immunolocalization was found mainly in Leydig cells, and a little amount was observed in the cytoplasm of peritubular myoid cells, Sertoli and germ cells.

It is not known whether apelin plays a protective or detrimental role in human sperm. The preliminary results of this research are promising to explore the behaviour of apelin in inflammatory conditions. The localization of APJ in human sperm and testicular tissue could help in understanding the exact role of the apelinergic system.
Background: Spinal and bulbar muscular atrophy (SBMA), is a rare disease characterized by the expansion of a CAG trinucleotide repeat (polyQ) in the androgen receptor gene (AR). Nuclear accumulation of ubiquitinated polyQ-AR upon ligand binding is a key step in SBMA pathogenesis, resulting in progressive lower motor neuron degeneration and muscle loss [1]. In addition to the ligand binding, the involvement of intra/intermolecular interactions with the AF-2 domain and the recruitment of coregulators upon DNA binding are mandatory in the pathogenetic commitment of polyQ-AR [2]. Importantly, apart from the muscle phenotype, SBMA patients do not always develop clear signs of hypogonadism [3]. Anti-androgenic drugs are currently under evaluation for the treatment of SBMA but the exposure risk to the long-term hypogonadism is a matter of concern. PROteolysis TArgeting Chimeras (PROTAC) molecules are gaining interest to obtain the therapeutic knockdown of proteins of interest (POI). PROTACs structurally involve an E3-ligase moiety and a POI binder-head. Provisional AF2-PROTAC prototypes were designed on the von Hippel-Lindau (VHL)-E3 Ubiquitin Ligase binder VH032, by changing anchor site to Triac, were designed and submitted to PRosettaC. The top-score prototype, according to PRosettaC results, had a linker length of C8. Accordingly, a panel of 3 readily synthesizable AF2-PROTAC candidates was proposed.

Methods: A screening on Protein Data Bank repository (https://www.rcsb.org/) was performed to identify suitable AF2 domain-binding warheads. Provisional AF2-PROTAC prototypes were designed on the von Hippel-Lindau (VHL)-E3 Ubiquitin Ligase binder VH032, by changing the length, in terms of carbon atoms, of a hypothetical linker chain consisting of only methylene units. In order to identify the optimal linker length, prototypes were then submitted to PRosettaC, a free web-server computational protocol for the prediction of PROTAC-induced ternary complexes [4]. A panel of readily synthesizable AF2-PROTAC was then generated on the basis of the reactants available on the market.

Results: 3,3',5-triiodothyroacetic acid (Triac)[5] was identified as a recognized AR ligand, suitable for the design of a AF2 domain-oriented PROTAC. Eleven AF2-PROTAC prototypes, differing for linker length (C2 to C11) and anchor site to Triac, were designed and submitted to PRosettaC. The top-score prototype, according to PRosettaC results, had a linker length of C8. Accordingly, a panel of 3 readily synthesizable AF2-PROTAC candidates was proposed.

Conclusion: AF2-PROTAC candidates will be tested on a cell model of SBMA, involving PC12 cells stably expressing highly expanded polyQ-AR [6], in order to address an efficient reduction of the ligand dependent-nuclear accumulation whilst preserving the expression of AR-responsive genes.

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Concentration of bioelements and heavy metals in serum and bone tissue in aging men

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Introduction: The relationship between the concentration of macro- and microelements in various tissues of the body and metabolic disorders requires in-depth research. Comparing the concentration of elements in bone and serum can provide valuable information on the relationship between these tissues in the human body. The aim of the study was to investigate the relationship between the concentration of bioelements (Zn, Cu, Cr, Mg, Se) and heavy metals (Pb) in serum and tissue and the values of VAI, LAP and BMI indicators.

Material and Methods: The study included 151 men aged 60 and 75 years qualified for hip arthroplasty. Bone tissue material was collected from patients during the arthroplasty procedure and venous blood was collected. The concentration of Mg, Zn, Cu, Cr, Fe, Se and Pb was determined in both tissues. For the purposes of carrying out the analyzes taking into account the indices of body mass and the accumulation of adipose tissue, the ROC curve was used in the first stage of the analysis to distinguish the research groups. The presence of MetS was the state variable in the analysis. The analysis showed that the cut-off point for the value of the body mass index (BMI) was 28.63 kg/m² (p < 0.007), for the visceral adiposity index (VAI) 2.27, and for the lipid accumulation product (LAP) 67.09.

Results: Analyzing the VAI index, it was shown that the Mg concentration in bone tissue was significantly higher in men with higher VAI values. Similarly, analyzing the relationship between the concentration in bone tissue and the LAP index, it was shown that both Mg concentration and Zn concentration were higher in patients with higher values in the analysis of this index. A multivariate logistic regression analysis with age adjustment was performed. It has been shown that there is a correlation between the serum Zn concentration and the VAI cut-off point. The relationship between the concentration of the examined elements in the bone tissue was observed in relation to the LAP cut-off point analysis. The value of this index was related to the concentration of Mg, Zn and Cu in bone tissue.

Conclusions: In our research, we have shown that the relationship between the concentration of selected bioelements and heavy metals in serum and in bone, and the values of VAI, LAP and BMI indicators is not the same in both analyzed tissues. Searching for simple and diagnostically easy indicators that may indicate the impact of metabolic disorders on the structure and functions of bone tissue is an important factor from the point of view of the health of the society and quick and effective prevention of bone tissue diseases. The conducted research shows that the value of the LAP index may be a good predictor of changes in the concentration of elements in the bone tissue in men. It is a metabolic index that is easy to determine on the basis of basic blood tests, therefore it deserves attention as the most reliable of the analyzed indexes.

Male infertility coexists with oxidative stress in semen and decreased sperm genomic integrity irrespective of leukocytospermia

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Because oxidative stress (OS) in semen results in low sperm quality and therefore in many cases contributes to male infertility (30–80% of cases) and can be caused by a different factors...
Strategically positioned resident immune cells shape medium-related effects on hypothermic storage of rat testicular cells

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Spermatogonial stem cells (SSCs) are responsible for maintaining spermatogenesis. Any damage to these cells, caused by genetic diseases or gonadotoxic radio- and chemotherapy doses, can lead to infertility. Fertility preservation methods, such as sperm cryopreservation, are effective techniques to preserve adult patients’ fertility. However, sperm cryopreservation is not an option for prepubertal patients, since they are not able to produce sperm. Thus, testicular tissue cryopreservation is a promising technique that can lead to fertility preservation for prepubertal patients. Testicular tissue cryopreservation is only available in a few hospitals worldwide. Moreover, testicular tissue biopsied in distance hospitals requires beneficial transportation and preservation conditions to protect the biopsied tissue quality. Thus, this study aims to investigate the impact of short-term storage (up to 24 hours), hypothermic conditions (4-8°C), and selected basal culture media or balanced salt solutions on prepubertal rat testicular tissue samples. The potential effects of various conditions were monitored using morphological tissue analysis, radioimmunooassay to measure testosterone levels as well as gene expression profiles via TaqMan Low-Density Array (TLDA) card analysis of 96 genes. Seven dpp-old prepubertal rat testicular cells were stored in six different basal culture media or phosphate-buffered saline for 12 and 24 hours in hypothermic conditions, which were assessed. A variation in energetic, apoptotic, and angiogenic expression levels was observed after 12 hours, and it became more prominent after 24 hours. However, no significant differences were seen in germ and testicular somatic cells’ tissue morphology and gene expression profiles. Moreover, testosterone production levels were not significantly changed in short-term storage in hypothermic conditions in any of the used basal media. In conclusion, this study shows that the transfer of testicular tissue in short-term hypothermic conditions for up to 24 hours does not affect the expression of testicular cell-specific genes; however, it has a moderate effect on the expression of certain genes specific to general cellular functions.
The epididymis constitutes an important transition zone for post-testicular sperm maturation in which both immunosuppressive mechanisms to protect immunogenic spermatozoa, and immunoreactive mechanisms to resist invading pathogens, are required. Previous investigations revealed that the proximal and distal regions of the epididymis react differently towards inflammatory stimuli. While the proximal regions (initial segment, caput) remain mostly unaffected, the distal regions (corpus, cauda) are more susceptible to inflammation-associated tissue damage, which severely affects male fertility. We hypothesized that strategic positioning of resident immune cells along the organ mediates this dichotomy of immune responses. Using a mouse model of acute bacterial epididymitis (elicited by intravasal injection of uropathogenic Escherichia coli), we analyzed disease progression (1-21 days) at cellular and transcriptional levels. The distally located cauda showed a strong immune response characterized by a massive influx of neutrophils (Ly6G+) and monocyte-derived macrophages (Ly6C+CD11b+F4/80+MHC-II+, assessed by flow cytometry) with concomitant fibrosis and loss of epithelial integrity, resulting in extravasation of spermatozoa (assessed by Masson-Goldner trichrome staining). RNASEq data indicated that a switch from innate to adaptive immunity occurred around day 10 post-infection within the cauda. In contrast, epithelial integrity remained intact in the caput and initial inflammatory responses were immediately resolved. To understand the physiological prerequisites that govern these distinctly different immune responses, we investigated the heterogeneity of extravascular immune cells that reside within epididymal regions by single cell RNA sequencing of CD45+ leukocytes and flow cytometry. In total, 12 distinct lymphocyte subpopulations were identified, displaying striking differences in their regional distribution. The proximal regions (initial segment, caput) are predominantly populated by intra- and peri-epithelial CX3CR1hi macrophage subsets (~80% of total CD45+ cells) that express numerous homeostatic signature genes that are crucial for maintaining tissue integrity. The distal regions (corpus, cauda) are populated by a heterogenous network of different immune cell populations (monocytes [10%], macrophages [40%], conventional dendritic cells 1 and 2 [10 and 10-20% respectively], αβ T cells, γδ T cells [10%], NK cells [10%], B cells [2-5% of CD45+ cells]) that coexist within the intraepithelial and interstitial compartment. This indicates a potential in situ crosstalk to maintain mucosal immune homeostasis and to facilitate rapid responses towards invading pathogens. These findings suggest that resident immune cells are strategically positioned along the epididymal duct to provide different immunological milieux required to maintain tissue integrity essential for sperm maturation, and to adequately resist invading pathogens ascending via the urogenital tract. Overall, these data provide the first atlas of CD45+ leukocytes at single cell resolution within the murine epididymis that will serve as an important platform for dissecting functional roles of individual populations in epididymal immune regulation.

Objectives: Male infertility is a multifactorial pathology suggested as the underlying factors in majority of cases of severe male infertility, especially non-obstructive azoospermia. The recent emergence of next-generation sequencing (NGS) offers an opportunity to analyze many genes at once and to develop various bioinformatic approaches, including variant burden investigation. Gene-based burden tests identify a set of rare variants in a given gene by comparing case and control cohorts. Another method – Gene Set Enrichment Analysis (GSEA) – analyzes gene networks, that share common regulation, biological function or chromosomal location. There is still a lack of reports on WES implementation with burden tests, gene network analysis and routine clinical diagnostics in male infertility research. The purpose of this study was to investigate the potential of exome sequencing in idiopathic azoosperma cases.

Methods: Whole exome sequencing was performed on 21 non-obstructive azoospermia patients. A gene set of previously described and novel candidate genes of azoosperma was compiled. Samples were sequenced using the Twist Comprehensive Exome Panel. The resulting sequences were mapped against the human genome GRCh38 reference sequence using BWAMEM. Copy number variations (CNVs) were annotated using AnnotSV. Samples were analyzed and filtered with the Illumina’s BaseSpace Variant Interpreter. Variants considered as pathogenic and likely pathogenic were confirmed by Sanger sequencing. Genetic burden test was performed with TRAPD. P value < 0.05 was considered significant. Protein interactions were investigated with ConsensusPathDB, STRING and CytoScape.

Results: SNV analysis detected two genetic variants of unknown significance (VUS) in genes, affecting the hypothalamic–pituitary–gonadal axis: NR5A1 and FGFR1. Clinical investigation did not demonstrate hypogonadism in the subject group. NM_004959(NR5A1):c.763C>T was interpreted at first as likely pathogenic (LP), according to ACMG guidelines. The patient’s phenotype did not reflect the expected phenotype. In CNV investigation, VUS deletion in AD genes TUBG1 and TUBG2 was found (seq[GRCh38] 17q21.2(42613632-42659961)x1).
No previously described known pathogenic genetic variants were found. Genetic variant burden was elevated in 1473 genes. 302 genes with increased loss-of-function (LoF) variant set were present in more than one sample. Variant burden of genes TKFC, DPM1, UBE2J2, MTCH2, GCLC, NPIP1B1, OR2T33, POTEG was elevated in > 50% of samples. Over-representation analysis with pathway based set of genes with high variant burden demonstrated 26 pathways, half of the pathways (13) being involved in sperm development, especially sumoylation (4). Over-representation analysis with protein complex-based sets obtained 14 protein sets, all involved in DNA repair and genomic integrity. STRING interaction analysis between genes with high variant burden showed two genome instability networks. **Conclusions:** Based on a preselected diagnostic gene panel, we identified four VUS in exomes, involved in hypothalamic–pituitary–gonadal axis. In patients with azoospermia, an increased burden of genetic variants is observed in interrelated genes involved in genome instability and spermatogenesis. These findings can add supporting information to the knowledge base of infertility diagnostics. WES as a routine diagnostics method in azoospermic patients calls for the further investigation.

P018  |  The role of mitochondria-dependent apoptosis in human sperm damage in men exposed to genital heat stress

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There are substantial premises that oxidative stress and apoptosis of the germ cells may be involved in the male gonad response to the genital heat stress. However, the significance of the local thermogenic factor for human ejaculated sperm has not been properly recognized in many aspects. In the present retrospective study, we investigated a link between sperm apoptosis and standard semen parameters in males exposed and not exposed to local hyperthermia. The studied cohort included 198 infertile males and volunteers 20–41 years of age. Based on the collected clinical and survey data, the men were qualified for one of the following research subgroups: professional drivers, infertile men with varicocele, infertile men not exposed to genital heat stress, and fertile men, which served as the control group. Freshly ejaculated semen samples were collected in sterile containers after 3–5 days of sexual abstinence. Within 60 minutes of ejaculation and liquefaction, standard semen analysis was performed manually according to the World Health Organization 2010 criteria. Besides, a set of classic (phosphatidylserine externalization, mitochondrial transmembrane potential, DNA fragmentation) as well as non-classic (phospholipid membrane scrambling, mitochondrial ROS generation) sperm apoptotic markers, were simultaneously investigated. In the present study, standard semen parameters including sperm density, motility, and morphology were statistically lower in all the studied subgroups compared to the control. As for other semen markers, a statistical difference was observed between all the studied subgroups and the fertile men in the percentage of sperm with damaged membrane architecture (merocyanine 540 negative cells), sperm with polarized mitochondria (JC-1 positive cells), and sperm with DNA fragmentation (TUNEL-positive cells). In turn, the sperm mitochondrial ROS generation (MitoSox Red positive cells) was significantly enhanced only in the groups exposed to genital heat stress compared to the control. A comparative analysis of the studied parameters allowed us to show some moderately strong or strong correlations of conventional semen parameters (motility, viability and/or morphology) with the percentage of merocyanine 540 negative sperm in the group of drivers and with the percentage of JC-1 positive sperm in the varicocele group. Moreover, only in the group of drivers, the percentage of MitoSox Red positive sperm was positively associated with the percentage of TUNEL-positive sperm. This study demonstrated for the first time a strong apoptosis-like phenotype initiated by increasing sperm mitochondrial ROS generation and decreasing sperm motility in men exposed to prolonged genital heat stress. These findings suggest that antioxidants may be useful as a potential treatment option for male subfertility/infertility caused by heat-induced oxidative stress. Financial support: National Science Centre, Poland (grants No 2015/19/B/NS5/02241, 2020/37/B/NS5/00549).

P019  |  Haploid selection causes allele frequency divergence among sperm from within the same ejaculate

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Males produce millions of sperm in a single ejaculate, but only very few reach the fertilisation site and only one sperm ultimately fertilises the egg. The bottleneck in sperm numbers from ejaculation to fertilisation offers the opportunity for selection to act on specific sperm phenotypes and genotypes. However, to what extent sperm traits are affected by the haploid sperm genotype is currently unknown. Using in vitro assays to separate sperm within an ejaculate and likelihood ratio tests to examine allelic frequencies at heterozygous paternal sites, we show that the genetic basis of natural variation in fertile sperm changes substantially under selection. Phenotypically diverse sperm pools differed genetically across the entire genome. Our results demonstrate that sperm phenotypes are affected by their underlying genotypes and that even a short window of haploid selection may favour advantageous genotypes to be passed down to the next generation. We anticipate that sperm genotyping, and selection might be advantageous tools to improve assisted reproductive technology and
the health and quality of domesticated animals and livestock as well as overcome fertility issues in humans.

P020  |  The role of Notch signaling pathway in mediating androgen-regulated cellular processes in seminiferous epithelium

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The Notch pathway is a conserved cell-cell signaling mechanism mostly limited to cells that are in direct contact, such as Sertoli and germ cells in seminiferous epithelium. It was previously demonstrated that in vivo inhibition of Notch signaling in adult mouse testes increased the apoptosis of spermatocytes and induced abnormal spermatid elongation and its premature release (Murta et al. 2014; PLoS One, 9:e113365). Further, our studies showed that blockade of Notch signaling up-regulates androgen receptor (AR) expression in Sertoli cells (Kamińska et al. 2020; Andrology; 8:457-472). To get insight into the molecular mechanisms involved in these effects, we explored the interaction between Notch pathway and AR signaling in Sertoli cells and examined its role in the control of genes encoding proteins involved in (1) ectoplasmic specializations: nectin cell adhesion molecule 2 (Nectin2) and afadin (Afdn), (2) actin remodeling during spermiogenesis: actin-related protein 3 (Arp3) and epidermal growth factor receptor pathway substrate 8 (Eps8), and (3) induction of germ cell apoptosis: Fas ligand (Fasl), Fas cell surface death receptor (Fas), B cell leukemia/lymphoma 2 (Bcl2) and BCL2 associated X (Bax) in seminiferous epithelium.

The study was performed using Sertoli cell line TM4 and rat testicular explants that were exposed to testosterone, immobilized recombinant ligands for Notch receptors: Delta-like (DLL1, DLL4) and Jagged (JAG1), or Notch pathway inhibitor (DAPT). Interaction between Notch target protein, Hairy/enhancer-of-split related with YRPW motif protein 1 (HEY1), and AR promoter was tested using chromatin immunoprecipitation. Gene expressions were determined with RT-qPCR. HEY1 preferentially binds to E-box elements and we found mouse AR promoter contains 3 E-boxes in a region spanning bp -1494 to -926. Chromatin immunoprecipitation followed by qPCR analysis demonstrated that HEY1 interacts with the AR promoter in TM4 Sertoli cells. Interaction was significant compared with IgG alone and blockade of Notch pathway with DAPT resulted in decreased binding of HEY1 in comparison to the vehicle-treated cells. Next, we found that in TM4 cells the expression of Nectin2, Afdn, Arp3, and Eps8 was up-regulated by testosterone, whereas Fasl was down-regulated, which confirmed androgen-dependent regulation of these genes. Further, it was demonstrated that the expression of these genes was down-regulated by exposure to immobilized Notch ligands: Nectin2 by DLL4, Afdn by DLL1 and JAG1, Arp3 by DLL4 and JAG1, Eps8 and Fasl by DLL4 and JAG1. The addition of DAPT abolished the effects induced by the ligands, increasing expression of the genes. These regulatory mechanisms were confirmed in physiological testicular environment. In rat testicular explants the expression of Ar, Nectin2, Afdn, Arp3 and Eps8 was up-regulated following testosterone exposure and Notch pathway inhibition. In addition, DAPT increased Fasl and Fas expression as well as the Bax:Bcl2 ratio. Taken together, our study provides novel data on the role of Notch pathway in the regulation of androgen-dependent processes in seminiferous epithelium. First, direct control of AR expression by Notch effector HEY1 was revealed. Second, several genes involved in spermiogenesis and germ cell apoptosis were identified as targets for Notch pathway.

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P021  |  Complete meiosis in rat prepubertal testicular tissue under in vitro sequential culture conditions

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Testicular tissue cryopreservation before gonadotoxic treatments allows fertility preservation in children suffering from cancer. Fertility restoration strategies, in particular in vitro maturation of prepubertal testicular tissue, are being developed mainly in animal models. The rat, widely used in biomedical research, including in reproductive biology, is a relevant model.

The aim of the present study was to determine whether sequential two-step culture protocols can improve the efficiency of rat in vitro spermatogenesis.

Rat prepubertal testicular tissues were cultured on agarose gels with either a one-step or two-step protocol, with or without PDMS ceiling chips. The progression of spermatogenesis, the ratio of germ cells to Sertoli cells, cell proliferation, seminiferous tubule area and intratubular cell density were assessed by histological and immunohistochemical analyses. TUNEL assays and PNA lectin labelling were performed to analyse the DNA integrity and the differentiation stage of in vitro produced spermatids.

Sequential two-step protocols allowed the production of spermatids with a higher efficiency compared to the one-step culture protocol. Most of in vitro produced spermatids contained unfragmented DNA and were at an early stage of differentiation. Rare elongating spermatids could be detected in the cultured explants. Although a complete in vitro spermatogenesis could not be obtained with PDMS ceiling chips, the entry into meiosis was promoted in one-step organotypic cultures.
A complete in vitro meiosis and the beginning of the elongation phase of spermiogenesis were obtained in the rat model using sequential culture methods. Further work will be necessary to identify the culture conditions allowing the completion of spermiogenesis, before considering potential clinical applications of this procedure.

P022  |  Vasovasostomy: long-term experience

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Introduction and Objectives: Vasovasostomy (VV) is the gold standard technique for vasectomy reversal. According to the published series, patency and pregnancy rates are on average 90% and 50%, respectively. The aim of this study is to analyze our VV series and compare them with the published literature.

Materials and Methods: We review 33 cases of VV procedures performed in our department between January 2001 and November 2021. In all cases, the procedure is performed with a single non-resorbable monofilament microsuture. SPSS v.28.0 was used for statistical analysis.

Results: The mean age of patients undergoing VV surgery was 43.3 years (29–62 years). The mean time to vas-recanalization was 8.8 years (1–30 years). In 32 of the 33 patients, the main reason for undergoing VV was change of partner (one remaining patient underwent surgery for post-vasectomy pain syndrome). Grade II complications of the Clavien-dindo classification were observed in four cases (three orchitis and one scrotal hematoma), no grade III or higher complications were described. Patency was achieved in 25 patients (92%). Nine of the 33 patients were lost during follow-up. After detailed analysis of the results obtained, 22 of the 24 patients followed up tried to achieve conception after VV, with a successful pregnancy rate of 41% (nine patients).

Conclusion: Our patency rate is similar to the previously published one; however, the successful pregnancy rate is lower. VV, even complex, is a widespread and a reproducible technique which can be successfully performed in centres with low prevalence of vasectomy reversal.

P023  |  Retinoid signaling in Sertoli cells regulates their immunoprotective function by controlling lymphocyte apoptosis

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Testis is an immune privileged organ, which prevents the immune responses against autoimmunogenic germ cells and inflammation. In the testis, the cells responsible for immune tolerance are mainly somatic Sertoli cells, which form the blood-testis barrier and also produce immunosuppressive factors and anti-inflammatory cytokines. It has been shown that Sertoli cells, due to the production of immunosuppressive factors, inhibit proliferation and induce the apoptosis process of lymphocytes, preventing inflammation in the testicle and maintaining testis immune tolerance. Lymphocyte apoptosis is led mostly by interaction of Sertoli cells expressing Fas ligand (FasL) with Fas-bearing lymphocytes and induction of Bcl-2-associated X protein/B-cell lymphoma 2 (Bax/Bcl-2) pathway. It has been shown that retinoic acid (RA), vitamin A derivative, blocks the ex vivo apoptosis of peripheral blood lymphocytes and thymocytes. However, the role of retinoid signaling in regulating the immune privilege of the testes remains unknown. The aim of this study was to determine whether retinoids, acting via the retinoid acid receptors (RAR) and the retinoid X receptors (RXR), control immunomodulatory functions of Sertoli cells by influencing the secretion of anti-inflammatory/pro-inflammatory factors and lymphocyte physiology.

Experiments were performed using in vitro model of co-cultures of murine Sertoli cells and T lymphocytes. Agonists and antagonists of retinoic acid receptors were used to inhibit/stimulate retinoid signaling in Sertoli cells. RT-qPCR was used for detection of the anti-inflammatory/pro-inflammatory genes expression in Sertoli cells. Next, to determine the indirect role of retinoids in the control of T cell physiology, proliferation (MTT assay) and apoptosis (TUNEL assay) was analyzed in lymphocytes co-cultured with stimulated Sertoli cells. Lastly, western blot and RT-qPCR were used for detection of the expression of apoptosis-related factors, Fas, Bax, Bcl-2, Caspase 9 and Caspase 8 in lymphocytes and FasL in Sertoli cells.

Our results demonstrate that RA inhibits the expression of immunosuppressive genes (transforming growth factor β, interleukin-10, gelatinase 1 and indoleamine 2,3-dioxygenase) and enhances the expression of pro-inflammatory factors (interleukin-1, interleukin-6, interferon γ and tumor necrosis factor α) in Sertoli cells. Significant increase in viability and decrease of the apoptosis rate in lymphocytes was found after RA treatment compared with control group. Moreover, our results indicate that RA blocks lymphocyte apoptosis acting through both RAR and RXR receptors. Western blot and qPCR analysis revealed that RA acting through RXR receptor regulate apoptosis by inhibiting FasL/Fas/Caspase 8 proteins. In addition, our results showed that RA also blocks apoptosis through Bax/Bcl-2/Caspase 9 pathway in RAR-dependent manner.

Collectively, the obtained results indicate that retinoid signaling negatively regulates immunologically privileged functions of Sertoli cells, crucial for ensuring male fertility. Retinoic acid acting through both receptors (RAR and RXR) inhibits lymphocyte apoptosis, which can be related to the development of autoimmunity and inflammation and in consequence infertility. Supported by a grant U1U/P03/NO/03.15 (Priority Research Area Bio5 under the program Excellence Initiative – Research University at the Jagiellonian University in Krakow).
The genetics of spermatogenesis are widely considered divergent across species. Yet, ancient biological programs like meiosis retain an evolutionarily-conserved genetic basis that can provide new insight into human reproductive disease. By screening 920 evolutionarily-conserved spermatocyte genes via in vivo RNAi in the fruit fly (Drosophila melanogaster) testis, we observed that the spliceosomal component RING finger protein 113 (dRNF113) is essential for spermatogenesis. The silencing of this gene, as insect male germ cells prepared to enter meiosis (driver: bam-GAL4), resulted in male sterility and a developmental block at the spermatocyte stage, with this cell type occupying an average of 64.1% of the entire testis area (vs. 12.3% in controls). The insect dRNF113 gene has two copies in the primate lineage, one of them (RNF113B) being testis-specific. By analysing a whole exome sequencing database consisting of 74 cases of human male meiotic arrest, we identified an infertile man, part of a consanguineous family of Middle-Eastern ancestry, with a homozygous loss-of-function (LoF) variant in RNF113B. Remarkably, the results from the testicular biopsy of this man revealed an equivalent meiotic arrest phenotype to that observed in dRNF113-silenced fruit flies. Spermatocytes were the most advanced cell stage in 89.0% of all assessed human seminiferous tubules (vs. 9.0% in controls). The expression pattern of dRNF113 and RNF113B were also largely concordant between the two species. Our single cell RNA-Seq dataset of normal human spermatogenesis indicated that RNF113B was predominantly expressed at meiotic entry, peaking at the diplotene stage of prophase I. This mirrored the localization pattern of the insect dRNF113 protein, which revealed a substantial nuclear accumulation in primary spermatocytes.

Insect dRNF113 also underwent a duplication event in the mouse lineage, independent to that that gave rise to RNF113B in humans. By generating, using the CRISPR/Cas9 system, a whole-body knockout (KO) mouse for the duplicated copy (Rnf113a2), we observed that male homozygous Rnf113a2KO mice were sterile, had visibly smaller testes than wildtype (+/+ ) littermate controls, and their seminiferous tubules were largely devoid of germ cells except for the rare presence of spermatogonia and spermatocytes.

In summary, by using comparative biology, we revealed a novel genetic cause of human male infertility (LoF variant in RNF113B) shared between three different species: humans, mice, and fruit flies. The retention of the key spermatogenic role of the RNF113 proteins across evolution suggests that this gene is part of the ancient molecular toolbox responsible for male animal germ cell development. Although the actual benefit of comparative biology for the identification of new genetic causes of human disease is often a contentious topic, here, we show how a merger between basic and clinical research can significantly expand our knowledge of fundamental reproductive processes.

Benign prostatic hyperplasia (BPH) is a commonly diagnosed disease among aging men. The exact mechanism of the pathophysiology and development of BPH has not yet been fully understood. However, there are many risk factors that increase the possibility of the onset and progression of BPH. These include age, metabolic syndrome (MetS), insulin resistance and hyperinsulinemia, obesity, steroid hormones, and genetic factors. There is growing evidence linking the development of BPH to the presence of inflammation. When inflamed, prostate stromal cells produce pro-inflammatory cytokines and chemokines. It is indicated that interleukins and interferon γ occurring in increased concentrations are potential mediators involved in the development of BPH. A key cytokine involved in the development of BPH is IL-17, which is practically undetectable in prostate tissue without hyperplasia. Interleukin-17 activates NFκB-mediated expression of other cytokines, including IL-6 and IL-8, which act as growth factors for glandular and stromal cells of the prostate. IL-6 is an activator for the human androgen receptor (AR), and thus regulates the expression and secretion of prostate-specific proteins. Another interleukin that is also involved in the development of BPH is interleukin-18, which is a stimulator of prostate stromal cell growth.

In our study, the immuno-expression and localization of IL-6 and IL-18 in the prostate tissue with benign hyperplasia (n = 59) were analyzed in men diagnosed with BPH with regard to MetS. The results showed that MetS significantly contributed to an increase in the percentage of cells showing the cytoplasmic expression of IL-6 in the prostate stroma. Moreover, MetS also significantly increased the percentage of cells showing high expression of interleukin-18 in the glandular part of the prostate. In addition, the relationship between...
short chain fatty acids (SCFAs) and tissue immunoexpression of IL-6 and IL-18 was analyzed. SCFAs are produced in the large intestine with the participation of the intestinal microbiota during anaerobic fermentation of exogenous components, such as dietary fiber and other undigested carbohydrates from food. These acids influence immune responses not only in the intestines but also in distant tissues. Their main function is to modulate immune mechanisms and provide immunity determined by the integrity of the intestinal mucosa. Our study has provided evidence that acetic acid is associated with the tissue expression of IL-6, in both the prostate stroma and epithelium of men with BPH and coexisting MetS. In contrast, it is not related to cells showing expression of IL-18 in the prostate of men with BPH. Disturbance of the intestinal microflora and its impact on inflammation and prostate diseases have not yet been thoroughly analyzed. Neither has been the influence of SCFAs on the development of BPH. Few publications can be found in the literature on the influence of the intestinal microflora on the prostate. They mainly concern the influence of intestinal bacteria on the synthesis of metabolites and androgens, which may contribute to the development of prostate cancer in humans.

P026 | Lipidomic profile of human sperm membrane identifies a clustering of lipids associated with spermatogenetic and reproductive function independently of lipidemia

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The reductions of sperm motility and/or count are among the major causes of reduced fertility or infertility in men. Lipid composition of spermatozoon is important in determining their functional characteristics, in particular on motility, capacitation and acrosome reaction. Despite some variations between different mammalian species, in general the sperm plasma membrane shares a unique lipid composition: (1) an unusually low content of cholesterol (Chol); (2) a high proportion of lipids containing a fatty acid chain attached; (3) the sn-2 position of the glycerol esterified mainly with long-chain polyunsaturated fatty acids (PUFAs); and (4) a high content of sulfogalactolipids1. Here we performed an untargeted analysis of membrane lipid composition of sperm from 41 infertile patients (mean age = 31.3 ± 8.3 years) undergoing standardised semen analysis2 and serum lipids evaluation by liquid chromatography-mass spectrometry (LC-MS) on methanol/dichloride-methane extracts from cell pellets. Twenty-one sperm membrane lipids were unambiguously identified by mass/charge ratio (m/z) and post source decay (PSD) spectra. Sulfogalactosylglycerolipid (SGG, aka seminolipid) was the most abundant component in the membrane, in agreement with previous studies3. In addition, we observed a significant proportion of PUFAs, such as C20:4 (arachidonic acid) and C22:6, representing 12% and 25% of total PUFAs in sperm membrane, respectively. Principal component analysis was computed for subsequent statistical analysis: the 21 lipids were grouped into five principal clusters, accounting for 76% of total variance. We then included these clusters as independent variables in multilinear regression analysis with following parameters: serum total Chol, HDL, LDL and triglycerides, sperm progressive motility, normal morphology, viability and semen concentration. Interestingly, none of sperm lipid clusters was associated with serum lipid markers. Conversely, one of the five clusters (including SGG, phosphatidic acid and phosphatydilcholine C18:1 16:0) was significantly associated with increased sperm motility, viability and concentration. A second cluster (including Chol sulphate, phosphatydilcholine C14:0 18:1 and C22:6 16:1) was associated with sperm progressive motility. To further characterize these associations, we performed Pearson’s correlations analysis between the components of the two aforementioned clusters and semen parameters. We found that SGG was positively associated with sperm concentration (p = 0.002) and motility (p = 0.001), and phosphatydilcholine C22:6 16:1 was positively associated with sperm concentration (p = 0.005), motility (p < 0.001) and viability (p = 0.011). On the other hand, Chol sulphate was negatively associated with sperm motility (p = 0.04). Altogether these results underline the important role of seminal lipids, which act independently of serum lipids levels and could rather represent an independent marker of spermatogenetic and reproductive function. Dietary PUFAs and SGG, in particular, have acknowledged antioxidant functions4 and could therefore represent the most sensitive markers of sperm quality and testicular function. SGG, the most representative component, is mostly synthesized during the mitotic phase of spermatogenesis (primary and secondary spermatocytes), and to a lesser extent in round spermatids5, thus providing a reliable proxy of the spermatogenic process. 1. Lopalo P, 2019. doi: 10.3389/fphys.2019.01344 2. WHO, 2021. 3. Tanphaichitr N et al., 2018. doi: 10.1016/j.pplipres.2018.08.002 4. Shan S, 2021. doi: 10.3390/iijms22168767

P027 | Transcriptional profiles of mitochondrial dynamics markers in human spermatozoa are associated with different types of spermiograms

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Infertility has become one of the greatest health issues today, affecting millions of people worldwide, with a significant contribution of
the male factor in many reported cases. Having in mind the increasing number of unexplained cases of infertile men in the peak of the reproductive period and the lack of an accurate test for assessment of spermatozoa functionality, World Health Organization expressed the need for the development of a new prognostic/diagnostic tool for detection of male infertility. Since mitochondria play important role in spermatozoa, regulating their homeostasis and functionality, it is reasonable to presume that they could be involved in these types of abnormalities and markers of their dynamics could be used as “mitochondrial-sperm-signature,” to test spermatozoa functionality. Regardless of that, little is known about mitochondrial dynamics markers in human spermatozoa. Thus, the main objective of this research was to investigate the transcriptional profile of main mitochondrial dynamics markers in spermatozoa from the population of men, diagnosed with the most common types of sperm abnormalities. For that purpose, spermatozoa obtained from men participating in the National program of in vitro fertilization, diagnosed with normozoospermia, teratozoospermia, asthenoteratozoospermia and oligoasthenoteratozoospermia, were separated from seminal plasma, capacitated and incubated with acrosome reaction inducer – progesterone. After RNA isolation and complementary DNA synthesis, samples were subjected to real-time PCR analysis. During quantification, all results were normalized to a normozoospermic group, used as a control and GAPDH, as a reference gene. Results showed a significant increase in the level of PPARGC1A transcript in spermatozoa from teratozoospermic group compared to normozoospermic. Conversely, the levels of PPARGC1B and MFN1 transcripts significantly decreased. The levels of NRF2, TFAM, MFN2, OPA1, FIS1 DRP1, PINK1 and PRKN in asthenoteratozoospermic and oligoasthenoteratozoospermic group, the levels of changes were not significant. In the same samples, the levels of transcripts of other mitochondrial dynamics markers remained consistent with a normozoospermic group, used as a control and GAPDH, as a reference gene. Results showed a significant increase in the level of PPARGC1A transcript in spermatozoa from teratozoospermic group compared to normozoospermic. Conversely, the levels of PPARGC1B and MFN1 transcripts significantly decreased. The levels of NRF2, TFAM, MFN2, OPA1, FIS1 DRP1, PINK1 and PRKN in asthenoteratozoospermic and oligoasthenoteratozoospermic group, the levels of changes were not significant. In the same samples, the levels of transcripts of other mitochondrial dynamics markers remained unchanged. Based on the obtained results it is evident that the markers of mitochondrial dynamics in human spermatozoa exerted different patterns depending on the type of spermogram and the most remarkable changes were observed in the teratozoospermic group. However, it is important to point out that research was conducted on a small number of the samples (3–10 individuals per group), so the results should be considered preliminary.

The sperm cell acquires a unique chromatin structure, tightly compacted and transcriptionally silent, thanks to the presence of the small and extremely basic protamines. This exceptional nuclear compaction occurs during the spermiogenesis through the synchronized process of nucleohistone-to-nucleoprotamine transition, and is essential for a proper sperm functionality. The uniqueness of this structure has attracted interest over the years, and the application of a wide range of methodologies allowed stabilizing the current model of sperm chromatin packaging. In mature human sperm, an 85–95% of the DNA is packaged by protamines, while the rest remains associated with histones. Electron and atomic force microscopy revealed that protamine-DNA complexes organize into supramolecular toroidal structures. However, there is not enough evidence about how the human sperm chromatin structure behaves in solution within the context of the whole nuclear compartment.

In the present work, we apply, for the very first time, SAXS from synchrotron light to describe how is the sperm nucleus constituted in solution, in purified nuclei (n = 10) and histone-depleted nuclei (n = 11) from human control patients. Besides, we studied how different naturally impaired chromatin statuses could impact the chromatin structure, such as altered protamine ratio (P1/P2, n = 4) and double-strand DNA (dsDNA) breaks (n = 6). SAXS was performed in the BL11 NCD-SWEET beamline from the ALBA Synchrotron Light source - beam energy 12.4 keV, detector distance of 6.7 m, Pilatus 1M detector (Dectris, Switzerland), 60 frames of 0.5 s.

Remarkably, we provide evidence of in vivo fractal-like supramolecular aggregates in the human sperm chromatin, arising from well-defined small subunits (radius r ~7.5 nm) which were stable between all groups, even in histone-depleted nuclei. The slightly bigger size compared to measurements already reported of in vitro reconstituted nucleoprotamine complexes (fractal-like supramolecular aggregates from small subunits of r = 2.5 nm) could be the result of a more complex in vivo conformation of the chromatin fiber. At the supramolecular level, patients with altered P1/P2 did not show significant differences on the supramolecular structures compared to controls. This could indicate that the protamines would have redundant functions, compensating the alteration to properly compact the sperm chromatin. Strikingly, a significant increase of the Radius of gyration (Rg) of the supramolecular aggregates was found in patients with dsDNA breaks compared to controls. Although it should be deciphered whether the dsDNA breaks are cause or consequence of this alteration, our findings reinforce the idea that genomic and chromatin integrities are closely related.
To conclude, SAXS data from human sperm nuclei in solution suggest a clearly well-defined and monodisperse structure of ∼10-15 nm size, not described up-to-date, as construction brick of larger supramolecular chromatin structures. Further studies are needed to unravel what is behind these highly-specific substructures packaging the paternal DNA, as well as to achieve the best adjusted model of supramolecular aggregation.

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P029 | Correlative study between molecular techniques for the detection of oxidative stress in semen samples of infertile patients

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Infertility represents a global growing health problem, the causes of which remain largely unknown in most cases. It currently affects about 186 million people worldwide. Recent studies estimate that global fertility has declined by 51% in the last century and sperm quality, in particular, has worsened in the last three decades. While socio-demographic factors may explain the decline in global fertility, other studies suggest that environment and lifestyle could impact both male and female fertility in a negative way. Although the male factor is involved in approximately half of the infertile couples, the current clinical evaluation of male fertility is mainly limited to the analysis of the semen parameters. However, it only exposes obvious alterations, such as those affecting sperm production or motility. Recent studies have highlighted oxidative stress (OS) as a potential factor involved in the 30–80% of men with unexplained infertility. Nevertheless, it is still unknown which methodologies would be optimal for its evaluation.

In this work, we carried out a pilot study with the aim of comparing different methodologies to detect OS and their correlation with DNA fragmentation. These results will allow the identification of complementary parameters to the semenogram that could be useful for the study of male infertility.

In particular, we have analyzed and correlated semen quality values with the reduction-oxidation potential of the semen (with MiOXSYS system), the amount of intracellular reactive oxygen species (EROS) (by using DCFH-DA colorant and flow cytometry) and the presence of cells with single and double strand DNA fragmentation (by performing alkaline and neutral comet assay, respectively). A total of 77 idiopathic infertile patients, including oligozoospermic, asthenozoospermic and oligoasthenozoospermic males, were enrolled in the study after informed consent.

A significant positive correlation between reduction-oxidation potential and the percentage of leukocytes in the semen (Spearman: 0.4144, p-value 0.0002) has been detected. These results highlight the critical impact of no spermatogenic cells in the semen, supporting previous evidences showing that leukocytes produce several orders of magnitude more EROS than mature spermatozoa. Furthermore, reduction-oxidation potential was found positively correlated with the values obtained by the alkaline comet assay (Spearman: 0.9048, p-value 0.002).

No correlation between extracellular and intracellular EROS in the semen samples was detected, suggesting that both EROS determinations are complementary and not a substitute for one another. Moreover, despite single strand DNA fragmentation seems to be correlated with reduction-oxidation potential of semen, double strand DNA fragmentation is suggested to be independent of OS. Nevertheless, further analyses increasing the number of samples are required to confirm the correlations found in this study.

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P030 | Evaluation of spermatogonial cell populations in male childhood cancer patients

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Spermatogenesis is a continuous process that relies on spermatogonial stem cells (SSCs) and offers a male the opportunity to generate sperm until late in life. If the SSC population is not formed or destroyed, permanent infertility follows. As a result, diseases or medical treatments might affect the SSC population, thereby causing male fertility (1). Historically, SSCs were characterized by their morphology as type Adark and A pale spermatogonia (2). However, advanced research techniques like single-cell RNA-sequencing have challenged this concept (3, 4). As a result, several SSC sub-populations have been identified, with expression profiles specific to stage (0 to stage 4) SSC sub-populations.
Nevertheless, detailed studies on protein expression of these spermatogonial markers have not been evaluated in human prepubertal testicular tissue.

To study human SSC subpopulations in postnatal testes, we included testicular tissue samples of boys who participated in the NORDFERTIL fertility preservation program. Patients were diagnosed with juvenile myelomonocytic leukemia (JMML; n = 4; age range: 1.3 ± 1.1 years) or acute myeloid leukemia (AML; n = 4; age: 8.7 ± 3.9 years). None of the patients had been exposed to alkylating agents. First, genes expressed by specific spermatogonial cell subtypes were identified using published single-cell RNA sequencing data. Then, based on this data, we used immunofluorescence staining for anti-PIWIL4 (spermatogonia (spg) at state 0), anti-ID4 (spg at state 0-1), anti-
GFRα1 (spg at state 1), anti-UTF1 (spg at state 0-1), anti-FGFR3 (spg at state 0-2), and anti-C-KIT (spg at state 2-3) in 4% PFA-fixed testicular tissue sections (5 μm thickness) to distinguish the different SSC subtypes.

In agreement with previous published single cell RNA sequencing data (4), we were able to identify age-dependent expression of spermatogonial markers in human testicular tissue. The proportion of spermatogonia in the older AML group was higher compared to the juvenile JMML group (PIWIL4: 0.46 ± 0.23 cells/mm²; ID4: 0.29 ± 0.21 cells/mm²; UTF1: 0.37 ± 0.27 cells/mm²; FGFR3: 0.07 ± 0.02 cells/mm²; C-KIT: 0.06 ± 0.00 cells/mm²).

Although the data indicate an age-related effect related to the expression profile of spermatogonial markers, future research is needed to elucidate the potential impact of disease-and treatment-related effects on the spermatogonial subtypes and their functionality later in life.

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P031  Sperm DNA fragmentation measured by the alkaline and neutral versions of the comet assay in men with normozoospermia and pathozoospermia

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Sperm DNA fragmentation (SDF) analysis by the comet assay was included in the sixth edition of the WHO manual (WHO 2020). How-
ever, while the alkaline version of the assay (pH ≥ 13) is commonly used for the examination of human semen, the application of the neutral version of this method is somewhat limited due to insufficient data. At the same time, the neutral comet assay allows detection of DNA double-strand breaks (DSBs), which may have a higher potential for negative reproductive effects compared with DNA single-strand breaks (SSBs) or alkaline-labile sites measured by the alkaline comet. The latter also detects DSBs, but in common pool with other DNA damage measured, that does not allow to evaluate DSBs effects separately.

The key objectives of the present study were as follows (1) to compare the results of the alkaline and neutral comets applied to the sperm samples of men with normo- or pathozoospermia; and (2) to assess DSBs in human sperm as a diagnostic tool for male infertility.

The frozen sperm samples of patients with normozoospermia (the control group, n = 34) and pathozoospermia (the pathospermia group, n = 44) were provided by the Republican DNA Bank of a Human, Animals, Plants and Microorganisms (Minsk, Belarus). The groups were previously defined following the WHO 2010 methods and reevaluated in the recent study using the WHO 2020 recommendations. Spermatozoa were studied using the neutral version of the comet assay for the DSB detection, and the alkaline comet assay was used to detect the total number of SSBs, DSBs and alkaline-sensitive sites. Wherein, DNA damage levels were analysed by visual scoring in arbitrary units and the number of cells with damaged (fragmented) DNA was estimated as the DNA fragmentation index presented in percent.

This study showed that significantly higher (p < 0.0001) levels of DSB measured by the alkaline comet, as well as SSBs, are in the pathospermia group compared with the control. The data obtained were confirmed when the most numerous subgroup with astenozoospermia (n = 23) was statistically analysed. Thus, the conducted testing using the alkaline and neutral comets allowed discriminating men with reduced fertility from the fertile ones.

The proportion of DSBs was unexpectedly high, about 50 and 44% of the total pool of DNA damage in the control and pathospermia groups respectively. A preliminary analysis of DSBs induced in vitro in spermatozoa of the control group by the well-known radiomimetic bleomycin sulphate showed that the mean level of DSBs recorded in the pathospermia group correspond to the mutagenic effect of bleomycin sulphate at a concentration of 20-30 μg/ml. These data on DSBs demonstrate a high degree of DNA dis-integrity in human sperm, especially in association with reduced fertility.

In conclusion it is worthy to note that the results of this work draw attention to the fact that not only the SDF of different origin measured by the alkaline comet assay, but also DSBs analysed separately are quite common in human sperm, and are strongly associated with pathozoospermia and may be used for male infertility diagnosis after additional studies.
The male reproductive tract is comprised of the testis, epididymis, vas deferens, the accessory sex glands (seminal vesicles, prostate, ampullary, bulbourethral, and preputial glands), urethra, and penis. Infectious agents such as bacteria and viruses gain access through the urethral opening of the penis and can ascend to the more distal parts of the genitourinary (GU) tract. Leukocytes play a significant role in defence from ascending pathogens, as well as also play important functions in normal tissue homeostasis. Therefore, we aim to characterize the immune cell subsets, particularly macrophages, and their functions longitudinally throughout the male reproductive tract during development as well as under normal and inflammatory conditions in adult mice. In this regard, we used a combined approach of flow cytometry and immunofluorescence analyses. Flow cytometry-based analysis of single-cell suspensions from healthy male reproductive organs (MRO) of 9-10 week-old C57BL/6J mice revealed that ~60% (testis), ~55% (epididymis), ~17% (vas deferens), ~3% (seminal vesicles), ~35% (prostate), ~17% (urethra), and ~3% (penis) of all CD45+ leukocytes were macrophages (F4/80+CD11b+ cells). Other myeloid cells, including monocytes and dendritic cells, were observed in all MRO; however, the proportion varies significantly between the organs. Of note, among leukocytes, B cells (CD19+) comprise the predominant populations in the lower GU tract (penis ~14%), whereas macrophages are the dominant leukocyte population in the upper GU tract (testis ~60%).

Macrophages are the sentinel immune cells of most organs, and the proportion of macrophages amongst leukocytes changes during development. Thereby, we examined the postnatal development of macrophage populations in the entire MRO. All organs contain a distinct subset of F4/80hiCD11blo macrophages at the first week of age. At the 3rd week of age, in the epididymis, vas deferens, seminal vesicles, and prostate, a second population of F4/80loCD11bhi macrophages appears and numbers progressively increase until 9-10 weeks of age. However, in the testis and penis, the second population, which appears after three weeks of age, is different to the other organs, namely F4/80hiCD11bhi. Intriguingly, the expression of MHCII increases differently in the macrophage populations with age in the various organs of the MRO. Next, we examined the heterogeneity of macrophage populations by flow cytometry based on CD206 and MHCII expression. We observed four macrophage populations, namely CD206+MHCII−, CD206+MHCII+, CD206−MHCII−, and CD206−MHCII+, with varying proportions in the MRO. In conclusion, our results indicate that the MRO contains distinct subsets of macrophage populations, likely as a reflection of fulfilling organ-specific homeostatic functions.

In recent years, it has been emerging that not only oncological therapies but also cancer itself can induce abnormal spermatogenesis. In addition, several Authors reported that occurrence of malignancy provokes also increases of sperm DNA damage, although such finding was not confirmed by others and the possible mechanisms responsible for such damage are presently unknown. This was a prospective observational study, conducted from 2018 to today, in 102 patients affected by cancer (haematological, n = 40; testicular, n = 62) and in 62 control subjects (male partners of infertile couples), both recruited among the population afferent to the Andrology Clinic of University of Florence for routine semen cryopreservation and semen analysis, respectively. Control subjects were normozoospermic with absence of leukocytospermia, semen viscosity, smoking habit and recent antibiotic therapies., in the recruited men, We evaluated standard semen parameters according WHO guidelines and, When semen samples was enough both sperm DNA Fragmentation (with SCD, Sperm Chromatin Dispersion) Test and oxidative stress (with a double staining with MitoSOX™ Red and LIVE/DEAD Fixable Dead Cell Green Stain, then detected by Flow Cytometry). In particular, oxidative stress was calculated as percentage of viable spermatozoa with MitoSOX™ Red labeling on total viable spermatozoa. We found poorer standard semen parameters (sperm motility, concentration and number) in cancer patients (both testicular and hematological ones) with respect to control group, whereas no difference was seen in the other tested characteristics (sperm morphology, abstinence, semen volume and pH, BMI). However, patient with testicular cancer, were younger than control subjects. No difference was observed between the two types of cancer. Regarding sDF, we found higher median values [IQR] in cancer patients (total: 22.25[17.00-25.95], n = 68; hematological: 23.00[20.13-26.38], n = 28; testicular: 21.13[16.13-25.73], n = 40) vs control subjects (12.50[8.25-14.75], n = 53); p < 0.05, test U di Mann-Whitney. In addition, the amount of sperm oxidative stress was dramatically higher in patients with cancer (total:38.92[24.90-58.87], n = 79; hematological: 38.85[24.98-50.77], n = 34; testicular: 38.92[20.59-63.59], n = 45) vs control subjects (11.50[8.38-17.20], n = 62); p < 0.05, test U di Mann-Whitney. We also studied the occurrence of a correlation between levels of sDF and oxidative stress. We found a sharp correlation when both cancer patients and control subjects were analysed (Spearman
Morphology of epididymis and expression of aquaporin 9 in epididymal duct of adult rats after long-term treatment with immunosuppressive protocols based on calcineurin inhibitors

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Factors that contribute to the reduction of male fertility are still being searched for. It has been revealed that calcineurin inhibitors (cyclosporin A and tacrolimus), used in patients after organ transplantation, have an adverse effect on the male reproductive system. Recently, attention is also paid to aquaporins (AQP9), which play a crucial role in the secretion/reabsorption of fluids in the transepithelial transport in the epididymal duct. Therefore, our study was designed to determine the effect of immunosuppressive treatment protocols based on calcineurin inhibitors on the morphology of epididymis and immunoexpression of AQP9 in the epididymal duct of the rat.

For 6 months (which is analogous to approximately 15 years of human life), 18 male Wistar rats (sexually mature 14 week old) received immunosuppressants: cyclosporin A (CsA), tacrolimus (FK-506), mycophenolate mofetil (MMF), and prednisone (Pre) according to the three-drug protocols used in patients after organ transplantation. Rats were divided into 3 groups (n = 6 for each group): control, CMP (CsA, MMF, Pre), and TMP (FK-506, MMF, Pre). Morphological analysis of epididymis was based on hematoxylin and eosin staining, while immunohistochemical reaction for AQP9 was performed on paraffin-embedded epididymal tissue sections. The morphometric parameters of epididymal duct and intensity of AQP9 immunoexpression were assessed by means on quantitative computer analysis (ImageScope viewer v. 11.2.0.780; Aperio Technologies, Vista, CA, USA).

In the CMP and TMP groups the wall of the epididymal duct was collapsed, which made its lumen irregular (corpus and cauda epididymis), height of epididymal epithelium was significantly lower and immunoexpression of AQP9 in the apical membrane of epididymal epithelium was decreased (caput, corpus and cauda epididymis) vs. control group. It should be highlighted that in the experimental and control groups the sperm content in the lumen of the epididymal duct was comparable and irrespective of its anatomical segment.

The long-term treatment of adult rats with immunosuppressive protocols based on calcineurin inhibitors may lead to morphological alterations in epididymis and disturbances of transepithelial fluid and solute transport in epididymal epithelium. This research was funded by the National Science Centre of Poland MINIATURA 5 grant No. DEC-2021/05/X/NZ7/00721

Age-associated epigenetic changes in mammalian sperm: implications for offspring health and development

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Background: Modern reproductive behavior in most developed countries is characterized by delayed parenthood. Older gametes are generally less fertile, accumulating and compounding the effects of varied environmental exposures that are modified by lifestyle factors. Clinicians are primarily concerned with advanced maternal age, while the influence of paternal age on fertility, early development, and offspring health remains underappreciated. There is a growing trend to use assisted reproductive technologies (ART) for couples of advanced reproductive age. Thus, the number of children born from older gametes is increasing.

Objective and rationale: We review studies reporting age-associated epigenetic changes in mammals and humans in sperm, including DNA
methylations, histone modifications, and non-coding RNAs. The interplay between environment, fertility, ART, and age-related epigenetic signatures is explored. We focus on the association of sperm epigenetics on epigenetic and phenotype events in embryos and offspring.

**Search Methods:** Peer-reviewed original and review articles over the last two decades were selected using PubMed and the WOS. Searches were performed adopting the two groups of main terms. The first group included 'advanced paternal age', 'paternal age', 'postponed fatherhood', 'late fatherhood', 'old fatherhood' and the second group included 'sperm epigenetics', 'sperm', 'semen', 'epigenetic', 'DNA methylation', 'chromatin', 'non-coding RNA', 'assisted reproduction', 'epigenetic clock'.

**Outcomes:** Age is a powerful factor in humans and rodent models associated with increased de novo mutations and a modified sperm epigenome. Age affects all known epigenetic mechanisms, including DNA methylation, histone modifications, and profiles of ncRNA. While DNA methylation is the most investigated, there is a controversy in the direction that dominates the age-dependent changes of differentially hypo- or hypermethylated regions with age. Successful development of the human sperm epigenetic clock based on cross-sectional data and four different methods for DNA methylation analysis indicates that at least some CpG exhibit a linear relationship between methylation levels and age. Rodent studies show a significant overlap between genes regulated through age-dependent differentially methylated regions and genes-targeted by age-dependent ncRNA. Both age-dependent epigenetic mechanisms target gene networks enriched for embryo developmental, neurodevelopmental, growth and metabolic pathways. Thus, age-dependent changes in the sperm epigenome cannot be described as a stochastic accumulation of random ‘epimutations’ and can be linked with autism spectrum disorders. Chemical and lifestyle exposures and ART techniques may affect epigenetic aging of sperm. Although most epigenetic modifications are erased in the early mammalian embryo, there is growing evidence that altered offspring epigenome and phenotype is linked with advanced paternal age from a father’s sperm accumulating epigenetic changes with time. It has been hypothesized that age-induced changes in the sperm epigenome are profound, physiological, dynamic over the years, stable over days/months, and likely irreversible.

**Wider implications:** This review raises a concern of delayed fatherhood and age-associated changes in sperm’s epigenome that may compromise reproductive health of fathers and transfer of altered epigenetic information to subsequent generations. Prospective studies using healthy males that consider confounders are recommended. We suggest a broader discussion focused on the regulation of father’s age in natural and ART conceptions is needed.

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**P037 | New kids on the block: testicular capsule macrophages come into play**

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Macrophages are sentinel cells present in almost all organs and play an essential role in tissue homeostasis, tissue repair, inflammation resolution as well as being a key player in innate immune responses. Akin to other organs, macrophages are the dominant immune cell population of the testicular parenchyma. They are supposed to play a critical role in maintaining the immune-privileged status of the testis, besides protecting against inflammatory stimuli. Until now, testicular parenchyma macrophages (TPM) were thought to be the only resident macrophage population present in the testis. However, few studies indicate the presence of macrophages also in the testicular capsule. The ontology, localization, phenotypes and functions of TPM are begun to be understood, however, information about testicular capsule macrophages

**P036 | Constant light during maturation: a challenge to the endocrine function of the Leydig cells**

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Factors influencing Leydig cell maturity and the acquisition of functional capacity are incompletely defined. Here we analyzed the constant light (LL) influence on Leydig cells’ endocrine function during reproductive maturation. Rats were exposed to LL from P21 to P90. Data were collected at juvenile (P35), peri/pubertal (P42, P49), and adult (P90) stages of life. Results proved the effect of LL on rats’ physiology by changing of bimodal voluntary activity pattern into free-running. Additionally, the peripheral clock in Leydig cells changed in LL condition, indicating disturbed rhythm: the positive element (Bmal1) increased in pre-/pubertal but decreased in the adult period while negative elements (Per2 and Reverba) were increased. The effects of LL were most prominent in puberty: pituitary genes encoding gonadotropic hormones (Cga, Lhb, Fshb) decreased; serum androgens and mass of testicular and sex accessory organs reduced; markers of Leydig cells maturity/differentiation (Ins13, Lhcgr) and steroidogenesis-related genes (Scarb1, Star, Cyp11a1, Cyp17a1) decreased; the steroidogenic and energetic capacity of the Leydig cell mitochondria decreased; the mtDNA copy number reduced, and mitochondrial dynamics markers changed: fusion decreased (Opa1 and Mfn2), and mitophagy increased (Pink1).

In adults, the negative effect of LL on mitochondrial function and steroidogenic capacity persists in adult Leydig cells while other parameters reached control values. Altogether, results indicate that LL slows Leydig cells’ maturation by reducing the endocrine and energy capacity of cells leading to the delaying of reproductive development.
(TCM) is completely lacking. We hypothesize that TCM will be distinct from TPM in ontology, phenotype and function.

Our flow cytometry data revealed that the testicular capsule contains a heterogeneous immune cell population consisting of monocytes, macrophages, dendritic cells and T cells. However, the proportion of macrophages amongst all leukocytes in the testicular capsule is significantly lower (30%) than in the testicular parenchyma (60%). Of note, the testicular capsule harbors more dendritic cells and T cells than the parenchyma. TCM can be distinguished from TPM by their morphology as they display very long dendrites, low expression of Cx3Cr1, and CD68, while levels of Ccr2 and MHC II expression are high. During inflammatory conditions, infiltrating Ly6G+ neutrophils, the first immune cell to migrate at the site of infection, are entrapped mainly in the testicular capsule rather than the testicular parenchyma. This result suggests that entrapment of Ly6G+ neutrophils in the testis capsule is a plausible mechanism to protect the testis from a strong inflammatory reaction and thus maintains immune privilege. Moreover, our results also suggest that TCM arise from blood monocytes rather than embryonic precursors by using Ccr2−/− mice. These results indicate that the testicular capsule contains a macrophage population distinct from TPM with different ontology and function.

**P038 | Effect of prenatal exposure to α-cypermethrin on DNA methylation patterns in rat sperm**

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 α-cypermethrin belongs to pyrethroids, one of the most used groups of insecticides that can act as endocrine disruptive chemicals (EDCs), known to impact gene expression at the epigenetic level. Testicular exposure to EDCs in foetal development may impair PGC differentiation causing decreased sperm quality and number and increased apoptosis of germ cells, as well as infertility in adults. However, such an effect has not still been established for α-cypermethrin. One of the most studied epigenetic mechanism is DNA methylation, which plays a major role in the de novo silencing of retrotransposon sequences in germ cells, as well as in the establishment of sex-specific imprinting.

In this study, we investigated the effect of prenatal exposure to α-cypermethrin on DNA methylation patterns in rat sperm of F1 offspring. Pregnant female Wistar rats were divided into 6 groups. The first three experimental groups (α-cyp1, α-cyp10 and α-cyp19) were treated per os from the 6th up to 21st gestational day with three different doses of α-cypermethrin (1, 10 and 19 mg/kg bw/day). In the same period, the positive control group (PC) was treated with diethylstilbestrol, solvent control group (SC) with corn oil and negative control group (NC) with water. DNA was isolated from the sperm of F1 generation pubertal pups. Subsequently, the methylation level of 3 different CpG sites within repetitive element LINE-1 was analysed for the assessment of global methylation by pyrosequencing method. Additionally, methylation analysis was performed for 7 different CpG sites within the differentially methylated regions (DMRs) of the imprinted genes Igf2 and H19.

The results showed no significant difference in the methylation levels of examined DNA sequences of LINE-1 between the experimental groups and the controls. Also, exposure to α-cypermethrin had no effect on the methylation levels of the examined DNA sequences of Igf2 and H19. This study implies to the possibility that prenatal exposure to α-cypermethrin does not affect the de novo DNA methylation process in the rat sperm of F1 offspring and indicates a need for further research of epigenetic changes caused by this endocrine disruptor at the genome-wide level.

**P039 | Unique cytogenetic variant of Klinefelter syndrome with double Y-autosomal translocation**

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**Abstract:** Klinefelter syndrome (KS) is one of the most frequent chromosomal abnormalities and commonest genetic cause of male infertility. About 85% KS patients have 47,XXY karyotype, other patients presented other mosaic and non-mosaic variants.

**Materials and Methods:** We reported on KS patient with unique double Y-autosomal translocation. The proband is 15 y.o. male patient (height 180 cm, weight 50 kg, normal IQ) admitted for cytogenetic examination and genetic counseling because of puberty delay. Testicular hypoplasia, hypergonadotropic hypogonadism, pituitary microadenoma and left-side varicocele were diagnosed in him. The proband was born in non-consanguineous marriage after IVF/ICSI procedure because of infertility. The father of proband is oligozoospermic man having Robertsonian translocation 13;15 – 45,XY,der(13;15)(q10;q10), the mother showed normal karyotype 46,XX.

Chromosome analysis was done on cultured peripheral blood lymphocytes using standard cytogenetic method (G-TG-staining) and FISH analysis with DYZ1, DYZ3, D15Z, SE13/21 and LSI UBE3A(15q11)/PML(15q24) probes. The Y chromosome loci (SRY, ZFY, AZFa,b,c) were analyzed by multiplex PCR. CAGn polymorphic locus of AR gene was evaluated in the proband and his parents to determine parental origin of the X-chromosome in the patient.

**Results:** Complex cytogenetic examination revealed 46,XX,der(Y)t(Y;15)(q12;q11.1),der(13)t(Y;13)(q12;p11.2) karyotype in the proband. Molecular analysis showed that the proband is SRY-positive, homozygous for CAGn polymorphic locus of AR gene (n = 24 allele), and that both X chromosomes are maternally inherited
because of X-X non-disjunction during meiosis II; no the Y chromosome microdeletion was found. Detected double Y-autosomal translocation is independent chromosomal abnormality from KS. Apparently, the der(13) and der(Y) chromosomes are resulted from abnormal meiotic recombination in the paternal meiosis between the Yq12 heterochromatic region and centromeric/pericentromeric heterochromatin of chromosomes 13 and 15, involved in robertsonian translocation in the father.

**Conclusion:** Rare cytogenetic variants of Klinefelter syndrome are associated with Y-autosomal translocations, originated as independent chromosome mutations.

**Keywords:** Klinefelter syndrome, AZF locus, AR gene, sex chromosomes, Y-autosomal translocations, robertsonian translocations.

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**P040** | Enhanced frequency of the L138ins variant of the CFTR gene in Russian infertile men

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**Actuality:** Pathogenic variants of the CFTR gene are one of common genetic factor of male infertility, resulted to Cystic Fibrosis (CF) and CBAVD syndrome, obstructive azoospermia. L138ins (c.411_412insCTA; p.Leu138dup) variant of the CFTR gene is CF-causing mutation, which is relative common mutation in CF patients from Slavic populations. The prevalence of this CFTR gene variant in Russian infertile men previously was not evaluated.

**Materials and Methods:** We examined large cohort of 6033 Russian infertile men. Genomic DNA extracted from peripheral blood lymphocytes was screened for 22 common pathogenic variants (CFTRdele2,3, 394delIT, 394delTG, L138ins, R508del, I507del, 1677delTA, G542X, 2143delT, 2184insA, 621+1g>T, 604insA, 621+1g>t, E92K, S1196X, W1282X, N1303K, 4022insT, 4015delA, G542X, 2143delT, 2184insA, 3821delT, 3849delTT, 3944delTG, L138ins, R334W, F508del, I507del, 1677delTA, G542X, 2143delT, 2184insA, 621+1g>T, 604insA, 621+1g>t, E92K, S1196X, W1282X, N1303K, 4022insT, 4015delA, and 3272-26A>G), and IVS9Tn (IVS8Tn) polymorphic locus of the CFTR gene. Molecular analysis was performed using by AFPL, MLPA, DNA sequencing by Sanger or massive parallel sequencing methods.

**Results:** Pathogenic CFTR gene variants were detected in 3.9% patients. Commonest variants were F508del and CFTRdele2,3(21kb), which present 61.0%, and 7.1% of detected CFTR mutations, respectively. A presence of two CF-causing CFTR gene variants was found in 61.0%, and 7.1% of detected CFTR mutations, respectively. A presence of two CF-causing CFTR gene variants was found in 61.0%, and 7.1% of detected CFTR mutations, respectively. A presence of two CF-causing CFTR gene variants was found in 61.0%, and 7.1% of detected CFTR mutations, respectively. A presence of two CF-causing CFTR gene variants was found in 61.0%, and 7.1% of detected CFTR mutations, respectively.

**Conclusion:** The results show a relatively high frequency of L138ins variant among Russian infertile men. This pathogenic variant of the CFTR gene is third in frequency after the two most common mutations, F508del and CFTRdele2,3(21kb). Some infertile men with two CF-causing pathogenic variants of the CFTR gene may have undiagnosed non-severe forms of CF or CFTR-RD.

**Funding:** The present study was performed within the framework of the project Multicenter Research Bio resource Collection “Human Reproductive Health” no. 15.BRK.21.0008 of the Ministry of Science and Higher Education of the Russian Federation.

**Keywords:** male infertility, CFTR gene, Cystic Fibrosis, CBAVD, azoospermia.

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**P041** | Use of mass spectrometry to identify proteins related to impaired sperm morphology and motility in infertile men

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**Introduction:** Genetic causes are likely involved in about 30% of male infertility cases with approximately 2000 genes specifically expressed in spermatogenesis. Next generation sequencing aided in identifying genes likely to be involved in impaired spermatogenesis and therefore male infertility. By complementing next generation sequencing approaches, mass spectrometry (MS) is a powerful tool to identify differentially expressed proteins (DEPs) between control and infertility groups. This study focused on identifying proteins in human ejaculated spermatozoa with impaired sperm morphology and motility to identify proteins associated with defects in sperm function.

**Material and Methods:** Human ejaculates classified as normozoospermia (NORM, n = 3) or presenting defective morphology and/or motility (asthenoteratozoospermia; AT, n = 3) were subjected to mass spectrometry (Orbitrap Eclipse). In silico analysis of DEPs were performed. Selected proteins were examined by immunohistochemistry (IHC) in testicular biopsies showing normal spermatogenesis (NSP) or spermatid arrest (SDA) phenotype.

**Results:** 36 proteins were significantly higher or lower in AT compared to NORM. In silico analysis identified several interesting candidate proteins of which five were selected for further investigations; of these, IPO4, ELSPBP1, and IFT57 were higher whereas CCDC105 and ACTR2 were lower. ACTR2, IPO4, CCDC105, IFT57 are present in testis, and IHC revealed their localisation in human germ cells with
a stronger signal for ACTRT2, IFT57, IFO4 in round/elongated spermatids. Importantly, ACTRT2 and CCDC105 were markedly lower in spermatids from SDA samples, confirming MS results. An epididymal-specific protein, ELSPBP1, identified as higher in AT, showed minimal expression in testis tissues. IHC showed nuclear localised IPO4 in all germ cells, with an enhanced cytoplasmic expression from pachytene spermatocytes to round/elongating spermatids compared to spermatogonia and leptotenes in NSP samples. Spermatogonia showed relatively low nuclear and no cytoplasmic IPO4 signal. Interestingly, IHC of SDA showed clear nuclear IPO4 signal in late germ cells, with reduced cytoplasmic expression in spermatocytes and no cytoplasmic signal in spermatogonia.

**Conclusion:** By mass spectrometry analysis of normal and defective ejaculated sperm, we identified 36 proteins that show quantitative changes in AT compared to NORM human sperm. These may have important functions in spermiogenesis, and altered expression levels might be linked to abnormalities in sperm function of infertile men. In particular, IPO4 is a member of the importin family involved in nuclear transport that is more abundant in AT sperm, and its differential localization in NSP and SDA patients suggests its role in protein transport could affect spermatid development. Current studies are focused on to quantify IPO4 subcellular localization in testis sections and to characterise its localisation in sperm to understand its functional role in human spermatozoa.

P042 | DNA-FISH analysis in testicular tissue cells from prepuberal patients with Klinefelter Syndrome

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Prepuberal boys with Klinefelter Syndrome usually have fertility problems in adulthood, mainly azoospermia. Due to the symptoms cause by the disease, Spermatogonial Stem Cells (SSCs) are less abundant and spermatogenesis does not occur. These patients do not have any alternative to restore the fertility in the future. When a boy has both XY and XXY cell lines, this patient is mosaic (46,XY/47,XXY). Meaning some cells could undergo spermatogenesis and generate gametes with normal sex-chromosomes number. The aim of this work is to test if KS patients diagnosed as pure can have testicular cell lines with XY chromosome number (mosaic).

We obtained samples from human prepuberal patients diagnosed with pure Klinefelter Syndrome (47,XXY) for the last 5 years. Testicular biopsy fragments are fixed for histological studies and other fragments are cryopreserved. Some fragments are used for immunofluorescence and subsequently processed by DNA-FISH to determine the sex-chromosomes content of testicular cells.

In this study, we used 10 prepuberal patients with KS and 5 prepuberal patients with other fertility problems with normal chromosone set as controls. We performed immunofluorescence to determine expression of germ cells (VASA) and SSCs (MAGEA4) markers and somatic cells markers such as Leydig cells (STAR) and Sertoli cells (SOX9). Afterwards, we perform DNA-FISH, with probes specific for chromosomes X and Y and chromosome 18 as a control.

The methodology used allows cytogenetic characterization of testicular tissue in paraffin embedded sections. Testicular mosaicism has been observed in all patients diagnosed as pure KS. We have observed a degree of mosaicism of 66-80% in SSCs, of 20-50% in Sertoli cells and of 30-50% in Leydig cells. We pursued a protocol with a good FISH efficiency that allows colocalization of previous immunohistochemically stained testicular cells.

We succeed to demonstrate the mosaicism of testicular cells in prepuberal patients that are diagnosed with KS. Thus, the better understanding of the SSCs with normal chromosome set (XY) could be useful for future in vitro expansion and stem cell therapies.

P043 | Brain hyperexcitability in an experimental model of chronic prostatitis/chronic pelvic pain syndrome: modulation by CO

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**Introduction:** The most common cause of chronic pelvic pain in male population is urological entity Chronic Prostatitis/Chronic Pelvic Pain Syndrome (CP/CPPS). Besides chronic and spontaneous pain, uroanatological symptoms, psychiatric comorbidities and sexual dysfunction, it is also accompanied with brain hyperexcitability. Sometimes, antiepileptic medications are potent in the CP/CPPS treatment, but there is no proper investigation in experimental models. Carbon monoxide (CO), gas neurotransmitter, has been identified as anti-inflammatory and immunomodulatory agent. Experimental modulation of the endogenously synthesized CO levels can be done in vivo
by parenteral application of CO-releasing molecules (CORMs). The aim of this study was to investigate effects of parenterally administered CORM-A1 on brain excitability in rats with experimentally induced CP/CPPS.

**Methods:** Adult male Wistar albino rats (n = 32) were randomly divided into: CP/CPPS (n = 16, intraprostatic injection of 3% λ-carrageenan) and Sham (n = 16, 0.9% NaCl) group. Additionally, after operation, animals from both groups were treated daily by 7 consecutive days by CORM-A1 (2 mg/kg, i.p. forming the Sham-CORM and CP/CPPS-CORM groups, n = 8 in each) or its solvent, phosphate buffer (PBS, forming Sham-PBS and CP/CPPS-PBS groups, n = 8 in each). To confirm CP/CPPS development, mechanical pain thresholds in the scrotal skin were determined by electrical von Frey aesthesiometer prior to, as well as, 2, 3 and 7 days upon operation. Seventh day upon intraprostatic injection, we challenged rats with subconvulsive dose of lindane (4 mg/kg). Hereupon, we assessed rats’ convulsive behavior (seizure incidence, latency and severity) and EEG manifestations (number and duration of ictal periods).

**Results:** Scrotal pain threshold was significantly decreased in all postoperative days in CP/CPPS-CORM and CP/CPPS-PBS animals compared to corresponding Sham-CORM and Sham-PBS animals (p < 0.001). In addition, CORM-A1 treatment in CP/CPPS-CORM rats led to statistically significant (p < 0.001) analgetic effect in all postoperative days, compared to CP/CPPS-PBS rats. Animals with prostatitis (groups: CP/CPPS-CORM and CP/CPPS-PBS) revealed significantly higher incidence (p < 0.01), decreased latency time (p < 0.01) and augmented severity (p < 0.01) of lindane-induced seizures compared to corresponding controls (groups: Sham-CORM and Sham-PBS). Further analysis showed that CORM-A1 treatment in CP/CPPS-CORM rats has partially reversed brain hyperexcitability and led to significantly lower incidence (p < 0.05), increased latency time (p < 0.05) and lowered severity (p < 0.05) of seizures, compared to the CP/CPPS-PBS rats. EEG analysis showed increased duration (p < 0.05) and number of ictal periods (p < 0.01) in CP/CPPS-CORM and CP/CPPS-PBS rats. CORM-A1 treatment has significantly reduced number of EEG ictal periods (p < 0.05) in CP/CPPS-PBS rats with no statistically significant influence on the duration of EEG ictal periods.

**Conclusion:** CO is potent modulator of brain hyperexcitability developed in male rats with CP/CPPS.

**Keywords:** chronic prostatitis, chronic pelvic pain, epilepsy, lindane, CORM-A1, rats

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**Therapeutic effect of lipid extract of solanum lycopersicum in combination with selenium in wistar rats with benign prostatic hyperplasia**

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Benign prostatic hyperplasia (BPH) is the increase in the size and/or number of stromal cells and the epithelium of the prostate at a point where urinary flow is obstructed causing bladder obstruction, generating lower urinary tract symptoms (LUTS). BPH is the most frequent benign tumor in men over 60 years of age and its prevalence increases linearly with age. Its etiology is attributed to the action of dihydrotestosterone (DHT) by activating a series of cellular processes that lead to the activation of cell growth and proliferation genes. DHT is generated from testosterone by the action of 5α-reductase. The use of 5α-reductase inhibitors such as finasteride, one of the main treatments used in BPH, has reported several adverse effects due to its prolonged use, which has led to the search for safe and effective therapeutic alternatives. For decades, phytotherapy has been used as a therapeutic alternative for BPH. S. lycopersicum, commonly known as tomato, is rich in vitamins, phenolic compounds and carotenoids such as lycopene, which together have been attributed antioxidant, anti-proliferative and pro-apoptotic effects, as well as the decrease of DHT by inhibition of 5α-reductase, on the other hand, Selenium, has also been attributed antioxidant, anti-proliferative and pro-apoptotic effects in prostate tissue. Fourteen-month-old Wistar rats were divided into six groups (Control, BPH, Finasteride, Extract, Selenium and Selenium-Extract). Except for the control group, they were administered testosterone (10 mg/kg/week) subcutaneously to mimic BPH. For 30 days, finasteride (5 mg/kg/day), tomato lipid extract (5 mg/kg/day) according to lycopene concentration), sodium selenite (10 μg/kg/day) were administered orally. Lipid extract in combination with Selenium had a ~60% decrease in prostate size compared to separate administration of the extract and selenium (~35%) and finasteride (~15%) and with respect to rats with BPH. In addition, the combination also improved the decrease in oxidative stress markers (Malondialdehyde and total nitrites), increased the activity of major antioxidant enzymes (Superoxide dismutase, Catalase and Glutathione Peroxidase), while also decreasing prostatic levels of testosterone, DHT and prostate specific antigen (PSA). The combination of tomato lipid extract in combination with selenium reversed cell proliferation, increased antioxidant activity, regulated androgen and PSA levels and reduced prostate weight and size to normal values.

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**Withdrawn**

**PELP1 and its possible interactions with proteins involved in ESR-mediated signaling in human sperm cells**

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**Purpose:** A key role of estrogens in the male reproductive tract is spermatogenesis regulation and functional gamete maturation.
These events can be disrupted by the cumulative effect of endogenous and exogenous estrogens. Characterization of estrogen signaling pathway-related genes’ expression in sperm cells could aid in better understanding sperm biology and quality.

Materials and Methods: In this study, we obtained samples from 119 male participants of Caucasian descent. We analyzed four genes in the sperm of men who donated semen for standard analysis. Expression levels were analyzed for estrogen receptors (ESR1 and ESR2) as well as their coregulators – proline-, glutamic acid-, and leucine-rich protein 1 (PELP1), and cellular kinase c-Src (SRC). Protein expression was confirmed with western blot and immunocytochemistry techniques.

Results: Expression of both ESRs differed in case of sperm normal and abnormal morphology and for ESR2 additionally in case of motility ($p < 0.05$). Gene expression ratios revealed significant, moderate, and negative correlations for ESR1/ESR2 and weak, negative ESR2/PELP1 correlations in the subgroup of patients with abnormal sperm values. Additionally, SRC/PELP1 were moderately and positively correlated in the subgroup with all parameters within the WHO reference range.

Conclusions: As known, ESR1 and ESR2 influence sperm biology, but our study showed that other genes are engaged in estrogen-signaling-pathway. Both PELP1 scaffolding protein and SRC kinase may be important factors influencing sperm biology via ESRs, but it would require further functional analyzes. Disrupted estrogen signaling in sperm cells may be associated with the deregulation of certain sperm cell functions.

Keywords: estrogen receptors (ESR1 and ESR2); proline-, glutamic acid-, and leucine-rich protein 1 (PELP1); proto-oncogene tyrosine-protein kinase c-Src (SRC); steroid hormones; sperm; This research was funded by National Science Centre Poland, grant number UMO-2016/23/D/NZ5/02604

P047  |  Stress changed the Leydig cell’s transcriptional activity depending on the diurnal time

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The increasing amount of data points to the circadian timing system as an essential part of processes regulating androgen homeostasis. However, the relationship between stress response, timekeeping-, and steroidogenesis-related systems is unexplored. Here, the stress-response of the testosterone-producing rat Leydig cells depending on the time of stressful events was studied. The study analyzes the effects of 3-hour immobilization (IMO) applied at different periods during the day. The IMO is performed once (1xIMO) or repeated in 10 consecutive days (10xIMO). Both types of IMO increased corticosterone and decreased testosterone blood level. However, the effect of 10xIMO occurring in the active phase on blood testosterone was less pronounced. This is related to different sensitivity to IMO-events depending on the diurnal time. Most steroidogenesis-related genes (Lhgr, Cyp11a1, Hsd3b1/2, Cyp17a1) were down-regulated in the inactive but unchanged or even up-regulated in the active phase of the day. Both types of IMO stimulated the expression of clock elements Bmal1/BMAL1, Per1/PER1 regardless of the day’s stage and reduced Rev-erba in the inactive phase. The principal-component-analysis (PCA) confirmed a major shift, for both IMO-types, in the transcription of the genes across the passive/active stage. Further, 10xIMO changed a diurnal pattern of the glucocorticoid receptor (Nr3c1/GR) expression while the observed time-dependent IMO-response of the Leydig cells correlated with different corticosterone engagements. Altogether, the Leydig cell’s stress-response depends on the daytime of the stressful event, emphasizing the importance of the circadian system in supporting androgen homeostasis and male fertility.

P048  |  The effect of sex steroids on RhoA/ROCK pathway in the rat distal vagina

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The RhoA/ROCK calcium-sensitizing pathway plays a role in clitoral contractility, and therefore we deemed interesting to investigate its involvement in other urogenital districts. This study investigated the sex steroid regulation of the vagina contractility through the RhoA/ROCK pathway, using a validated animal model. Ovariectomized Sprague-Dawley rats (OVX) were treated with 17β-estradiol (E2), testosterone (T), with (T+L) or without letrozole, and compared with intact animals. Contractility studies were performed on vaginal strips to test the effect of ROCK inhibitor Y-27632 and NO synthase inhibitor L-NAME. In vaginal tissues, ROCK1 immunolocalization was investigated, mRNA expression was analyzed by semi-quantitative RT-PCR, and RhoA membrane translocation was evaluated by Western blot (Wb) analysis. Finally, rat smooth muscle cells (rvSMCs) were isolated from distal vagina of intact animals and the quantification of RhoA inhibitory protein RhoGDI was performed by Wb after stimulation with NO donor SNP, with or without administration of ODQ (soluble guanylate cyclase inhibitor) or KT5823 (PKGR1 inhibitor).

The results show that ROCK1 was immunolocalized in the smooth muscle bundles and in the blood vessel walls of vagina, while a weak positivity was detected in the epithelium. Y-27632 induced a dose-dependent relaxation of noradrenaline pre-contracted vaginal strips, decreased by OVX and completely restored by E2, compared to
controls, while T and T+L further decreased it, even below OVX level. Accordingly, in Wb analysis OVX significantly induced RhoA activation compared to controls, as revealed by its membrane translocation, with both decreasing it at a level significantly lower than in controls. This effect was not exerted by E2. Abolishing the NO formation via L-NAME increased Y-27632 responsiveness in the OVX+T group; L-NAME had only a partial effect in controls, whilst it did not modulate Y-27632 responsiveness in OVX. Finally, stimulation of rSMCs with SNP significantly increased RhoGDI protein expression, an effect counteracted by ODQ and, partially, by the protein kinase inhibitor KT5823 incubation.

In conclusion, in vagina, androgen administration in OVX functionally decreases RhoA/ROCK activity by hampering RhoA membrane translocation and plays a critical role in the inhibitory mechanism of the smooth muscle distal vagina contractility. Accordingly, androgens, by inhibiting the RhoA/ROCK pathway, could positively contribute to vaginal smooth muscle relaxation, favoring the sexual intercourse.

**PO49 | PELP1 and SRC as key factors participating in the ESR1-mediated pathway in human testis and epididymis**

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**Background:** The effect of estrogens on the male reproductive system is well characterized in many studies. However, as estrogens are pleiotropic hormones, their transduction signaling pathways can be modulated or modulate various cellular processes. Proline-, glutamic acid-, and leucine-rich protein 1 (PELP1) is among the proteins engaged in the estrogen social network.

**Tissue samples:** Twenty-six tissue samples obtained from thirteen organ donors were used in this study.

**Materials and Methods:** The mRNA levels of both estrogen receptors (ESR1 and ESR2) and their coregulators, including PELP1 and SRC kinase, were analyzed using quantitative PCR. The protein presence was confirmed by western blot. The cellular localization was determined with the use of immunocytochemistry.

**Results:** The expression of both SRC and PELP1 was significantly lower in the epididymis when compared to the testis (p < 0.05). Both genes were significantly, strongly, and positively correlated (p < 0.0001, R = 0.78) regardless of the tissue type.

In testis and epididymis, both gene expression levels were significant and positively correlated (R = 0.66, p = 0.014, and R = 0.80, p = 0.0019, respectively). Moreover, in the testis PELP1 level positively correlated with the ESR1 level (R = 0.6; p = 0.0367). No significant correlation was observed in the epididymis (p > 0.05).

**Conclusion:** Our study suggests a key role of both PELP1 and SRC in the ESR1-mediated pathway in human testis and epididymis.

**Keywords:** estrogen receptors (ESR1 and ESR2); proline-, glutamic acid-, and leucine-rich protein 1 (PELP1); proto-oncogene tyrosine-protein kinase c-SRC (SRC); estrogen signal transduction coregulators; steroid hormones; human testis; human epididymis

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**PO50 | Characterization of lung impairment related to metabolic syndrome and protective effect of testosterone: an in vivo study in an animal model**

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Metabolic syndrome (Mets) is characterized by the co-presence of multiple physiopathological conditions including the onset, in the male population, of hypogonadotropic hypogonadism, characterized by low levels of testosterone (T) and gonadotropins. Recent studies pointed out a correlation between Mets and decreased lung function, although the mechanism underlying this effect remains unknown. A potential cause that could contribute to the link between Mets and the decline in lung function is represented by systemic and tissue-specific inflammation induced by obesity, the paramount marker of Mets.

In order to investigate the mechanisms determining the onset of lung dysfunction, we used a non-genomic animal model of Mets, which recapitulates the main characteristics of the human Mets phenotype, obtained by exposing rabbits to a high-fat diet (HFD).

Previous studies have shown how T is able to exert powerful anti-inflammatory effects on different organs in this experimental model and the potential protective action of this hormone on Mets-induced pulmonary alterations has been investigated.

In this study, we performed functional, biomolecular, and histomorphological analysis on lung samples isolated from control group (RD, regular diet, 12 weeks) animals or rabbits fed with HFD (standard diet supplemented with 0.5% cholesterol and 4.0% peanut oil, 12 weeks), alone or in combination with T for the last 6 weeks (HFD + T6W group). The inflammatory and pulmonary tissue remodeling was analyzed by immunohistochemical techniques (Picrosirius Red assay for collagen deposition) and molecular analysis (semi-quantitative RT-PCR). Spirometry was used to assess the resistance to airway ventilation (pressure of the airway opening, PAO), a functional parameter related to the reduced pulmonary compliance.

At the end of treatment, the results showed the presence of a clear impairment of lung function in HFD rabbits, demonstrated by the significant increase in PAO compared to RD. Accordingly,
immunohistochemical and molecular analysis confirmed the presence of a significant increase in pro-inflammatory and pro-fibrotic status in the lungs of HFD animals. T administration significantly improved not only some metabolic parameters but also lung ventilation, compared to the HFD group, while also counteracting pro-inflammatory macrophage activation and peribronchiolar fibrosis. The gene expression analysis of the main inflammation and fibrotic markers confirmed a positive effect of treatment with T.

This study clearly shows the validity of the experimental model employed for the development of inflammation and pulmonary fibrosis, associated with metabolic disease, and the preliminary results highlight how treatment with T is able to mediate anti-inflammatory and antifibrotic effects in this model.

P051 Does Honeybee work for human male health? ‘The effect of CAPE on erectile function’

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Erectile dysfunction (ED) is the inability to reach and maintain an erection necessary for sexual performance (1). The oxidative stress is one of the main reasons for ED (2). Hydrogen sulfide (H2S), the third gasotransmitter, have antioxidant and relaxant effects and plays a role in penile erection. H2S donors and H2S synthesis enzymes (CBS and CSE) substrate L-cysteine causes relaxation in penis corpus cavernosum dose dependently. A flavonoid isolated from honeybee propolis Caffeic acid phenethyl ester (CAPE) has also antioxidant, antiinflammatory and dose-dependent vasodilator effects in the aorta and coronary arteries just like H2S (3,4). CAPE increases expression of the CSE enzyme in fibrotic liver tissue (5). Preincubation of spermatozoa with the CAPE protects against oxidative DNA damage in vitro (6). However, the relaxant effect of CAPE in penile tissue and the role of H2S has not been investigated yet. Thus, we investigated the effect of CAPE and the role of H2S in its possible effect in erectile function and dysfunction.

Strip myograph (DMT) were used in all experiment to get contraction relaxation responses in mice strips. ANOVA was used in all statistical analyses (n = 5). Endothelial integrity was tested in all strips by more than 40% relaxation to Ach. CAPE and L-cysteine concentration response curve were obtained in phe-precontracted (30μM) strips in the presence or absence of aminoxyacetic acid (AOAA,10mM), H2S synthesis inhibitor. CAPE (10-4-10-3M) caused concentration dependent relaxation in mice penile (p < 0.001) and AOAA inhibited these relaxations significantly. Besides CAPE (10 μM) increased L-cysteine-induced endogenous H2S dependent relaxation in healthy mice penile tissue (p < 0.01) and this augmentation is inhibited by AOAA. Thus, for the first time we showed that CAPE caused relaxation in penile tissue through H2S.

Beside the effects of endogenous H2S-induced relaxations in mice penile tissue in oxidative stress induced by pyrogallol (100μM) in control and CAPE-incubated penile strips. Pyrogallol, decreased L-cysteine-induced relaxations significantly (p < 0.001). CAPE ameliorated impairment of endogenous H2S dependent relaxations in oxidative stress in mice penis (p < 0.001) and this beneficial effect of CAPE were reversed back by AOAA. These results demonstrate that oxidative stress reduced endogenous H2S-induced relaxations and CAPE ameliorates impaired relaxations through H2S.

Our study suggest that CAPE may contribute to erectile function and could be protective effects on erectile dysfunction under oxidative stress through H2S and targeting endogenous H2S pathway may prevent ED associated with oxidative stress.

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P052 Molecular mechanisms underlying bisphenol A-associated decline in human sperm quality

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Every day we are exposed to environmental chemicals that affect our endocrine system commonly called endocrine disruptors, such as bisphenol A (BPA). BPA leaks from polycarbonate plastics used to line food and drink containers and seems to negatively affect male fertility. High urine and seminal plasma BPA levels have been associated with low sperm quality and production, and paternal exposure may contribute to diseases in their offspring. However, only a few studies reported the deregulation of sperm proteins or RNAs associated with this risk factor for male infertility. This work aimed to investigate the correlation between seminal plasma BPA concentration and seminal quality in Portuguese men living in the Aveiro region. Additionally, we intended to study the alterations in human sperm proteins and small non-coding RNAs to recognize potential biological markers of exposure, sperm quality decline and poor fertility to be used to diagnose and manage male infertility. To do so, 102 Portuguese men from the Aveiro region (Portugal), aged between 19 and 56 years old were included in this study. Basic semen analyses were performed...
Effects of lifestyle and mercury exposure on male reproductive health: a cross-sectional study

Over the past few decades, there has been increasing evidence on the global decline in male reproductive health. Many studies have reported that lifestyle factors (e.g., alcohol, cigarette and drugs consumption) and exposure to environmental pollutants (e.g., mercury (Hg) and bisphenol A) may affect the male reproductive function and consequently, decrease the semen quality. Hg is one of the most prevalent contaminants, generated by anthropogenic activities such as industrial, pharmaceutical, and agricultural activities. Increased Hg levels were associated with male infertility or subfertility status, increased sperm DNA damage and abnormal sperm morphology and motility. Several studies showed that infertile subjects with unexplained infertility had higher levels of Hg in hair, blood, and urine than fertile ones. However, the molecular mechanisms underlying the effects of exposure to Hg and lifestyle on male fertility decline remains unknown.

Thus, the main goals of this cross-sectional study were to: i) assess male exposure to Hg in the Aveiro region using hair as non-invasive biological matrix; ii) examine the influence of variables that may contribute to Hg exposure; and iii) study the impact of Hg exposure and lifestyle on the male reproductive health. For that, the study was carried out in thirty eligible men who attended the Urology service at Centro Hospitalar do Baixo Vouga (located in Aveiro). A detailed questionnaire regarding sociodemographic, diet, lifestyle and reproductive data was completed by participants. Samples of semen, hair, and urine were collected in the normal setting of the hospital from participants. Semen samples were analysed according to the World Health Organization criteria by experienced technicians and spermatozoa extracts were performed. Total Hg (THg) levels were quantified in hair samples by atomic absorption spectrometry after thermal decomposition of the sample using the Advanced Mercury Analyzer (AMA-254, LECO). According to hair THg levels, participants were divided into three groups and urine metabolomic profiling was performed by nuclear magnetic resonance (NMR) spectrometry.

This study demonstrated Hg bioaccumulation in biological samples from participants living in the Aveiro region: 47% of all individuals had THg levels higher than Hg levels considered acceptable by United States Environmental Protection Agency (US EPA) (1000 ng/g) and 20% presented THg levels higher than Hg levels considered acceptable by WHO (2000 ng/g). Moreover, significant positive correlations between THg levels in hair and percentage of tail defects and THg levels and teratozoospermia index were found. Thus, our results yielded additional information for conducting Hg risk assessment for the male reproductive health. Further and continuous monitoring of Hg exposure and male lifestyle behaviour should be required in order to prevent possible adverse effects in male reproduction.

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ABSTRACT

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Over the past few decades, there has been increasing evidence on the global decline in male reproductive health. Many studies have reported that lifestyle factors (e.g., alcohol, cigarette and drugs consumption) and exposure to environmental pollutants (e.g., mercury (Hg) and bisphenol A) may affect the male reproductive function and consequently, decrease the semen quality. Hg is one of the most prevalent contaminants, generated by anthropogenic activities such as industrial, pharmaceutical, and agricultural activities. Increased Hg levels were associated with male infertility or subfertility status, increased sperm DNA damage and abnormal sperm morphology and motility. Several studies showed that infertile subjects with unexplained infertility had higher levels of Hg in hair, blood, and urine than fertile ones. However, the molecular mechanisms underlying the effects of exposure to Hg and lifestyle on male fertility decline remains unknown.

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ANDROLOGY

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Epigenetic alterations in retrotransposable elements and the dysfunction in their silencing pathways in male germ cells have been recently associated with severe form of male infertility (e.g. azospermia). In addition, possible alterations of these elements in male germ cells can be transmitted to the next generation with an effect on the offspring’s phenotype. Retrotransposon silencing by DNA methylation has been extensively studied in mouse spermatogenesis, but limited achievements are observed in humans, due to the rarity of the appropriate samples.

The aim of this study was to investigate overall LINE-1 DNA methylation in human foetal testis samples which span mid- and late-gestation periods in relation to cell cycling activity, as it was predicted in rodents that DNA methylation takes place in the mitotically quiescent cells. Hence, RB1 gene methylation in human tissues along with the distribution of tumor suppressor protein pRB1 and its phosphorylated forms in relation to the proliferation marker Ki-67.

DNA methylation was studied in FFPE samples of human foetal testis using the sequencing-by-synthesis approach (e.g. pyrosequencing) which allows accurate quantification of the examined CpG sites. The protein expression was studied by immunofluorescence while also quantified on the immunohistochemically stained FFPE sections.

We observed statistically significant LINE-1 DNA methylation increase in mid-gestation period while also a slight reduction at the beginning of puberty. RB promoter region was hypomethylated until the end of pregnancy. Regarding the cell cycle, Ki-67 expression in testicular tubules showed a significant decline in the 2nd and 3rd trimester, with the highest decrease in 20-21w. Interestingly, pRB expression also decreased until the end of pregnancy, but with value oscillations.

We next searched for more specific expression of RB-phosphorylated forms, as phosphorylation of its carboxyl-terminal domain at S780 and S795 directly inhibits RB association with E2F. First, we showed that the number of pRBser780-positive cells per tubule was significantly reduced in mid-gestation period (20w), but later oscillates with the increasing trend. pRBser795-positive cells followed the similar pattern of expression as previous form, such as downturn in the mid-gestation (20w) period.

Although more research is needed, it seems that, in humans, epigenetic silencing of L1 retrotransposon occurs during mid-gestation period and is coupled with the changes in the activity of cell cycle regulators, such as RB protein and Ki-67. More work on these events is needed to clearly define the sensitive periods of testis development in humans, whose perturbations might lead to infertility or germ cell cancer later in life.
The etiopathogenesis of male infertility is a multifactorial medical problem and is correlated with many congenital and acquired defects of the urogenital tract, cancers, urogenital infections, heat stress in the scrotum, hormonal disorders, genetic abnormalities and immunological factors. It is estimated that approximately 30–50% of male infertility cases are recognized as idiopathic, very often associated with low-quality of spermatozoa. On the other hand, unexplained infertility (couples where male patients have normal basic semen parameters and female patients have normal ovulation and fallopian tube potency) is diagnosed in approximately 15% of cases. Therefore, the comprehensive evaluation of male fertility status should be developed using scrotal ultrasonography (USG) and assessment of the key reproductive hormone as well as advanced seminological tests.

Our study was designed to clarify the relationship between standard semen parameters, testicular volume, levels of reproductive hormones and the fragmentation of sperm nuclear DNA (SDF) in a group of fertile and infertile participants.

Patients (n = 130) were clustered as subjects: 1) with an abnormal volume (ultrasonography) of at least one testis (<12 mL) or with a normal volume of testes and 2) with abnormal levels of at least one of the reproductive hormones (FSH, LH, PRL, TSH, total T – electrochemiluminescence method) or with normal hormonal profiles and 3) with high level of SDF (>30%), moderate (>15–30%) or low (≤15%) (sperm chromatin dispersion test).

In subjects with a decreased testicular volume (vs. subjects with normal testicular volume) and in subjects with abnormal levels of reproductive hormones (vs. subjects with normal levels of hormones), decreased basic semen parameters (sperm count, morphology and progressive motility) were found. Additionally, participants with abnormal testicular volume had a higher percentage of SDF (medians: 27.00% vs. 17.00%) and a higher level of FSH (medians: 8.05 mIU/mL vs. 5.29 mIU/mL). In turn, men with a high level of SDF had lower testicular volume (left testis volume – medians: 13.00 mL vs. 16.00 mL; right testis volume – medians: 12.00 mL vs. 16.00 mL) and conventional sperm parameters (sperm count, morphology, progressive motility, total motility and vitality) than men with a low level of SDF. On the other hand, there were no significant differences between men with SDF >30% and men with SDF >15–30% in any study parameters. Analysis of the Spearman’s rank correlation coefficient showed negative relationship between SDF and sperm count, morphology, motility, vitality as well as with volume of the left testis. Moreover left and right testis volumes were negatively correlated with the level of FSH and positively with sperm count. Additionally, volume of left testis positively correlated with sperm progressive motility. Furthermore, the LH level was negatively correlated with sperm count.
Due to the heterogeneous distribution of seminiferous tubules in the testes of patients with non-obstructive azoospermia (NOA), retrieving enough good quality sperm for ICSI may require a complete testicular dissection. According to the only available study in this field, sperm may be found in the testis surface in 34.2% of patients, while a deeper testicular dissection is able to provide sperm for ICSI in 28% of those without sperm in the testis surface. The present study sought to determine the probability of finding enough sperm for ICSI at the initial wide incision of the testis in another cohort of patients with NOA undergoing microdissection testicular sperm extraction (mTESE).

We retrospectively evaluated 276 patients, aged 37 (20-62) years, who underwent unilateral (86; 31.2%) or bilateral (190; 68.8%) mTESE from January 2018 through December 2021. During mTESE the entire surface of the testicular parenchyma was explored first in search for dilated seminiferous tubules: if no/ not enough sperm were retrieved, the deeper portion of the testicular parenchyma was explored. Histopathology demonstrated Sertoli-cell only syndrome in 65.6% of operated testes, while maturation arrest was found in 19.5%, hypospermatogenesis in 12.7% and hyalinosis in 2%.

Sperm was retrieved in 137 patients (49.6%). Sperm were obtained at the initial wide incision in 46 out of 174 testes (26.4%) and only in patients who underwent unilateral mTESE, with a consequent probability of 16.6% in the whole cohort of patients with NOA (46/276 patients). In the remaining patients (91, 66.4% of those with SSR, 32.9% of the whole cohort), a deeper testicular dissection was required to obtain enough good quality sperm for ICSI: in some of them sperm were even found at the initial wide incision, but their number and/or quality was not sufficient for ICSI. On multivariate logistic regression, only the histopathological subcategory hypospermatogenesis was predictive of the chance of retrieving sperm from the surface of the testis (OR 3.24, 95% CI 1.37-7.69, p = 0.007).

The results of the present study suggest that most patients with NOA, particularly those with unfavorable histopathological patterns (such as Sertoli-cell only syndrome or maturation arrest), require a complete dissection of the testicular parenchyma to obtain enough good quality for ICSI. Since mTESE enables the complete exploration of the testicular parenchyma, it is to be preferred to conventional TESE, which allows the retrieval of the seminiferous tubules from the testis surface only, to retrieve sperm in patients with NOA.

**Conclusions:** The present study identified clinical conditions in which it might be appropriate to supplement standard seminal analysis with testing for IgG-ASA. Further studies are needed to clarify the pathogenetic mechanisms underlying the revealed associations and to produce an external validation of our predictive model.

**Materials and Methods:**

We retrospectively evaluated 2712 consecutive men who attended our university/hospital andrology clinic for the evaluation of fertility potential was carried out. Immunological screening with the IgG-MAR test had been performed on all ejaculated. Clinical data were retrieved from medical records.

**Results:** An IgG-MAR test positivity between 10% to 49% and ≥50% was found in 30 (1.1%) and 199 men (7.3%), respectively. Of all the possible information reported in the medical history, when compared to ASA-negative group (n = 2483), men with ≥50% IgG-MAR test positivity more frequently reported a history of ureaplasma urealyticum infection (2.5% vs. 0.6%, p = 0.009), post-pubertal parotitis (4.0% vs. 1.4%, p = 0.011), hernioplasty (7.0% vs. 2.3%, p = 0.0001) and hemmorhoidectomy (1.0% vs. 0.1, p = 0.04). At the multiple logistic regression analysis, all these variables were independently associated with IgG-ASA occurrence. A nomogram was then constructed that could predict the probability of IgG-ASA occurrence as a function of the different combinations of the four independent predictors identified at the multiple logistic regression. The variable with highest predictive power was hemmorhoidectomy surgery (OR:7.4; 95%CI: 1-46.8), followed by ureaplasma urealyticum infection (OR:4.29; 95%CI: 1.37; 11.3), hemmorhoidectomy (OR:3.2; 95%CI: 1.7-5.8) and post-pubertal parotitis (OR:2.8; 95%CI: 1.2-5.8). The internal calibration plot revealed a significant agreement degree between the predicted and observed probability.

**Conclusions:** The present study identified clinical conditions in which it might be appropriate to supplement standard seminal analysis with testing for IgG-ASA. Further studies are needed to clarify the pathogenetic mechanisms underlying the revealed associations and to produce an external validation of our predictive model.
P060 | Unsupervised datamining of orchiopexy surgical reports for unilateral cryptorchidism reveals unnoticed profiles of genital anomalies

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Introduction: Cryptorchidism is the most common birth defect in newborn boys and the current standard therapy for undescended testes is inguinal or scrotal orchiopexy. This surgical procedure provides an accurate insight of anatomical variations and anomalies of the male genital tract. Analysis of cryptorchidism subphenotypes that comprise these associated anomalies may lead to a better recognition of disease heterogeneity, a deeper understanding of risk factors roles and a better further follow-up for cryptorchid boys.

Patients and Methods: A final study population of 422 boys with unilateral cryptorchidism was gathered and a set of nine data were extracted from orchiopexy reports. An unsupervised clustering was performed using the Gower distance function and the partition around medoids (available in R). We defined clusters number through silhouette analysis and reported graphical and statistical descriptions for each cluster.

Results: Median age of orchiopexy was 46.8 months. Undescended testes were 52.4% on the right side, 65.3% in the external ring, 31.4% within the inguinal canal and 3.3% in an intra-abdominal position. Amongst them, 29.1% presented a small size and 32.2% an abnormal epididymo-testis connexion. According to these variables, the Gower algorithm identified 10 different clusters of boys. Statistical and graphical analysis confirmed that these anomalies were significantly associated with higher testes’ positions (i.e. abdominal and intracanal position vs external ring). However, for testes at the external ring, the frequency of anomalies was significantly higher when the peritoneal-vaginal duct remained open i.e. testis size was more often smaller and the epididymis more often disconnected (respectively 33.1% vs 13.6%, p < 0.001 and 33.1 vs 14.3%, p < 0.001). To date, this finding has not been reported and quantified. It raises the question of the link between peritoneal-vaginal duct patency, testes anomalies and migration and distinguishes an unnoticed group at risk amongst cryptorchid boys.

Conclusion: This study confirms the effectiveness of Gower algorithm to mine orchiopexy surgical data and reveals an anatomical pattern that opens new pathophysiological and clinical perspectives.

P061 | Features of Leydig cells in patients with non-obstructive azoospermia

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Non-obstructive azoospermia (NOA) is a form of male infertility characterized by disorders of the testicular parenchyma and impaired spermatogenesis. This study aimed to investigate the nature of Leydig cell changes in patients with NOA, especially whether their actual proliferation occurred. The study included a total of 72 patients with azoospermia. 24 patients (control group) were diagnosed with obstructive (OB) and 48 with non-obstructive azoospermia. Qualitative histological analysis of the control group of patients (men with obstructive azoospermia and fully preserved morphology of the testicular parenchyma) and biopsies of patients with NOA showed a significant difference in the degree of preservation of spermatogenesis and morphology of Leydig cells. In the group of infertile men with NOA Leydig cells sometimes displayed an abundant cytoplasm and were organized into larger clusters. However, in some samples from patients with NOA, significant fibrosis of the interstitial compartment has been demonstrated, often with the presence of inflammatory cells (mononuclear leukocytes). The results of the stereological analysis showed that there was no increase in the number of Leydig cells; on the contrary, the comparison of the examined groups of patients showed a slight decrease in their number in the biopsy samples of patients with NOA. This decrease in the number of Leydig cells can be explained by previous inflammatory changes within the testicular interstitium that cause consequent interstitial fibrosis.

P062 | Sperm retrieval using mTESE in patients with Klinefelter syndrome and azoospermia

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Introduction: Azoospermia is caused by genetic abnormalities occur up to 25%. Klinefelter syndrome (KS) is the most frequent sex chromosomal aneuploidy, occurring in 1-2/1,000 live male births. Less than 50% of individuals with KS are diagnosed in their lifetime. KS is 13.7% in the azoospermic group. Sperm obtaining and inclusion in reproduction men with KS is still a controversial topic in the fertility clinic. The second problem is the protection of fertility in adolescents with KS, based on knowledge of present complete spermatogenesis in a younger group of patients.

Objectives: Analysis results of mTESE in patients with Klinefelter Syndrome and azoospermia.

Materials and Methods: Based on our experience (from 2012 – 467 mTESE), during 2017-2022 36 patients with KS 47.XXY / per total
Human spermatozoa express TNF-a receptors

321 mTESE (11.2%) had testicular biopsy mTESE after the diagnosis of azoospermia, according to EAU Guidelines. In 6 patients additional genetic abnormalities were noticed: 3 (CFTR gene): IVS8-5T + (TG)11 / -; IVS8(TG)13(TJ) / -; delFS08 / - and 3 (karyotype): 47,XXY,9qh-.21ps+; 47,XXY[4]/46,XY[46]; 47,XXY[23]/46,XX[9]. Patients were divided into 2 groups: 1) childless couple 28/36 (77.8%), age 25-42 years (mean 33); 2) fertility protection 19/36 (22.2%), age 16-22 years (mean 18.5). Before the mTESE, hormone therapy was given to normalize LH, FSH and testosterone levels, as a known positive correlation factors in sperm obtaining. All mTESE procedures were done as the one-day-surgery protocol, under general anesthesia, with antibiotic prophylaxis. The Leica M860 2x2 microscope was used, magnification 20-25 x. The collected specimens (3 from each testes) were placed in Bouin’s fluid for standard histopathological evaluation by pathologist according to the Johnsen score (1970). At the same time, the testicular tissue was transferred to the IVF LAB for cryopreservation, for future IVF/ICSI. No significant surgical and anesthetic complications after surgery were noted.

Results: The childless couple group (28): tubules with only few spermatozoa (<5-10) – 2/28 (7.1%). In others: Sertoli Cell Only Syndrome – 19/28 (67.9%); Maturation Arrest 7/28 (25%) : spermatogonia – 2; only few (<5) spermatocytes – 1; several or many spermatocytes – 1; only few spermatids (<5-10) – 2; many spermatids – 1.

The fertility protection in adolescents group (8): tubules with sperm – 2/8 (25%): only few (1) and only few/many (1). In others: Sertoli Cell Only Syndrome – 4/8 (50%); Maturation Arrest 2/8 (25%): only few spermatids (<5-10) – 2; many spermatids – 1.

In all cases, hyperplasia of Leydig cells with the formation of nodules (Leydigoma) were recorded, without any signs of malignancy. In 1 patient (17 years), in the left gonad intratubular pre-invasive neoplasm Germ Cell Neoplasia In Situ (GCNIS) was diagnosed and unilateral orchietomy was done.

Conclusion: A group of patients with KS can be included in reproduction based on sperm obtaining using mTESE. In the fertility protection adolescent group, the histological findings are better than in the group of older men from childless couple, so this is suggestion that fertility protection should be proposed immediately after the diagnosis of KS. According to high risk of neoplasm oncological evaluation of testicular specimens should be routine.

Human spermatozoa express TNF-a receptors

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Cytokine concentration such as TNF-a in human seminal plasma has been connected to male subfertility. However, only little is known about the expression of respective on spermatozoa. Thus, the aim of this study was to evaluate expression of TNF-a receptor 1 (TNFαR1) and TNF-a receptor 2 (TNFαR2) on human spermatozoa from patients referring to andrology department for semen analysis (n = 72) by immunofluorescence and flow cytometry. In this context TNFαR1 (10.88% +/- SD 5.58) and TNFαR2 (30.31% +/- SD 12.34) could be detected by flow cytometry on the surface of human spermatozoa. Immunofluorescence staining demonstrated that both receptors were expressed on the mid-piece and post-acrosomal segment. TNFαR1 and TNFαR2 expression did not correlate to TNF-a concentration in seminal plasma. However, significant correlation of TNFαR1 and TNFαR2 expression could be demonstrated to spermatozoa count and concentration while TNF-a concentration in seminal plasma inversely correlated to motility. Activation of TNFαR1 and TNFαR2 on spermatozoa led to an increase of apoptosis in spermatozoa. In conclusion, human spermatozoa express TNFαR1 and TNFαR2. Its activation might be involved in altered spermatozoa function.

The real-time elastography can predict sperm retrieval in non-obstructive azoosperma

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Background: In non-obstructive azoospermia (NOA), surgical sperm retrieval (SSR) success rate of testicular sperm extraction (TESE) did not exceed 50%. Unfortunately, available “pre-surgical” parameters exhibit a poor predictive value. The real-time elastography (RTE) is an ultrasonography (US) technology assessing the mechanical elasticity of tissues. In the hypothesis that a higher elasticity could reflect a wider preserved functional testicular parenchyma, we evaluated the potential role of RTE in predicting SSR in NOA patients undergoing microdissection TESE (mTESE).

Patients and Methods: 30 consecutive infertile patients attending our Andrology Unit for azoospermia underwent mTESE. Conventional grey-scale US and RTE were performed using an Esaote MyLab alpha US machine, equipped with the software ElaXto® for RTE (Esaote S.p.A., Florence, Italy). RTE images were obtained on the largest longitudinal scan to get a representative picture of the whole parenchyma elasticity: the colour mapping scale included blue (stiff tissue), green (intermediate strain), and red (soft tissue). To quantify the percentage of different colour shades, we used the software ImageJ®, providing, for each RTE image, the green to blue ratio (G/B ratio) as a global measure of tissue elasticity.
**ABSTRACT**

**P066** | **Testosterone serum levels are related to sperm DNA fragmentation index reduction after FSH administration in males with idiopathic infertility**

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**Results:** Six patients (20% of the study population) received a diagnosis of obstructive azoospermia (OA), where, as expected, mTESE resulted in successful SSR in all cases. When compared to NOA, the group with OA exhibited significantly lower levels of FSH, LH, ejaculate volume and seminal pH, as well as a significantly higher US testicular volume. In OA series, the histological confirmation of preserved spermatogenesis allowed to define the “normality features” of testicular RTE images. Quantitative analysis revealed a prevalence of green (median: 55.2%) over blue pixels (29.6%) with a G/B ratio regularly ≥1.5. In the series of 24 patients with NOA, testicular histopathology revealed different spermatogenesis disorders, included hypospermatogenesis, meiotic arrests, Sertoli Cell-Only Syndrome and scleroyalinosis. In the NOA group, 15 patients (62.5%) yielded successful SSR. They exhibited RTE images similar to those observed in OA. Whereas, in the 9 cases where mTESE yielded negative SSR, RTE images predominantly displayed centripetal, blue-colored pixels with a low G/B ratio, in no case higher than 1.4 (median: 0.78, range: 0.5-1.4). When compared to NOA patients without SSR, those yielding successful SSR exhibited RTE images with significantly lower percentages of blue pixels (42.1 ± 12.0% vs. 52.7 ± 6.6%, p = 0.01) and higher G/B ratio (1.54 ± 1.49% vs. 0.78 ± 0.26%, p = 0.02). No significant difference was found in endocrine, seminal, and conventional US parameters.

**Conclusions:** In our series of patients with NOA, quantitative analysis of testicular RTE images was demonstrated to be a non-invasive, easily applicable, and repeatable method to predict SSR at mTESE. In particular, a G/B ratio higher than 1.4 excluded an unsuccessful SSR.

**P067** | **Real-world evidence of follicle stimulating hormone effectiveness in male idiopathic infertility**

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**Background:** Testicular overstimulation is the pursued therapeutic goal when exogenous follicle stimulating hormone (FSH) is empirically administered to men with idiopathic infertility. Although a robust physiological rationale supports the FSH use in male idiopathic infertility, useful biomarkers to monitor and evaluate its efficacy are still far from being detected. While pregnancy rate remains the strongest outcome in couple infertility management, the identification of reliable and possibly early biomarkers of therapeutic response to FSH in males is mandatory. Sperm DNA fragmentation (sDF) index, even if supported by many evidences, is not yet recognized as valid endpoint of hormonal treatment efficacy. Moreover, no suitable biomarkers of FSH effectiveness during the therapy are available.

**Aim:** The primary aim of the study was to evaluate if testosterone serum levels are related to sDF change after FSH administration. The secondary aim was to confirm the sDF index validity as biomarker of FSH administration effectiveness in male partners of infertile couples using a prospective, controlled dataset.

**Methods:** A retrospective post-hoc re-analysis was performed on raw data of clinical trials in which idiopathic infertile men were treated with FSH and both testosterone serum levels and sDF were reported among primary and/or secondary endpoints. Additional data regarding couple infertility history, age, anthropometric variables, FSH treatment scheme and semen parameters were included in a single dataset. Logistic regression analysis was performed using responders to FSH treatment as dependent variable and all parameters detected after FSH administration as cofactors/covariates.

**Results:** Three trials were included accounting for 251 patients (median age 35, 28-59 years). The comprehensive analysis confirmed the FSH beneficial effect on spermatogenesis detected in each single trial. Indeed, increasing the sample size, this re-analysis highlighted an overall significant sDF decrease (p < 0.001) of 20.2% of baseline value. Although sDF resulted not related to testosterone serum levels at baseline, a significant correlation was highlighted after three months of FSH treatment (p = 0.002). Moreover, testosterone serum levels and patients’ age significantly correlated with sDF (p = 0.006). Dividing the cohort in responders/not responders to FSH treatment according to sDF change, the FSH effectiveness in terms of sDF improvement was related to testosterone and sex hormone binding globulin serum levels (p = 0.003).

**Conclusion:** The re-analysis of published trials data investigating FSH administration in male idiopathic infertility highlights the beneficial effect of exogenous FSH stimulation with a 20% relative decrease of baseline sDF index. In terms of sDF reduction, a 59.2% of FSH-responders was here detected. Although sDF index does not correlate with testosterone serum levels at baseline, after three months of FSH administration a significant inverse correlation appears, suggesting an association between the FSH administration-related sDF improvement and testosterone serum levels increase. This is the first time in which a communication/interaction between the two cell compartments of the testis (i.e. Sertoli and Leydig cells) can be hypothesized in response to FSH administration.
ABSTRACT

A novel diagnostic test to identify patients suffering from loss of CatSper function

Background: Exogenous follicle-stimulating hormone (FSH) administration in male idiopathic infertility showed the most convincing physiological rationale in the face of a clinical efficacy below expectations. Accordingly, it was calculated that 10 to 18 men have to be treated with FSH to achieve one pregnancy.

Aim of the study: The aim of the study is to assess the effectiveness of FSH administration in male idiopathic infertility in a real-world clinical setting.

Materials and Methods: A retrospective real-world study was carried out, including all consecutive male partners of infertile couples attending the Andrology Unit of Modena (Italy) from June 2015 to May 2022. Medical history, physical and andrological examinations, hormonal and seminal parameters, therapeutic management and pregnancy data were collected. Primary endpoints were semen parameters, while the number of pregnancies was the secondary outcome.

Results: 197 on 362 (54.4%) infertile men were treated with FSH (mean age 37.9 ± 6.1 years). After FSH administration (therapy duration 9.1 ± 7.1 months), a significant increase in sperm concentration (9.9 ± 12.2 versus 18.9 ± 38.9 million/mL, p = 0.045) was detected. Also, treatment led to a significant increase in normozoospermia (from 1.0 to 4.8%, p = 0.044) and decrease in azoospermia rate (from 9.6 to 6.5%, p = 0.044). Forty-three pregnancies were recorded (30.5%), 22 spontaneous and 21 after assisted reproduction. Dividing the cohort in FSH-responders and non-responders considering obtaining or not a pregnancy, a higher sperm concentration (15.7 ± 26.6 versus 22.2 ± 25.7 million/mL, p = 0.033) and progressive sperm motility (18.0 ± 18.2 versus 27.3 ± 11.3, p = 0.044) were found in pregnancy group.

Conclusion: Our experience suggests that FSH empirically administered to men with idiopathic infertility improves sperm concentration and leads to pregnancy in 1 of 5 patients. Although the expected limits due to a real-world data study, the number of FSH-treated patients required to achieve a pregnancy seems to be lower if compared to previously published data.

There is a growing body of evidence that human sperm require the activity of the sperm-specific Ca2+ channel CatSper (cation channel of sperm) to fertilize the egg. Therefore, we developed the prototype of a novel in vitro diagnostic test to assess the function of CatSper in sperm from patients undergoing semen analysis. Using this “CatSper-Activity-Test”, we identified in a cohort of 576 men seeking medical advice for suspected male infertility seven patients suffering from a loss of CatSper function. According to standard semen analysis, the CatSper-deficient patients are (with one exception) normozoospermic, feature no other pathological condition, and were diagnosed with unexplained infertility. Most notably, their sperm not only failed to fertilize the egg naturally but also upon intrauterine insemination (IUI) and in vitro fertilization (IVF), whereas intra-cytoplasmic sperm injection (ICSI) was successful. Two additional CatSper-deficient patients were identified among patients visiting our clinics for other reasons. We show that the loss of CatSper function is predominantly caused by a homozygous deletion of the CATSPER2 gene; one patient featured compound heterozygous variants of the CATSPERE gene.

The CatSper-deficient sperm from these patients were characterized using a battery of techniques, including motility analysis, electrophysiology, and Ca2+-fluorimetry. We show that CatSper-deficient sperm fail to hyperactivate and, thus, to penetrate the egg coat, explaining the failure of natural conception, IUI, and IVF. In fact, considering the total IVF failure rate of our institute, and given that each of the seven patients would have initially undergone IVF before attempting ICSI, we assume that CatSper-deficiency might account for nearly half of all male-factor IVF failures.

We conclude that loss of CatSper function is a common cause of unexplained male infertility. Affected patients are prone to experience failing cycles of medically assisted reproduction (MAR) using IUI and IVF – if the infertility is identified at all. The novel CatSper-Activity-Test faithfully identifies CatSper-deficient patients with a hands-on-time of only a few minutes and no special equipment or training required. Therefore, we envisage the CatSper-Activity-Test as a novel tool for the early diagnosis of male infertility allowing evidence-based treatment decisions in MAR, thus, sparing patients the burden of unnecessary medical and financial risks.

P068 | A novel diagnostic test to identify patients suffering from loss of CatSper function

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P069 | Epididymal organ sparing microsurgery – Adenomatoid tumors of epididymis

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Introduction: The majority of scrotal tumors are malignant, whereas rare epididymal lesions are slow-growing asymptomatic scrotal masses, mostly benign in nature. In order of frequency adenomatoid tumors of mesothelial origin are the most common (73%), leiomyomas (11%) and cystadenomas (9%) can also occur.
Case presentations: Our first case is a 29-year-old male who presented with a palpable scrotal mass and negative tumor markers. Physical examination revealed a 1 cm in diameter, firm mass at the tail of the epididymis. A 12x8x12 mm diameter, fully demarcated, vascularised, echo-dense area of the right epididymis was shown by scrotal ultrasound without any testicular alteration. The next case was a 49-year-old male with a 1cm size mass on the right side of the scrotum. Ultrasonography revealed a well-defined, vascularized, 10x7x9mm hyper-echogenic lesion on the tail of the epididymis.

In both of the cases microsurgical epididymal tumor resection was performed, where Frozen Section Examination confirmed its benign nature, thus an organ sparing approach became possible. Final histological examinations confirmed adenomatoid epididymal tumors.

Discussion: Only 1 case report in the last 5 years, and totally less than 100 cases of epididymal adenomatoid tumors have been reported in the literature.

Adenomatoid tumor is the most common paratesticular neoplasia in middle-aged males, usually incidental findings, asymptomatic and most commonly located on the tail of the epididymis.

In the case of palpable epididymal masses the correct diagnosis, fertility preservation and avoiding radical surgery have the utmost importance, especially in reproductive-aged patients.

Background & Objectives: There is growing evidence showing that pharmacological agents given to women, before and after conception, may have a negative impact on their fertility and pregnancy outcome. However, such information is much more limited when it comes to the male partner. Most attention has been given to chemo- and radiotherapy, men receiving such treatment being counselled to avoid making their partner pregnant during the first 6 post-treatment months. Hypertension and antihypertensive treatment (AHT) are common even among young adults and due to delayed parenthood, in many parts of the world, one can assume the incidence of hypertension in fathers might also increase.

The objective of this study is to examine the association between paternal use of AHT and adverse outcome of the offspring.

Materials and Methods: This study was based on the use of Swedish national registries. The characteristics of all children born alive in Sweden 2006-2014 and their parents were extracted from the Swedish Medical Birth Register, which covers more than 99% of all births in Sweden. The data on prescribed medication given to fathers was derived from the Swedish National Prescribed Drug Register. A total number 885,730 children were included. In this cohort a total of 14,886 (1.7%) fathers had dispensed AHT at least one time during the 6 months prior to conception. The odds ratios (OR) of low birth weight (LBW), small for gestation age (SGA), prematurity, low Apgar score (LAS) and major malformations were analyzed using binary logistic regression.

The model was adjusted for parental age and educational level, maternal BMI, smoking-status and parity, and the use of intracytoplasmic sperm injection.

Results: Children to fathers using AHT were more likely to have a LBW (adjusted odds ratio [aOR] 1.14, 95% CI 1.06 to 1.24) and to be SGA (aOR 1.18, 95% CI 1.06 to 1.31) compared to children of fathers without AHT. Children to fathers prescribed only angiotensin-converting enzyme inhibitors (ACEi) were more likely to be with LBW (aOR 1.20, 95% CI 1.00 to 1.44). Children to fathers who were prescribed only beta blockers (BB) were more likely to be SGA (aOR 1.28, 95% CI 1.07 to 1.54).

Conclusions: According to our results there are a higher risk for adverse outcome among children to fathers using AHT in the 6 months prior to conception. This is the first study to establish a link between AHT and adverse outcome of the offspring. However, we cannot differentiate weather the association is due to the AHT or underlying disease. Thus, further research is needed to clarify the causation before clinical recommendations can be given to prospective fathers using AHT.

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analysis was performed, according to the WHO manual (WHO, 2010 & 2021) and the ESHRE-NAFA recommendations. The subjects’ fertility history was also recorded. 

**Results:** 5.06% of the samples exhibited reductive stress, in contrast to 51.8% showing oxidative stress. 43.04% of the examined samples were evaluated at the state of redox balance (values within the reference range, i.e. \(<1.34 \text{ mV/10}^6\text{ spermatozoa})

Men with reductive stress were between 31 and 51 years (median: 43, average: 41.2). 35% of these subjects reported smoking on a daily basis by conventional cigarettes, electronic or vaporizer. 65% reported alcohol consumption 1-4 times per week. 10% of the patients had been on therapy with tamoxifen before the examination, 5% with antibiotics and 5% had used muscle relaxants.

In almost all of the above cases, at least one basic semen parameter was found below the WHO reference limits. Only one sample exhibited all conventional parameters within the reference ranges. Typical morphology was found below the reference limits in all but one of the examined samples (5%), while sperm motility and concentration were found below the reference limits in 69% and 55% of the cases, respectively. Also, 40% of these subjects had previously used antioxidant supplementation.

None of these men reported the achievement of paternity by the time of examination. Interestingly, 40% of them had undergone one or more failed ART attempts, ending to absence of pregnancy or miscarriage.

**Conclusions:** Although oxidative stress is the most studied condition related to sperm redox imbalance, reductive stress may also constitute an etiological factor of male fertility, with negative repercussions on the basic seminal profile. Excessive use of antioxidants can lead to the impairment of oxidative mechanisms which are important for sperm function. Thus, baseline sperm redox levels must be taken into account before antioxidant administration in the context of the therapeutic management of male infertility.

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**P072 | Hormone levels, semen quality and sperm function in unselected young men from Lodz, a city in central Poland**

Renata Walczak-Jedrzejowska, Radoslaw Zajdel, Joanna Jurewicz, Wojciech Hanke, Jolanta Slowikowska-Hilczer

In 1992, Carlsen et al. published a meta-analysis of 61 studies that showed a decrease in sperm concentration in male semen over the past 50 years. Since then, many prospective studies have been conducted around the world to assess the fertility status of young men from the general population, which revealed the presence of geographical differences in semen quality. Thus, the aim of the study was to analyze the semen quality and hormonal profile in young men from general population, inhabitants of Lodz, central Poland.

The study was conducted over a period spanning from December 2015 until March 2018 and 288 men aged 18-30 took part in it. Each participant underwent a physical examination, anthropometric measurements, and provided semen and blood samples. Additionally, participants completed questionnaires on demographic data, current health status, past diseases and lifestyle factors. Semen was tested using the manual method according to the WHO recommendations 2010. Apart from basic semen analysis sperm chromatin dispersion test (SCD) was performed to determine sperm DNA fragmentation and sperm hyaluronan binding test (HBA) – to determined sperm maturity. The concentration of FSH, LH, testosterone, estradiol was determined by the chemiluminescent method, while inhibin B (InhB) by the microimmunoenzymatic method.

Previously diagnosed diseases, not related to reproductive system, were reported by 15% of men. They were mainly allergies (4%), asthma, thyroid disease and hypertension (2% each). The varicocele was observed in 9% of men and 2.8% reported childhood cryptorchidism.

Seventeen percent of participants smoked cigarettes, and 52% consumed alcohol 1-3 times a week. In the last 3 months prior to the study, 44% of participants were taking medications unrelated to the treatment of their underlying disease, 30% were taking vitamins and supplements, 11% - sports nutrition products, 12% - drugs, and 1% - anabolic androgenic steroids. Two percent of the respondents were underweight, 26% were overweight and 5% were obese (1st and 2nd degree).

The vast majority of participants have normal hormone levels. The FSH level was above 7.0 mIU/mL in 2.4% of men while 3.4% of them had it below 1.0 mIU/mL. The LH level was above 8 mIU/mL in 1.7% of participants and below 1.0 mIU/mL in 0.7%. Inhibin B level below 100 pg/mL and testosterone below 8 nmol/L were observed in 6.9% and 1.7% of men, respectively. Only 1 men had estradiol level above 220 pmol/L. According to the WHO 2021 reference values, oligozoospermia was found in 14%, asthenozoospermia - in 6% and teratozoospermia - in 40% of participants. Overall, in 46% of men, at least one of the above parameters was suboptimal. Additionally, 24% of them had abnormal HBA results, and although only 8% had an index of sperm DNA fragmentation above 30%, 38% had it between 15-30%.

The obtained results indicate that a quite high percentage of young men from the general population present suboptimal semen and sperm quality.

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**P073 | New profile of Globozoospermia: Sperm parameters, cytogenetic and molecular investigations**

MB. Ben Khedher, A. Achile, S. Dimassi, S. Mougou, A. Saad M. Gribaa, S. Ibala-Romdhane

In 1992, Carlsen et al. published a meta-analysis of 61 studies that showed a decrease in sperm concentration in male semen over the past 50 years. Since then, many prospective studies have been conducted around the world to assess the fertility status of young men from the general population, which revealed the presence of geographical differences in semen quality. Thus, the aim of the study was to analyze the semen quality and hormonal profile in young men from general population, inhabitants of Lodz, central Poland.
associated with total globozoospermia. It was reported in the literature that mutations in these gene influence the acrosome formation during spermiogenesis, whereas the meiosis was not disturbed. Recently a new SPATA16 mutation was identified in Tunisian patients. This mutation was shown to be related with a specific phenotype of total globozoospermia. In this work, we described the sperm parameters, sperm DNA fragmentation, molecular and cytogenetic findings of this new globozoospermia phenotype.

**Patients and Methods**: Five unrelated Tunisian globozoospermic patients were included. They were recruited during routine infertility treatment at Farhat Hached University Hospital’s Laboratory of Human Cytogenetics, Molecular Genetics, and Reproductive Biology (Sousse, Tunisia). Semen parameters were assessed according to the World Health Organization guidelines. Sperm morphology was assessed according to David’s modified classification (Auger, Eustache, & David, 2000). Sperm DNA fragmentation was evaluated by TUNEL assay. Karyotyping and Yq chromosome microdeletion were performed. Mutation screening was assessed by polymerase chain reaction (PCR) and Sanger sequencing of the SPATA16.

**Results**: Semen analysis revealed severe oligozoospermia (sperm concentrations averaging 3.2 10⁶/ml) and a complete absence of sperm motility (akinezoospermia). Morphology examination indicated total globozoospermia consisting of 100% round headed acrosomeless spermatozoa with a significant presence of double or multiplehead (35%) and multitailed (25%,2%) spermatozoa associated to high index of multiple abnormalities (MAI) with an average of 3.58. They all showed DNA fragmentation indexes higher than 30%, indicating that their spermatic DNA had been altered. All of the patients had normal somatic karyotypes and no Y microdeletions were found. For the molecular study, two patients were found to be homozygous for the new SPATA16 exon 2 deletion; screening for other mutations cases is ongoing.

**Conclusion**: In this study, we report a particular morphological sperm defect (double/- multi-headed and multi-tailed spermatozoa) frequently observed in five globozoospermic Tunisian patients. Two of them, were found homozygous for the rare and newly identified SPATA16 exon 2 deletion. The results confirm again the pathogenicity of SPATA16 mutations in globozoospermia and this anomaly could lead to an abnormal meiosis, explaining this particular phenotype of total globozoospermia.

**Keywords**: globozoospermia, multiple round-headed, multi-tailed spermatozoa, SPATA16.

**P074** | Macrocephalic spermatozoa syndrome: study of a cohort of Tunisian patients

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**Introduction**: Macrocephalic spermatozoa syndrome has been described as a rare phenotype of severe male infertility. Different AURKC mutations severely affecting the protein function have been identified and associated to the typical phenotype form. The homozygous c.144delC mutation has been identified as the most frequent variant causing macrozoospermia in North African patients. It’s frequency of 1/50 was established on individuals from the Maghrebian general population. We reported, in this study, the spermiological and molecular characteristics of patient’s cohort with this syndrome.

**Patients and Methods**: Thirty-seven patients were diagnosed with Macrocephalic spermatozoa syndrome during a fifteen years period in the Laboratory of Human Cytogenetics, Molecular Genetics and Reproductive Biology, Farhat Hached University Hospital (Sousse, Tunisia). Sperm analysis was performed in accordance with the World Health Organization recommendations. Sperm DNA fragmentation was evaluated by TUNEL assay. Karyotyping and Y-chromosome micro-deletions were also performed. Genomic DNA was extracted from peripheral blood and PCR was performed to amplify the seven exons of AURKC gene. Sequencing analysis was carried out using the Big Dye Terminator v3.1 sequencing kit and an ABI 310 Genetic Analyzer (Applied Biosystems).

**Results**: The patients’ average age was 36.6 years and all suffered from primary infertility with a mean duration of 5.3 years. Twelve patients were issued from a consanguineous marriage (31.6%). A spermcytogram indicated on average of 100% atypical forms, with 84.5% macrocephalic and irregular heads, 93.3% abnormal acrosomes, and 42.9% multiple flagella which correspond to the typical phenotype form. The multiple anomaly index (MAI) is extremely high, with a mean value of 3.52. This trait is almost often accompanied with oigoasthenozoospermia. The fragmentation index was evaluated in ten patients, and all of them had an index higher than 30%, indicating an altered spermatic DNA. All patients had a normal somatic karyotype and none of them was positive for Y-chromosome microdeletions. Molecular analysis of AURKC gene revealed that 34 (91.89%) out of the 37 patients studied were homozygous for the c.144delC mutation at exon 3 of the gene. One patient (2.7 %) was homozygous for the Y248X mutation at exon 6 of the same gene, and two patients had neither of these mutations.

**Conclusion**: Macrocephalic sperm syndrome is a rare morphological defect commonly encountered in North Africans. The Maghrebian mutation c.144delC in the homozygous form of the Aurora kinase C gene would be the most major cause of this condition. This highlights the necessity of AURKC molecular analysis for macrozoospermic patients in reducing unnecessary ICSI attempts.

**Keywords**: infertility, teratozoospermia, macrocephalic spermatozoa, AURKC mutations.

**P075** | Whole Exome Analysis in idiopathic non-obstructive azoospermia: identification of a novel candidate gene involved in meiosis

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**Introduction**: Whole Exome Analysis in idiopathic non-obstructive azoospermia: identification of a novel candidate gene involved in meiosis.
Abstract

High prevalence of HPV infection in young males and in subjects with risk factors: the need for a universal vaccination in males

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The human papillomavirus (HPV) infection is very common among men and women across all geographic, racial, and socioeconomic areas. The role of HPV in the etiology of cancer at different genital sites is well documented, and growing evidence suggests that the virus also acts in the causation of oral cancer. In men, the presence of HPV has been well documented in the anal region, perineal area, scrotum, glans, penile shaft, urethra, and even semen. Furthermore, its persistence at these sites has been related both to male factor infertility and to cancer development at different genital and nongenital areas. Our previous studies described the possible role of HPV in male infertility. In July 2021 ESHRE published the guidelines for medically assisted reproduction in patients with a viral infection or disease, analyzing the impact of the most frequent viral infections on reproductive health in couples candidate to ART, including HPV. These guidelines referred for the prevalence of the infection at 24% in <25 years old women.

The aim of the present study was to evaluate the prevalence of human papillomavirus (HPV) in a population of unselected young males and in a population of males with risk factors for HPV.

150 male subjects, aged 18-25 y.o., participating to a surveillance programme conducted by our centre, have been enrolled for this study. We moreover enrolled 450 patients 18-45 y.o. with risk factors for HPV infection (partner with diagnosed HPV infection, history of HPV infection or HPV-induced lesions, infertility, unprotected sexual intercourses with different partners).

In all subjects we performed polymerase chain reaction and fluorescence in situ hybridization (FISH) for HPV detection. We moreover performed immunofluorescence for HPV 16-L1 and immunoglobulins (IgA, IgG, and IgM) determination. Flat penile lesions (FPL) were detected by penoscopy.

We observed a high prevalence of HPV infection in unselected young males: condylomas have been observed in 4.7%, HPV-associated FPL in 7.3%, seminal detection of HPV-DNA in 38.6% and anti-HPV antibodies in 27.3% of the studied population.

In male subjects with risk factors the prevalence raised to 94%: HPV-DNA was detected in 36.6%, Ab anti-HPV in 30.7%, HPV-associated FPL in 7.8% and condylomas in 18.9%.

In many cases we reported the presence of high-risk HPV genotypes or genotypes included in HPV vaccines.

Our data underline the high prevalence of HPV in an unselected population of young males and the presence of HPV in almost all subjects belonging to a population with risk factors, thus underlining the importance of HPV vaccination as a pivotal strategy aimed to prevent both infertility and cancer.
**Introduction & Objective:** To investigate whether a subpopulation of non-obstructed azoospermic (NOA) men are positive for the presence of spermatozoa in vas deferens (VD) and evaluate the fertilizing capacity of spermatozoa recovered from the VD.

**Patients and Methods:** Ninety-nine NOA-men underwent microscopic testicular biopsy and microsurgical aspiration of fluid from the VD. Testicular tissue was minced and evaluated for the presence of spermatozoa. Another piece of testicular tissue was processed for hematoxylin-eosin (H-E) stain. Fluid aspirated from the VD was also microscopically observed. Forty-eight men were positive for testicular spermatozoa. Nine men were positive for spermatozoa in the lumen of VD. Frozen thawed testicular spermatozoa or VD-spermatozoa from the latter nine men were injected into approximately half of the oocytes of each one respective female partner. Fertilization rates and embryonic development rates after ooplasmic injections of spermatozoa (VD-spermatozoa vs testicular spermatozoa) and oocyte culture procedures were compared.

**Results:** A percentage of NOA-men equal to 48% or 9% were positive for testicular spermatozoa or VD-spermatozoa, respectively. Among NOA-men positive for testicular spermatozoa, a percentage equal to 18% were positive for VD-spermatozoa. There were no significant differences in the percentage of fertilized oocytes and embryonic development rate after ooplasmic injections of testicular spermatozoa vs VD-spermatozoa (p > 0.05; chi-square test was used). Among four NOA-men with late maturation arrest (i.e., arrest at the round spermatid stage), three NOA-men were positive for spermatozoa in the testis and the VD.

**Conclusions:** Spermatozoa from the VD can be recovered from NOA-men avoiding the complications of testicular surgery. The latter spermatozoa have acceptable fertilizing capacity. In addition, ooplasmic injections of VD-spermatozoa can trigger early embryonic development. Seventy-five percent of NOA-men with arrest at the round spermatid stage in H-E stain were positive for spermatozoa in VD.

**Material and Methods:** Patients were examined for fertility disorders, identified genetic abnormalities, or due to pregnancy planning. Examined sample consist 16 men with Y chromosome mosaicism identified by cytogenetic study. Chromosome analysis was performed using standard cytogenetic examination. Fluorescence in situ hybridization (FISH) was performed to identify/verify mosaicism and determine the structural Y chromosome anomaly. Y chromosome microdeletions were detected by multiplex PCR with primers for loci SRY nZFX/ZFY (Yp11.3); sY84, sY86, sY615 (AZFa), sY127, sY134 (AZFb), sY254, sY255 (AZFc). The semen examination was carried out according to the recommendations of the WHO Guidelines.

**Results:** Examined cohort were divided into two groups: group I - patients with AZF deletions: AZFb+c, n = 7 and AZFc(b2/b4), n = 1 (n = 8); group II - without AZF deletions (n = 8). Cytogenetically identifiable unbalanced Y chromosome abnormalities were found in 13 patients, including 5 of 8 patients of group I, and all patients of group II. There was no statistically significant difference between the groups for the average age (33.1 ± 11.8 y.o. and 32.0 ± 3.8 y.o.), ejaculate volume, pH and ejaculate viscosity. A higher concentration of spermatozoa was found in patients without AZF deletions (group I - 15.9 ± 31.0 mln/ml, group II - 0.003 ± 0.009 mln/ml; p = 0.026). Prominent difference was found between groups in the structure of pathozoospermia: various forms of pathozoospermia were revealed in group I (azoospermia, n = 3; oligoasthenoteratozoospermia, n = 3; asthenoteratozoospermia, n = 2); in group II shown only severe forms of pathozoospermia (azoospermia, n = 7; severe oligozoospermia, n = 1), as well as in the frequency of oligospermia ~ 37.5% and 12.5%, respectively. Both mosaics with normal sperm counts showed no unbalanced abnormalities and AZF deletions, low percentage (%) of cells without the Y chromosome.

**Conclusions:** Patients with Y chromosome mosaicism have high frequency structural Y chromosome abnormalities and AZF deletions. Unbalanced cytogenetic Y chromosome rearrangements and pathogenic Yq11.2 microdeletions are characterized by a severe degree of spermatogenesis disorder in male patients with Y chromosome mosaicism (azoospermia and extremely severe oligozoospermia). In men with Y chromosome mosaicism the preservation of fertility is possible in absence of unbalanced rearrangements (ring (Y), Yp isochromosomes, and dicentric Yq and Yp chromosomes, terminal Yq11.2 deletions), dramatically disrupting meiosis and "severe" types of AZF deletions, especially AZFb+c and AZFa+b-c deletions, as well as severe disorders of gonadal development.
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Introduction: Testicular germ cell tumors (TGCT) can present as seminoma (SE) and non-seminoma, e.g. embryonic carcinoma (EC). Tumor development, progression, and prognosis are poorly understood, but infiltrating immune cells and associated cytokines/chemokines are involved. Of note, T cells represent the major component of tumor infiltrating lymphocytes (TIL) in TGCT, however the presence of rare subtypes, such as regulatory (Treg) and follicular helper T (Tfh) cells has not been studied.

Material and Methods: As part of an ongoing prospective study including a comprehensive clinical database collected since 2017, testicular tissue was obtained from 91 patients aged 18-69 years undergoing orchietomy due to TGCT. To identify key cell and molecular components, we analyzed tissue samples from different areas of tumor (tu)-bearing and contralateral testes. Analysis of infiltration density and distribution of immune cells including TILs was performed using immunohistochemistry (IHC) in specimens from patients with SE (n = 47), EC (≥ 50%; n = 16), and GCNIS-derived TGCT (n = 13) then compared to specimens with normal spermatogenesis (n = 10). In addition, immune cells were analyzed by flow cytometry (FC) using fresh human testis samples (n = 24) from different areas of tumor-bearing and contralateral testes.

Results: General histopathology of all patients showed considerable heterogeneity. Semi-quantitative scoring of IHC revealed immune cell infiltrates mainly comprised of T cells and macrophages with an increasing trend from tumor-distant to tumor-central biopsy sites. Interestingly, T cells, including Treg and Tfh cells, were more abundant in SE compared to all other groups as shown by IHC. FC analysis confirmed the highest abundance of T cells and respective subsets within the tumor in SE compared to other localizations. Two-way hierarchical cluster analysis revealed a high homogeneity of the immune cell infiltrates within the SE group with no marked differences between localized and metastatic TGCTs.

Conclusion: This cohort study demonstrates the complexity and interindividual variability of TIL and provides suggestive evidence for the possible importance of rarer T cell subtypes in the immune environment of TGCT. Future experiments will interrogate Treg and Tfh functions to identify novel prognostic factors and/or immune-therapeutic concepts for human TGCT.

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Azoospermia is a condition where there are no spermatozoa in the ejaculate and men who wish to have children undergo testicular sperm extraction (TESE). The success rate of TESE in nonobstructive azoospermia (NOA) is only around 50%. Histological analysis is the only method used for predicting sperm retrieval. The shortcomings are that histology in NOA is heterogeneous and the method is invasive. Magnetic resonance imaging (MRI), on the other hand, can provide whole testis imaging and parameter mapping and is also a non-invasive method. The aim of this study was to assess which parameters obtained with ex-vivo MRI could be useful in predicting testicular histology. Thirty-five samples of testicular tissue were obtained via TESE and all of them underwent 7T MRI, specifically diffusion tensor imaging (DTI), magnetization transfer imaging (MTI), and magnetic resonance spectroscopy (MRS). Images were analyzed in ImageJ while the concentrations of metabolites were determined using the QUEST algorithm of the jMRUI software and tetramethylsilane (TMS) as a reference. Samples were then histologically processed, analyzed, and divided into groups based on their mean Johnsen score: JS < 2, JS ≥ 2, JS ≤ 4, 4 < JS

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Objective: Current female sexual dysfunction diagnostic criteria require an assessment of both symptoms and symptom-associated distress. Female sexual dysfunction can lead to infertility due to decreased intercourse frequency. On the other hand, primary infertility is a stressful condition that affects female life’s mental, physical, social, and personal aspects. It is a cause of wasting time and money and
background of marital disruption and divorce. There is limited information about the direct impact of infertility on female sexual function and especially on female sexual distress. This study aimed to evaluate the effect of primary infertility on female sexual function using both the FSFI and FSDS-R questionnaires, according to the female sexual dysfunction definition.

**Materials and Methods:** A hundred and sixty infertile women with primary infertility and 160 healthy female controls within 18-45 years were included. None of them received treatment for IUI, IVF, or ovulation induction. All women were assessed with FSFI-Gr, FSDS-R, and a short questionnaire about the frequency of sexual intercourse and sex-life satisfaction using the Likert visual scale. Based on the data features, suitable statistical analyses were performed, including percentage, Chi-square test, student t-test and Pearson’s correlation test.

**Results:** There were no observed significant differences in the general characteristics of the two groups. Using both questionnaires, FSFI and FSDS-R, 32 women in the infertility group (29.09%) and 13 females in the control group (11.82%) presented sexual dysfunction. In all individual domains and total FSFI scores, the infertility group showed statistically significantly lower scores (p < 0.05) than the control group. Also, the frequency of intercourse (infertility group vs control group, mean value ± SD: 3.52 ± 1.05 vs. 4.61 ± 1.27) and the sex life satisfaction visual scale (2.46 ± 0.32 vs. 3.54 ± 0.42) were statistically significantly lower in infertile women group.

**Conclusion:** Women with primary infertility are at considerable risk of sexual dysfunction based on the FSFI and FSDS-R questionnaires. The infertile person loses self-esteem by repeatedly attempting to achieve the desired goal (having a baby) but failing to make it. The problem can be significantly worse when the female has been highly successful in other areas of life. Furthermore, there is a natural or feared loss of marital relationships and relationships with family and friends.

**ABSTRACT**

A hundred and sixty infertile women with primary infertility and 160 healthy female controls within 18-45 years were included. None of them received treatment for IUI, IVF, or ovulation induction. All women were assessed with FSFI-Gr, FSDS-R, and a short questionnaire about the frequency of sexual intercourse and sex-life satisfaction using the Likert visual scale. Based on the data features, suitable statistical analyses were performed, including percentage, Chi-square test, student t-test and Pearson’s correlation test.

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**Conclusion:** Women with primary infertility are at considerable risk of sexual dysfunction based on the FSFI and FSDS-R questionnaires. The infertile person loses self-esteem by repeatedly attempting to achieve the desired goal (having a baby) but failing to make it. The problem can be significantly worse when the female has been highly successful in other areas of life. Furthermore, there is a natural or feared loss of marital relationships and relationships with family and friends.
Background: Testicular Germ Cell Tumor (TGCT) is a multifactorial and polygenic neoplasia. Despite epidemiological and Genome-Wide Association Studies highlighted its high inheritability and the increased familial cancer risk among TGCT patients’ relatives, the genetic basis for TGCT remains unclear. Germine mutations in DNA repair genes have been reported in several autosomal dominant cancer predisposition syndromes, such as Lynch syndrome (LS) and Hereditary Breast and Ovarian Cancer syndrome (HBOCS). Recently, TGCT has been linked with LS, but no association with other cancer syndromes has been proposed so far.

Objective: To elucidate the role of DNA repair genes in the etiology of TGCT in the context of familial history of multiple cancers.

Methods: DNA was extracted from peripheral blood lymphocytes of 63 TGCT patients with more than one family member suffering from different cancer types. WES was carried out using the Agilent Sure-Select_V6 kit and the Illumina NovaSeq6000. After standard quality check, variants in the coding sequences and splice-sites were filtered for Minor Allele Frequency (MAF ≤ 0.01) and for an in-house Pathogenic Index based on six in silico prediction tools. Genes carrying variants have been crossed with a list of 547 genes involved in DNA repair mechanisms obtained from Gene Ontology. Variants were classified based on the American College of Medical Genetics and Genomics (ACMG) guidelines and those having “Pathogenic” (P) or “Likely Pathogenic” (LP) verdict were selected. Further bioinformatic analysis included: expression profile, cancer databases, animal models, and literature search. Selected variants were validated by Sanger sequencing.

Results: We identified 29 heterozygous and 1 hemizygous LP and P variant in 28 DNA repair genes in 25/63 patients (40%). 10/30 heterozygous variants were located in genes already known to be associated with several cancer types present in the family members of the probands. We have identified the following variants in genes associated with: i) HBOCS (ERCC3:c.2065-2A>C; FANCC:c.455dup POLK:c.1577A>G; BRIP1:c.1941G>C; and DCLRE1C:c.1903dup); ii) LS (MSH6:c.2906_2907del, MLH3:c.3222_3223del); iii) colorectal cancer (MUTYH:c.1640del) iv) breast cancer (MMS19:c.346C>G; BRIP1:c.1941G>C; FANCC:c.455dup); v) leukemia (SETMAR:c.401A>T).

Conclusions: P and LP variants in DNA repair genes are present in 40% of patients in our highly selected cohort, suggesting their potential role in the pathogenesis of TGCT. Our findings provide further evidence for the link between TGCT and LS and proposes novel associations with HBOCS and other type of cancers. Additional experiments are ongoing in order to confirm the causality of these variants.

P085 | Obstructive azoospermia as first presentation for Von Hippel Lindau disease

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Introduction: Von Hippel-Lindau disease (VHL) is one of the most common inherited neoplasia syndromes and is characterized by highly vascular tumors of the eyes, brain, and spine, as well as benign and malignant tumors and/or cysts of the kidneys, adrenal medullae and sympathetic paranganglia, endolymphatic sac, epididymis, and broad ligament. This disorder is caused by highly penetrant mutations of VHL gene (3p25.3).
Presentation: We report a case of an infertile /azoospermic 38 years old man, seeking diagnostic investigations and eventually sperm cryopreservation. The semen analysis showed an ejaculate pH 7.2, a volume of 2cc and azoospermia confirmed after centrifugation.

In his medical history the only relevant data was the findings of bilateral epididymal cysts when he was 18 years old. On physical examination we highlighted abdominal obesity, absence of gynecomastia, normotrophic testis associated with bilateral epididymal hard mass. We performed scrotum ultrasonography which identified a epididymis bilateral enlargement due to several cysts (right maximum diameter 14 mm). To further investigate this condition the patient underwent a scrotal and pelvic MRI with contrast that confirmed the presence of multiple epididymis cysts. Biochemical tests showed negative testicular cancer biomarkers and a subclinical slight normogonadotropic hypogonadism. On June 26, 2020 the patient underwent bilateral biopsy of testis (with sperm cryopreservation), tunica vaginalis’eversion and partial enucleation of epididymal cysts bilaterally. Histology concluded for bilateral papillary cystadenoma of the epididymis.

Given the rarity of epididymis bilateral papillary cystadenoma, in order to exclude the presence of Von Hippel-Lindau disease the patient underwent abdominal ultrasound and ophthalmology evaluation.

These medical examinations showed multiple pancreatic cysts with maximum diameter of 60mm at the pancreatic head, kidney cysts and at the middle third of the right kidney a solid mass with 2 peripheral calcific spots. These findings were confirmed at MRI evaluation; one of the pancreatic cysts (because of the reduction of DWI) and the renal cystic mass (33 mm maximum diameter with solid content) were highly suspicious for malignancy. Partial nephrectomy was performed and the histology confirmed the diagnosis of a clear cell renal cell carcinoma. Further evaluations for pancreatic lesions are ongoing.

The patient had been tested positive for VHL gene mutations (c.464-1G/A;t.?). Screening for pheochromocytoma was negative.

Discussion: to our knowledge this is the first report of a male infertile/azoospermic condition leading to a previously unknown VHL syndrome, presenting with life-threatening renal, retinal and pancreatic lesions.

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Introduction: Male infertility is commonly characterized by severe pathozoospermia, especially azoospermia and severe oligozoospermia. These forms are often caused by genetic defects, and very heterogeneous. There are numerous copy number variations (CNVs) and pathogenic variants in genes associated with male infertility, therefore it’s detection requires the use of complex approach and genomic methods.

Materials and Methods: The sample included 200 patients with male infertility associated with azoospermia (n = 172) or severe oligozoospermia (n = 28). The age of the patients ranged from 18 to 48 (30.5 ± 5.7) years. We performed clinical and genetic examination, karyotype analysis, FISH-analysis on peripheral blood lymphocytes and buccal epithelium cells using centromeric probes for X- and Y-chromosomes (DXZ1, DYZ3), detecting of Y-chromosome microdeletions ( locus AZF), analysis of common pathogenic variants and polymorphic IVS9Tn locus of the CFTR gene. 42 patients underwent whole-exome sequencing (WES) on the Illumina NextSeq500 device (Illumina Inc., USA).

Results: Karyotype abnormalities were found in 52 (26.8%) patients: Klinefelter syndrome (47.XXY; n = 34), dysomy Y (47.XYY; n = 6), 46,XX-male syndrome (n = 3), balanced autosomal abnormalities (n = 9). FISH-analysis shown no hidden gonosomal mosaicism in patients with the sex chromosomes aneuploidy. Complete AZF deletions were detected in 16 (8%) patients: AZFb (n = 3), AZFc(b2/b4) (n = 8), AZFb+c (n = 5). Monogenic forms of male infertility were diagnosed in 24 (12%) patients, among them: Congenital Hypogonadotropic Hypogonadism (CHH)/Kallmann syndrome in 15 (7.5%) patients, cystic fibrosis in 6 (3%) men and CBAVD syndrome in 3 (1.5%) men. Thus, genetically determined forms of fertility disorders were found in 92 (46%) patients, including 75 (37.5%) azoospermic men and 19 (67.8%) oligozoospermic men. A group of 42 patients (idiopathic non-obstructive azoospermia – NOA, n = 40; severe oligozoospermia, n = 2) underwent WES. Variants of the nucleotide sequence were detected in 18 (42.8%) patients in 29 genes (EP300, AR, MCM8, ANOS1, CYP21A2, CFTR, CATSPER1, LHCGR, TEX15, GNGT1, DNAH11, DNAH17, FSHB, DUSP6, HS6ST1, SMAD3, TEX14, VWA2, FSHB, CFAP44, MEIOB, GNRHR, RNF216, QRICH2, PLCZ1, CYP11B1, KMT2D, SLC4A1) involved in the spermatogenesis. In one patient, a heterozygous 7q21.3 microdeletion, capturing gene GNGT1, was detected. GNGT1 gene is involved in controlling the migration of primordial germ cells (PGCs) to genital ridges. Another patient was found to have 410 bp-microdeletion capturing the DUSP6 gene (12q21.33), pathogenic variants in which lead to the development of CHH, type 19. However, this CNV was not detected by the reference method (MLPA). Some detected heterozygous variants in the genes responsible for autosomal recessive forms of male infertility, and also did not combine with clinical and spermatological picture. The identified variants in the genes require segregation and functional studies to confirm their pathogenicity. The remaining 24 out of 42 patients (57.1%) did not have genetic variants associated with severe forms of pathozoospermia. However, this does not exclude genetic factors of male infertility and requires further genetic examination.

Conclusion: Comprehensive genetic approach and using genomic methods in examination of infertile patients increase the effectiveness of the diagnosis of genetically determined pathozoospermia.
ABSTRACT

Chromosome 8 And Non-Obstructive Azoospermia: a report of two cases

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text

Introduction: Non obstructive azoospermia (NOA) can be caused by several genetic factors, including chromosomal abnormalities, Y chromosome microdeletions and gene mutations. Cytogenetic anomalies, especially structural chromosomal aberrations were found in 10-15% of azoospermic males. Nonetheless, new research has shed light on the impact of autosome gene unbalanced translocations in spermatogenesis. In this study, we report genetic exploration by karyotype, Fluorescence in-situ hybridization (FISH) and molecular analysis of chromosome Y of two patients addressed for exploration of their azoospermia.

Patients and Methods: Two cases of azoospermic patients were addressed to the laboratory of Human Cytogenetics, Molecular Genetics, and Reproductive Biology (Sousse, Tunisia) for genetic testing.

For both patients, physical examination was normal. Follicle stimulating hormone levels in the blood were increased for both patients while plasma level of testosterone was reduced for patient P2. Ultrasound showed a bilateral testicular hypotrophy for patient P1 and unilateral hypotrophy for patient P2. Analyses of sperm revealed non obstructive azoospermia (NOA). Chromosomal analysis was obtained from cultured blood lymphocytes using R-banding technique. FISH analysis was carried out with commercial probes to characterize chromosome rearrangements. Multiplex Ligation Probe Amplification was used to determine the chromosome Y microdeletion.

Results: Karyotype revealed that all metaphase cells, for P1, had a 45,X,der(8)t(Y;8) formula. FISH analysis using SRY gene-specific probe localized on Yp11.31 showed a positive signal on the short arm of chromosome 8. Signals for the SHOX gene localized on Yp11.32 and for the Y centromere (DYZ3) were detected on the short arm of chromosome 8. For patient P2, the karyotype revealed an apparently balanced reciprocal translocation t(8;14)(p12;q12). Investigations of AZF region microdeletions revealed in P1 the loss of the long arm of the Y chromosome involving AZF and Yq heterochromatin regions.

Discussion: Azoospermia in patients with structural chromosome abnormalities can be explained by the fact that spermatogenesis failure was caused by chromosome mis-segregation during meiosis. For patient P1, the causative factor of male infertility and spermatogenetic failure in Y-autosome translocation may be caused by the disruption, loss of AZF loci by the translocation breakpoint or by a positional effect. For patient P2, recent research have identified genes at 8p12 such as KAL2, TEX15, and NRG1 as being involved in spermatogenesis and their loss in unbalanced translocations can result in azoospermia. Also, a position effect of unknown spermatogenesis regulatory gene(s) at 14q12 could explain NOA for P2 moreover, further investigations are needed.

Conclusion: Our study, discussing two new cases of a rare (Y;8) and (8;14) unbalanced translocations in NOA, highlights the necessity of genetic testing in NOA before any investigation, as well as the need for future research in order to improve genetic counselling in male infertility therapy.

P088 The European Academy of Andrology (EAA) ultrasound study on healthy, fertile men: clinical, seminal and biochemical characteristics

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Background: Infertility affects 7-12% of men and its etiology is unknown in half of cases. To fill this gap, use of the male genital tract colour-Doppler ultrasound (MGT-CDUS) has progressively expanded. However, MGT-CDUS still suffers from lack of standardization. Hence, the European Academy of Andrology (EAA) has promoted a multicenter study ("EAA ultrasound study") to assess MGT-CDUS characteristics of healthy-fertile men to obtain normative parameters.

Objectives: To report (a) the development and methodology of the "EAA ultrasound study", (b) the clinical characteristics of the cohort of healthy-fertile men and (c) the correlations of both fertility history and seminal features with clinical parameters.

Methods: A cohort of 248 healthy-fertile men (35.3 ± 5.9 years) was studied. All subjects were asked to undergo, within the same day, clinical, biochemical, seminal evaluation and MGT-CDUS before and after ejaculation.

Results: The clinical, seminal and biochemical characteristics of the cohort have been evaluated. The seminal characteristics were consistent with those reported by the WHO (2010) for the 50th and 5th centile for fertile men. Normozoosperma was observed in 79.6% of men, while normal sperm vitality was present in almost the entire sample. Time to pregnancy (TTP) was 3.0 [1.0-6.0] months. TTP was
negatively correlated with sperm vitality (Adj. $r = -0.310, p = 0.011$), but not with other seminal, clinical or biochemical parameters. Sperm vitality and normal morphology were positively associated with FT3 and FT4 levels, respectively (Adj. $r = 0.244, p < 0.05$ and Adj. $r = 0.232, p = 0.002$).

Sperm concentration and total count were negatively associated with FSH levels and positively, along with progressive motility, with mean testis volume (TV). Mean TV was $20.4 \pm 4.0$ ml and the lower reference values for right and left testes were $15.0$ and $14.0$ ml. Mean TV was negatively associated with gonadotrophins levels and pulse pressure. Varicocele was found in $33\%$ of men.

**Conclusions:** The cohort studied confirms the WHO data for all semen parameters and represents a reference with which to assess MGT-CDUS normative parameters.

**P089** The European Academy of Andrology (EAA) ultrasound study on healthy, fertile men: scrotal ultrasound reference ranges and associations with clinical, seminal and biochemical characteristics

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**Background:** Scrotal colour-Doppler ultrasound (CDUS) still suffers from lack of standardization. Hence, the European Academy of Andrology (EAA) has promoted a multicenter study to assess the CDUS characteristics of healthy-fertile men (HFM) to obtain normative parameters.

**Objectives:** To report and discuss the scrotal organs CDUS reference ranges and characteristics in HFM and their associations with clinical, seminal and biochemical parameters.

**Methods:** A cohort of 248 HFM (35.3 ± 5.9 years) was studied, evaluating, on the same day, clinical, biochemical, seminal and scrotal CDUS following Standard Operating Procedures.

**Results:** The CDUS reference range and characteristics of the scrotal organs of HFM have been evaluated. CDUS showed a higher accuracy than physical examination in detecting scrotal abnormalities. Prader orchidometer (PO)- and US-measured testicular volume (TV) were closely related. The US-assessed TV with the ellipsoid formula showed the best correlation with the PO-TV. The mean TV of HFM was $\sim 17$ ml. The lowest reference limit for right and left testis was $12$ and $11$ ml thresholds defining testicular hypotrophy. The highest reference limit for epididymal head, tail and vas deferens was $12$, $6$ and $4.5$ mm, respectively. Mean TV was associated positively with sperm concentration and total count and negatively with gonadotrophins levels and pulse pressure. Subjects with testicular inhomogeneity or calcifications showed lower sperm vitality and concentration, respectively, than the rest of the sample. Sperm normal morphology and progressive motility were positively associated with epididymal head size/vascularization and vas deferens size, respectively. Increased epididymis and vas deferens sizes were associated with MAR test positivity. Decreased epididymal tail homogeneity/vascularization were positively associated with waistline, which was negatively associated with intratesticular vascularization. CDUS-varicocele was detected in 37.2% of men and was not associated with seminal or hormonal parameters. Scrotal CDUS parameters were not associated with time to pregnancy, number of children, history of miscarriage.

**Conclusions:** The present findings will help in better understanding male infertility pathophysiology, improving its management.

**P090** Extended ultrasound of the male urogenital tract

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**Background:** The andrological ultrasonography including transrectal and testicular sonography is largely unstandardized, and consensus on assessment and classification of several parameters is still lacking. Recently, the European Academy of Andrology (EAA) proposed the reference values for scrotal ultrasound. However, the reference values for prostate and seminal vesicles (SV) using transrectal ultrasound (TRUS) are still missing. The aim of this study was to describe the ultrasonographic differences in testicular, seminal ducts and prostatic-vesicular region among well-defined andrological patients groups.

**Methods:** During the period 03.06.2019–26.05.2022, fifty four (54) outpatient males passed the extended urogenital tract sonography for four different causes (16 patients with idiopathic azoospermia [FSH range 3.3–29.9 U/L, LH range 2.9–15.2 U/L]; 21 patients with oligo-asthenoteratozoospermia (OAT) [FSH range 2.0–22.7 U/L, LH range 2.5–11.6 U/L]; 8 patients with haematospermia; 9 patients with pelvic pain exacerbating after ejaculation.) in Andrology Centre of Tartu University Hospital, Tartu, Estonia. All the study participants were investigated by one clinician (S. T.), who has trained for ultrasound in the frame of EAA Ultrasound School. The ultrasound investigations consisted of scrotal ultrasound, pre- and post-ejaculatory TRUS. All the ultrasonographic studies were performed using the following ultrasound machines: FlexFocus 400 (BK Medical®) or bkSpecto.
ABSTRACT

The European Academy of Andrology (EAA) ultrasound seminal and biochemical characteristics

Purpose: The present study systematically collected and analyzed seminal, biochemical, and ultrasound parameters following Standard Operating Procedures.

Methods: One hundred eighty-eight men (35.6 ± 6.0 years) from a cohort of 248 HFM were studied, evaluating, on the same day, clinical, biochemical, seminal, TRUS parameters following Standard Operating Procedures.

Results: TRUS reference ranges and characteristics of the prostate and SV of HFM have been evaluated. The mean PV was ~25 mL. PV lower and upper limits were 15 and 35 mL, defining prostate hypotrophy and enlargement, respectively. PV was positively associated with age, waistline, current smoking (but not with testosterone levels), seminal volume (and negatively with seminal pH), prostate inhomogeneity, macrocalcifications, calcification size and prostate arterial parameters, SV volume before and after ejaculation, deferential and epididymal size. Prostate calcifications and inhomogeneity were present, while midline prostatic cysts were rare and small. Ejaculatory duct abnormalities were absent. Periprostatic venous plexus size was positively associated with prostate calcifications, SV volume and arterial peak systolic velocity. Lower and upper limits of SV anterior-posterior diameter after ejaculation were 6 and 16 mm, defining SV hypotrophy or dilation, respectively. SV total volume before ejaculation and delta SV total volume (DSTV) positively correlated with ejaculate volume, and DSTV correlated positively with sperm progressive motility. SV total volume after ejaculation was associated negatively with SV ejection fraction and positively with distal ampullas size. SV US abnormalities were rare. No association between TRUS and time to pregnancy, number of children or history of miscarriage was observed.

Conclusions: The present findings will help in better understanding male infertility pathophysiology and the meaning of specific TRUS findings.

Background: Transrectal ultrasound (TRUS) parameters are not standardized, especially in men of reproductive age. Hence, the European Academy of Andrology (EAA) promoted a multicenter study to assess the TRUS characteristics of healthy-fertile men (HFM) to establish normative parameters.

Objectives: To report and discuss the prostate and seminal vesicles (SV) reference ranges and characteristics in HFM and their associations with clinical, seminal, biochemical parameters.

Methods: One hundred eighty-eight men (35.6 ± 6.0 years) from a cohort of 248 HFM were studied, evaluating, on the same day, clinical, biochemical, seminal, TRUS parameters following Standard Operating Procedures.

Results: TRUS reference ranges and characteristics of the prostate and SV of HFM have been evaluated. The mean PV was ~25 mL. PV lower and upper limits were 15 and 35 mL, defining prostate hypotrophy and enlargement, respectively. PV was positively associated with age, waistline, current smoking (but not with testosterone levels), seminal volume (and negatively with seminal pH), prostate inhomogeneity, macrocalcifications, calcification size and prostate arterial parameters, SV volume before and after ejaculation, deferential and epididymal size. Prostate calcifications and inhomogeneity were frequent, while midline prostatic cysts were rare and small. Ejaculatory duct abnormalities were absent. Periprostatic venous plexus size was positively associated with prostate calcifications, SV volume and arterial peak systolic velocity. Lower and upper limits of SV anterior-posterior diameter after ejaculation were 6 and 16 mm, defining SV hypotrophy or dilation, respectively. SV total volume before ejaculation and delta SV total volume (DSTV) positively correlated with ejaculate volume, and DSTV correlated positively with sperm progressive motility. SV total volume after ejaculation was associated negatively with SV ejection fraction and positively with distal ampullas size. SV US abnormalities were rare. No association between TRUS and time to pregnancy, number of children or history of miscarriage was observed.

Conclusions: The present findings will help in better understanding male infertility pathophysiology and the meaning of specific TRUS findings.
Background: Infertility affects 8-12% of men of reproductive age. Although scientific literature reports several clinical parameters likely associated with male infertility/fertility, b)previous studies report gonadotropins and testosterone, thresholds suggestive of abnormal/normal testicular function and c)values of several seminal parameters > 5th centile of their distribution reported by the WHO Manual suggest a higher probability to be fertile, so far thresholds of clinical, seminal and biochemical parameters predicting a pregnancy with live birth are not available.

Objectives: To assess differences in clinical, seminal and biochemical characteristics of males of infertile couples (MIC) and males of fertile couples (MFC) and to identify clinical, seminal and biochemical predictors of a pregnancy with live birth evaluating several parameters in MIC and MFC with live birth.

Methods: We evaluated 604 MIC (37.5 ± 7.0 years) and 115 healthy MFC with live birth (36.6 ± 5.3 years). We compared the clinical, seminal and biochemical characteristics of the two groups. Subsequently, for clinical, seminal and biochemical variables with continuous distribution we performed iterative ROC curves to identify parameters predictive for a pregnancy with live birth, reporting the threshold with the best sensibility and specificity predicting a pregnancy with live birth. Then, dichotomous variables related to the thresholds found for each parameter were used as independent variables in iterative binary logistic regression analyses adjusted for confounders (male and female partner age) to evaluate the adjusted risk (‘odds ratio’, OR) to be a man of a fertile couple (dependent variable), i.e. to induce a pregnancy with live birth. Accordingly, clinical, seminal and biochemical dichotomous variables have been used as independent variables in iterative binary logistic regression analyses adjusted for confounders to evaluate the adjusted risk (‘odds ratio’, OR) to induce a pregnancy with live birth (dependent variable).

Results: MFC and MIC, as well as their female partners, showed a similar age. MCF had a lower prevalence of cryptorchidism and mumps history, less general and adrological physical examination abnormalities, better seminal and hormonal parameters, better sexual function, lower urinary tract symptoms and psychopathological traits compared with MIC. Female partner age < 34.5 years old, mild alcohol consumption, absence of cryptorchidism and mumps history; mean blood pressure < 93 mmHg, mean testis volume (Prader) ≥ 20 ml, absence of epididymal dilatation, absence of bilateral agenesia of vas deferens; sperm concentration ≥ 40 * 106/ml, sperm total count ≥ 128 40 * 106/ejaculate, progressive motility ≥ 52%, normal morphology ≥ 5%, seminal pH ≥ 7.5; FSH < 3.8 U/L, LH < 3.2 U/L, total testosterone ≥ 17 nmol/l, calculated free testosterone ≥ 0.335 nmol/l, SHBG ≥ 31 nmol/l; absence of erectile dysfunction (IIEF-15-EFD ≥ 26) and MHQ total score ≤ 20 were predictive for a pregnancy with live birth.

Conclusions: This study reports for the first time thresholds related to clinical, seminal and biochemical parameters predictive for a pregnancy with live birth. The application of these thresholds can be useful in the clinical practice to evaluate if a man consulting to evaluate his fertility potential, shows characteristics predictive for a pregnancy with live birth.

P095 I Fertility alterations of males with non-obstructive azoospermia due to hypogonadotropic hypogonadism after gonadotropin treatment

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Introduction: Hypogonadotropic hypogonadism (HH) is a rare cause of non-obstructive azoospermia (NOA). Treatment recommendations include GhRHa and gonadotropins for at least 12 months. In case of poor treatment results and persisting azoospermia, surgical spermatozoa extraction from the testes is the suggested solution. The present study aims to evaluate the fertility results of NOA males undergoing treatment for HH in the Urology Department of Ioannina University.

Methods: The present study includes twenty-five NOA males suffering from HH. Patients underwent hormonal evaluation and double sperm centrifugation to establish azoospermia and HH. After the recommended treatment period, sperm diagram results, sperm retrieval rates, pregnancy rate and live births were assessed.

Results: Twenty-five males suffering from NOA received treatment with gonadotropins for a median time of 13.1 months. After that period, 10 NOA males presented with spermatozoa (40%). The 15 azoospermic men followed a micro-TESE procedure to retrieve spermatozoa from testicles. This group of males underwent a gonadotropins treatment program for 14.1 months (range 6-21 months). In 13 men (86.7%), the micro-TESE process resulted in the presence of spermatozoa. After 12 cycles of ICSI with spermatozoa from testicles, we had 6 (50%) successful pregnancies and five live births (including two twins) and one abortion after nine weeks of pregnancy.

Conclusions: A significant number of males suffering from NOA due to HH will produce spermatozoa. The azoospermic NOA males with HH, after treatment, can retrieve spermatozoa with new surgical techniques and proceed to live births.

P096 I Is non-morbid obesity the cause of idiopathic male infertility?

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Introduction: After performing standard andrological tests, in about 40% of cases, male infertility has no obvious cause and is considered idiopathic. The role of obesity in male infertility in the absence of other morbid hormonal or cardiovascular changes is not fully elucidated and continues to be controversial.

Purpose: Determining the incidence of non-morbid obesity in male infertility and the results of management.

Material and Methods: The study included 207 men with male infertility who considered themselves healthy, with a BMI of less than 40, and standard andrological examination (medical history, physical examination, semen analysis, sex hormone profile, testis ultrasound) found no causes of infertility but confirmed alterations in semen analysis. Additional tests included: Lipid profile (Cholesterol, LDL, HDL Triglycerides), 25-OH-Vit.D and thyroid hormones.

Results: In 61 (29%) patients BMI was less than 25, including BMI up to 20 in 12 (6%) men, 78 (38%) patients were overweight and 68 (33%) had grade I-II obesity. Patients were divided into 3 groups. The total amount of sperm was 15.6 (± 4.5) mln - group 1, 17.1 (± 5.6) mln - group 2 and 14.2 (± 3.1) mln - group 3. The average progressive motility in groups was 13%, 16% and 11.4%, and the average normal morphology was 3.4%, 4.8%, 2.8% respective. Azospermia or OAT was detected in 7 patients (3 men in group 1 and 2 men in groups 2 and 3). Hyperthyroidism was found in 3 patients in group 3 and 1 in group 1. Hyperthyroidism found in 4 patient in group 1. In group 3, 55 (81%) patients had increased cholesterol and/or its fractions and 13 (19%) patients had normal lipid profiles. In group 1, 7 (11%) patients had increased lipid profiles. We administered antioxidant treatment to 196 patients. In patients from groups 2 and 3, we advised lowering BMI by changing the lifestyle and diet for a period of 6 months. Repeated evaluation at 6 months showed improvement in lipid profile in 22 (28%) patients in group 2 and 32 (48%) patients in group 3, and improvement in sperm analysis in 21 (34%) patients in group 1, 32 (48%) patients in group 2 and 39 (57%) patients in group 3. Vitamin D deficiency was determined in about 80% of cases, with no differences within the groups.

Discussions and conclusions: We cannot say for sure that MI considered idiopathic is more common in obese patients, but only 23% of patients had a healthy BMI (18.5-24.9). We did not report significant differences between the total sperm count, mobility or normal morphology in patients in different groups, but there is a tendency of decreased motility and normal morphology in patients with non-morbid obesity. BMI and lipid profile can be important parameters in the complex assessment of male infertility. Antioxidant treatment, concomitant with BMI reduction, showed an improvement in spermogram parameters at a 6-month interval in all patients, but more obviously in those with initial non-morbid obesity (BMI 30-39).

P098 | Hypogonadotrop hypogonadic fathers – a single centre experience

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Introduction: Hypogonadotropic hypogonadism is a relatively rare, congenital or acquired malfunction of the pituitary gland, characterised by low gonadotropin and consequently low testosterone levels and male infertility. Gonadotropin substitution is a potentially effective treatment to induce/restore fertility within 3-24 months.

Material and Methods: Between January 2012 and December 2021, 49 patients were diagnosed with hypogonadotropic hypogonadism. Classical sperm parameters, hormone levels and fertilisation
success rates were analysed retrospectively. Combined human chorionic gonadotropin (hCG) and recombinant follicle stimulating hormone (rFSH) treatment was initiated and tailored individually. Follow up was performed every 12 weeks.

**Results:** Thirty-seven patients (75.5%) were diagnosed with congenital disease (isolated form, Kallmann syndrome, panhypopituitarism, pituitary hypoplasia, and 21-hydroxylase deficiency in 28, 3, 3, 2 and 1 cases, respectively). Twelve patients (24.5%) suffered from the acquired form of the disease (previous pituitary macroadenoma surgery, anabolic steroid use, and recent diagnosis of pituitary cranio-pharyngeoma in 7, 4 and 1 cases, respectively). Baseline FSH and LH levels were below 1.5 IU/L (mean: 0.61 IU/L, range: 0.1-1.47 IU/L) and 1.95 IU/L (mean: 0.62 IU/L, range: 0.01-1.9 IU/L), respectively. Nine out of the 49 patients underwent testosterone replacement therapy previously. In the untreated group of 40 patients, 80% (32/40) showed low testosterone levels (below 8 nmol/L, mean: 3.15 nmol/L), 20% (8/40) of patients’ testosterone levels were in the grey zone (between 8-12 nmol/L, mean: 10.18 nmol/L).

The drop out rate was 38.8% (19 patients), a total of 30 patients’ data could be collected for the therapeutic database. Azoospermia was found in 70% of the enrolled patients (21/30). Cryptozoospermia, oligo-astheno-teratozoospermia and astheno-teratozoospermia was diagnosed in 3 (10%), 5 (16.7%) and 1/(3.3%) cases, respectively. During the treatment period spermatogenic improvement occurred in 83.3% of the cases (25/30).

Eighteen of the azoospermic patients (85.7%) had improved (average timespan until the appearance of sperm cells in the ejaculate took 7 months), 9.5% became normozoospermic. All three continuously azoospermic patients underwent microsurgical testicular sperm extraction, with a 100% sperm retrieval rate. Eight patients of the non-azoospermic group (88.9%) showed a significant spermatogenic improvement, 33.3% became normozoospermic.

Seven patients (23.3%) achieved spontaneous pregnancy (a total of 10 newborns). In the remaining 23 patients 10 cycles of ART resulted in five pregnancies therefore for the 30 treated patients 15 children were born during the study period.

**Conclusion:** Gonadotropin replacement is an effective method to treat hypogonadotropic hypogonadism to achieve fertility. In our series 12 of 30 patients achieved fatherhood (in three cases multiple times), and sperm cells could be harvested in all azoospermic patients after treatment (surgically in three cases) in an average of 7 months of the therapy. The high success rate of the treatment underlines the importance of appropriate diagnostic process, and the early recognition of hypogonadotropic hypogonadism.

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**ABSTRACT**

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Campania Region has been facing waste management crisis since 1980, characterized by urban, toxic and industrial waste illegal disposal, burying and incineration. Cadmium (Cd) is consistently shown to affect male reproductive function by multiple mechanisms, mostly elucidated in experimental models. The aim of the current single-center, observational, cross-sectional cohort study was to evaluate the prevalence of testis morphostructural alterations in a large cohort of men living in 3 municipalities of Campania Region (Acerra, Afragola, Giugliano) belonging to the high-environmental impact area “Land of Fires”, by addressing the potential association with seminal Cd (sCd) levels. Study cohort included 465 males (age range: 14-50 yrs; mean: 29.5 ± 7.23 yrs). Morphostructural testis characteristics were assessed by ultrasound and sCd determination was performed in 385 samples by inductively coupled plasma-mass spectrometry. Prevalences of testis morphostructural alterations, unilateral or bilateral, included varicocele (35.4%), hydrocele (34.8%), parenchymal structure inhomogeneity (19%), hypotrophy (14.6%), microlithiasis (2.5%), solid lesions >5 mm (0.2%). Participants with detectable sCd levels (N = 128) displayed significantly reduced mean testicular volume (16.56 ± 4.68 vs. 17.66 ± 4.34; p = 0.0153) and higher prevalence of hypotrophy (21% vs. 10%; p = 0.0059) and varicocele I-V grade (47.5% vs. 29.5%; p = 0.0008), but not clinically relevant varicocele III-V grade (18% vs. 11%, p = 0.09), together with a slightly higher prevalence of parenchymal structure inhomogeneity (25.8% vs. 16.7%; p = 0.059), compared to participants with undetectable sCd levels (N = 257). Furthermore, a significant difference in mean testicular volume was detected when comparing participants with sCd levels above (N = 49) and below median value (N = 79) and undetectable sCd levels, respectively (14.88 ± 3.79 vs. 17.22 ± 5.03 vs. 17.66 ± 4.34; p < 0.001). sCd level was persistently correlated with mean testicular volume after correction for the presence of clinically relevant varicocele (r = -0.185; p = 0.001). sCd levels was identified as the best predictor of mean testicular volume in linear regression analysis performed by setting sCd, smoking habit, age and BMI as independent variables. ROC curve analysis highlighted that a sCd level > 0.76 μg/L correctly identified testicular hypotrophy with a 60% sensibility and 70% specificity. In conclusion, the current study demonstrated for the first time, in a large cohort of adult males living in high-environmental impact areas of Campania Region, an inverse relationship between sCd levels and mean testicular volume and prevalence of varicocele, independently from age, BMI and smoking habit, therefore further strengthening the concept of gonadal toxicity exerted by Cd.
During the last 30 years, Campania Region has been characterized by waste management crisis, resulting in the largely documented illegal disposal of urban, toxic and industrial waste, and diffuse practice of illegal waste burning. Chronic exposure to environmental pollutants has been shown to adversely affect male reproductive function and semen quality; the heavy metal cadmium (Cd) is a human carcinogen ubiquitous in the environment consistently shown to affect male reproductive function by endocrine and non-endocrine actions. Testis is particularly susceptible to Cd poisoning, and in experimental studies in animals and human spermatozoa Cd exerted reproductive toxicity, mediated by structural damage to testis vasculature and blood-testis barrier, inflammation, Sertoli and Leydig cells cytotoxicity, oxidative stress, epigenetic actions, and disturbance of the hypothalamus-pituitary gonadal axis. No large investigations have been performed addressing semen quality of young men living within the high-environmental impact area “Land of Fires” (LF) and/or the potential implication of non-occupational exposure to heavy metals, particularly Cd. Within a population-based awareness and screening campaign for male infertility in Campania Region, the current study enrolled a large cohort (N = 493) of healthy men (mean age: 24 yrs) with at least 10 years of residence in 3 high-environmental impact municipalities (Acerra, Afragola, Giugliano in Campania; AAG) belonging to LF, for comparison with 410 age-matched men living in low-environmental impact municipalities (Other areas, OA) (ARPAC D.L. 136/2013). No “a priori” exclusion criteria were applied concerning andrological profile, Anamnesis, clinical and andrological examination, testis ultrasound, and 2 semen analyses (WHO 2010) were performed. Serum and semen concentrations of 23 trace elements were determined by ICP-MS. Participants from AAG had significantly lower sperm concentration (p < 0.001), count (p < 0.001) and normal sperm morphology (p = 0.035), compared to participants from OA; consistently, the prevalence of normozoospermia was significantly lower (p = 0.0022) and that of oligozoospermia was significantly higher (p = 0.0017) in AAG. Participants from AAG were grouped as being normozoospermic (N = 328) or having seminal parameters below reference range (N = 165); no difference was detected in serum trace elements concentrations between groups, whereas seminal Cd concentration was significantly higher (p = 0.031) in the pathological seminal parameters group. Moreover, seminal Cd concentration was negatively correlated to sperm concentration (r = -0.211; p = 0.017) and count (r = -0.177; p = 0.045). Since seminal Cd concentration was frequently below the limit of detection (LoD = 0.20 μg/L) across AAG cohort, participants were grouped as having seminal Cd concentration below (N = 364) or above (N = 129) LoD. Participants with detectable seminal Cd concentration had significantly reduced sperm count (p = 0.028) and normal sperm morphology (p = 0.036), compared to those with undetectable concentration. Compared to AAG, none of the participants from OA had detectable seminal Cd levels (0% vs. 26.2%; p < 0.0001). The current study supports a detrimental effect of the specific environmental exposure pattern of AAG polluted municipalities on semen quality, and higher seminal Cd levels were associated to worse seminal parameters, suggesting that cumulative Cd exposure might exert reproductive toxicity at environmentally relevant levels of exposure. Further studies including the objective assessment of Cd burden in environmental matrices are encouraged to corroborate the impact of Cd exposure on the risk of male subfertility/infertility.
P102  |  Decrease in sperm motility between one and three hours after ejaculation: association with the Hypo-Osmotic Swelling Test

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Background: The Hypo-Osmotic Swelling (HOS) Test has been associated with the membrane integrity of spermatozoa, as it determines the sperm’s membrane ability to maintain equilibrium between the sperm cell and its environment. It is used as an alternative to dye exclusion, especially when choosing spermatozoa for intracytoplasmic sperm injection (ICSI).

Aim: To assess the decrease in sperm motility over time (one and three hours after ejaculation) and to correlate sperm motility with membrane integrity using the HOS test.

Methods: Semen samples from 103 men with couple infertility (normozoospermia, n = 8; dyspermia, n = 95), aged 20 to 66 years, underwent conventional semen analysis. The effect of time (one and three hours after ejaculation) on three categories of sperm motility [rapid progressive (RP), total progressive (TP), total motile (TM)] was assessed.

Results: A significant decrease was observed in RP, TP and TM three hours after ejaculation compared with the baseline values (p < 0.001 for all categories). Sperm concentration, total sperm count, normal morphology and the HOS test results were positively correlated with RP, TP and TM three hours after ejaculation (p < 0.001 for all categories). In contrast, the abnormal sperm forms (head and tail) were negatively correlated with RP, TP and TM three hours after ejaculation (p < 0.001 for all categories). The HOS test result explained 51%, 68% and 79% of the decrease in RP, TP and TM, respectively, three hours after the ejaculation.

Conclusions: The decrease in sperm motility between one and three hours after ejaculation is significant and depends on several semen parameters. The HOS test may help in predicting the semen quality three hours after ejaculation, which might be of clinical importance in cases of assisted reproductive technique (ART) procedures.
ABSTRACT

Biochemical markers of spermatogenesis and fertility assessment are important in the practical management of infertile males and the determination of an individual’s prognosis. We performed an analysis on 100 males with a male infertility factor. The following study inclusion parameters were analyzed: semenogram, FSH, LH, testosterone, estradiol, prolactin, TSH, and inhibin B concentrations. The patients were subsequently treated by reproductive endocrinologists in accordance with AUA/ASRM and EAU guidelines. The reproductive status was evaluated over a period of 3 years. We found a strong correlation of sperm count with inhibin B ($r = 0.744$, $p < 0.001$) and FSH concentration levels ($r = -0.464$, $p < 0.001$). Among 95 patients at follow-up, pregnancies occurred for 59 of their partners (48 spontaneous, 5 after IVF–ET, and 6 after IUI). Thirty-six patients remained childless despite the therapy. Sperm count and inhibin B level were the best predictors of natural fertilization (ROC AUC: 0.86 and 0.84; cut-off: 2.7 min/mL and 45 pg/mL). Although inhibin B and FSH can be used to evaluate spermatogenesis and fertility, the initial sperm concentration appeared to be the best predictor of success. Pregnancy was achieved in a surprisingly large proportion of patients with a very low concentration of inhibin B and a low initial sperm count. It is noteworthy that 81% of the pregnancies were achieved without medically assisted reproduction.

P105 | Is oxidative stress evaluated in viable human spermatozoa a marker of good semen quality?

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Background: Oxidative stress (OS) is defined as unbalance between reactive oxygen species (ROS) production and antioxidant defences. Low levels of ROS are necessary for physiological sperm functions, whereas high levels impair fertility damaging membranes, proteins and DNA. Previous studies, performed by using different methods and probes for OS evaluation in semen or in spermatozoa, highlighted the negative role of ROS on sperm functions. However, such studies were not conclusive because of the small number of included subjects and of high variability in the cohorts. In the present study, we used two probes, CellROX® Orange Reagent and Dihydroethidium (DHE), which reveal different ROS in viable spermatozoa, to evaluate the association between ROS and standard semen parameters and DNA fragmentation.

Methods: Sperm ROS were evaluated by two fluorescent probes revealed by flow cytometry: CellROX® Orange, that reveals hydrogen peroxide, and DHE that shows distinct specificity toward both superoxide anion and hydrogen peroxide. Sperm DNA fragmentation (by TUNEL/PI method), sperm kinematic parameters and hyperactivated motility (by C.A.S.A. system) were concomitantly assessed. Phosphatidylserine membrane exposure was evaluated by Annexin V.

Results: To demonstrate that the two probes were sensitive to an induction of ROS in spermatozoa, we used Tert-Butyl hydroperoxide (TBHP, an analog of hydrogen peroxide) for CellROX® Orange and Menadione (which generates superoxide) for DHE. After incubation with TBHP for 30 min at 37°C, an increase of the percentage of positivity CellROX® Orange spermatozoa was observed. Similarly, when Menadione was added to spermatozoa for 30 min at room temperature, a noticeable increase in DHE positivity was revealed. We evaluated ROS levels in 121 semen samples, and we found that ROS in viable spermatozoa, assessed with the two probes, were positively associated with motility, morphology and number and negatively with DNA fragmentation. In unviable spermatozoa, ROS levels were negatively associated with semen quality. The positive correlations between ROS in viable spermatozoa and semen parameters, above described, indicate that the oxidative status revealed by the two probes is related to a better sperm quality. To further investigate this possibility, we double labelled spermatozoa with CellROX® Orange and Annexin V, a marker of early signs of apoptosis. We found that only 2.8% of Annexin V positive spermatozoa was also positive for CellROX® Orange, indicating that the probe mostly identifies spermatozoa without apoptotic features. To further verify that CellROX® Orange identifies spermatozoa with better quality, we evaluated CellROX® positivity in swim-up selected spermatozoa finding significantly higher levels of CellROX® Orange positivity respect to unselected samples.

Conclusion: Our results suggest that ROS detection with CellROX® Orange and DHE identifies viable spermatozoa with better performance.

P138 | Which sperm parameter limits could really guide the clinical decision in assisted reproduction?

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Background: The predictive role of sperm motility and morphology was recently detected in a large sample of more than 20000 assisted reproductive technology (ART) fresh cycles. However, the complete ART procedure consisted in both fresh and frozen-embryo transfers and only a comprehensive evaluation of the entire process could really confirm if these parameters really predict the ART success.
Aim of the study: To identify predictive parameters of ART success, applying a real-world data analysis (RWD) on the entire ART path, combining fresh and frozen cycles.

Materials and methods: A retrospective RWD analysis was performed, enrolling all couples attending a single ART centre from 2008 to 2021. The analysis included both fresh and frozen cycles, and both in vitro fertilization (IVF) and intra-cytoplasmic sperm injection (ICSI) procedures. Primary endpoints were strong ART outcomes, i.e., biochemical and clinical pregnancies and live birth rates (LBR).

Results: Fresh cycles success (considering LBR) was predicted by female age (OR: 1.04 [1.02-1.06]), injected oocytes (0.96 [0.93-0.99]), embryo number (0.79 [0.75-0.83]) and progressive sperm motility (0.98 [0.97-0.99]). On the contrary, frozen cycles outcomes were predicted only by sperm motility (0.97 [0.95-0.99]). This prediction was confirmed in IVF but not in ICSI cycles.

Conclusions: Both female and male’s parameters predicted the ART success considering entire path. However, frozen cycles success was predicted only by progressive sperm motility, suggesting that the potential amelioration of this male parameter is relevant to improve ART success. Those couples expected to obtain the highest embryos number after fertilization (low female age and better semen parameters) will have more attempts with frozen cycles and thus would benefit of a potential treatment focused to improve sperm parameters.

P139 | Diagnostic and management challenges of Leydig cell tumor in infertile man: a case report

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Introduction: Increasing referrals for testicular imaging during infertility investigation have led to an increase in findings of Leydig-cell tumors (LCTs). These tumors account for only 1–3% of testicular neoplasms. In general occur unilaterally, with only 3% of cases found bilaterally. LCTs are strongly associated with male infertility. Up to 20 percent of Leydig cell tumors in adults are classified as malignant based predominantly upon large size, vascular invasion and increased mitotic activity. The differential diagnosis for a Leydig cell tumor also includes adrenal testicular rest tumors that are found in men with congenital adrenal hyperplasia.

The diagnostic and treatment pathways can be challenging, because there are no evidence-based guidelines for the clinical management of LCT.

Case: 33-years-old male patient was referred to endocrinologist’s due to infertility, lasting for approximately one year with a normal sexual life.

After clarification of past medical history testicular tumors emerged. In 2019, the patient was consulted by the urologist, ultrasound revealed lesions in the testicles on both sides. It was decided to remove the right testicle on suspicion of a malignant process. Histopathological features were characteristic to testicular tumor associated with congenital adrenal hyperplasia/testicular adrenal rest tumors. In the Department of Endocrinology, additional investigation due to infertility was performed, elevated 17-OH-progesterone (11.4 nmol/l), elevated follicle-stimulating hormone (FSH) 23.4 U/l and slightly decreased testosterone (11.6 nmol/l) were determined. A routine semen test indicated azoospermia. Repeated ultrasound revealed 1.1x0.7 and 0.9x0.7 cm diameter hypoechoic lesion in the left testicle (an approximate volume of the testicle was 20ml). Other laboratory tests were within normal limits (CEA 1.8 mcg/l, AFP 0.83 kU/l, βHCG 0.6 U/l, LDH 189 U/l, LH 8.84 U/l, SHBG 29 nmol/l, inhibin B 47.19 ng/l). Depending on the patient test results, congenital adrenal hyperplasia (CAH) was suspected. Cosyntropin stimulation test, CYP21A2 gene mutation test and steroid profiling were performed. After analysis of laboratory tests, CAH was excluded. Remaining unclear diagnosis was decided to perform biopsy of the testicle. Histopathological analysis confirmed Leydig cell tumor with low proliferation index. Furthermore, paraffin samples with the right testicle postoperative material were re-examined, LCTs were confirmed bilaterally.

The patient was discussed at multidisciplinary team meeting, cause of azoospermia was confirmed and it was decided to continue active surveillance (testicular ultrasound twice-yearly, chest-abdomen-pelvis CT yearly) and refer patient to fertility and reproductive medicine center for mTESE (depending on the concentration of inhibin B, the chance of ongoing spermatogenesis remains).

Conclusion: Due to its low incidence, the management of these small tumors is still a challenge for urologists, endocrinologist, surgeons, and pathologists. The management options for these lesions include radical orchietomy, active surveillance and testis-sparing surgery. If the initial work-up is without pathological findings and no other risk factors for malignancy are detected, active surveillance appears a safe option, once the diagnosis is ascertained using the latest imaging approaches.

P140 | Translational medicine in Andrology

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The goal of translational medicine is to conduct research the results of which can be used directly at the bedside of the patient. This is how we chose the currently hot topic in andrology – sperm DNA fragmentation (SDF) and the risk factors associated with it.

Sperm DNA fragmentation is a marker of fertility, and it shows how many sperm cells have fragmented DNA. There are several factors influencing SDF – both modifiable and non-modifiable ones.

To get a clear picture of all risk factors involved, we conducted our search in the 3 main databases (PubMed, Embase and Central), which yielded almost 27,000 articles and we could extract data from over 200
of them. We were able to summarize the following and many other risk factors as part of our meta-analysis: regarding associated health conditions, the presence of varicocele compared with its absence increases SDF by more than 10%. Abnormal glucose tolerance compared with normal glucose tolerance has an even greater impact – almost 14%. From infections, HPV had close to no effect (0.16%) on SDF, the presence of Chlamydia had a moderate (4.87%), but statistically insignificant effect, whereas the presence of STIs had a moderate (5.54%), but statistically significant effect. Regarding tumors, testicular malignancies had moderate effect on SDF, slightly below 10%, Hodgkin-lymphomas had statistically significant, but clinically mild effect with an increase in SDF by less, than 4%, non-Hodgkin lymphomas had a higher – over 6% – increase, but statistically insignificant effect. Lymphomas in general, result in a moderate increase of 5.19%, whereas leukemias mean an increase of 6.07%, but a statistically insignificant one.

Regarding lifestyle factors, when comparing normal BMI with the other categories, only underweight vs. normal BMI came out to be statistically significant, but neither of the three comparisons (regarding WHO BMI categories) were clinically significant, though it was still obvious that as BMI increased, SDF also increased. The difference in SDF for alcohol consumption did not come out to be statistically significant either, but a dose-dependency could be observed when comparing heavy drinkers vs. abstainers and moderate drinkers vs. abstainers. Smoking also showed a dose dependency: the heavy smokers vs. non-smokers comparison came out to be both statistically and clinically significant though, with a difference of almost 10% of SDF.

In accordance with previously suggested short ejaculatory abstinence period, we looked at different abstinence times, but none of the results suggested that it would be beneficial to increase (or decrease) ejaculatory frequency.

Another risk factor I would highlight is age, for which we found that when considering SDF only, there’s a more drastic increase after the age of 50 with a mean difference of 12.58%.

Our results contradict some of the currently popular “beliefs” regarding the risk factors’ effect on SDF, like short ejaculatory abstinence time or the importance of HPV on SDF, but many other studies, especially the establishment of registries would be necessary to clarify the exact effects of all the risk factors.

**Introduction:** Fasting is likely to be a therapeutic way to cure or at least to help to cure some pathologies as obesity, endocrinologic disorders and even cancers. As an example of fasting, Ramadhan, a holy month, during which moslems abstain from eating, drinking, smoking, and sexual intercourse. The period of Ramadhan fast occurs during the day for 14 hours per day, and the subsequent 10 hours period is open for consumption of food, drink, and sexual intercourse. The aim of this study was to determine the potential impact of Ramadhan fasting on sperm parameters.

**Patients and methods:** This retrospective, monocentric study was conducted on 50 patients, who were addressed to the Laboratory of Cytogenetics and Reproduction Biology (Monastir, Tunisia) for semen analysis during the year of 2020 and 2021. Two sperm analysis were conducted before and after Ramadhan covering the spermatogenesis period (3 months). Semen parameters were assessed and interpreted according to the WHO 2021 guidelines.

**Results:** The median age was of 35±5.24 years, the sexual abstinence delay was similar before the first and the second analysis (3.06±0.52 and 3.12±0.64 days, respectively).

We have shown that the mean total sperm motility decreased after the fasting period, from 32.2% to 28% (p= 0.020). A significant increase in the percentage of dead spermatozoa, which was 24.8% before fasting and were 29.5% after fasting (p= 0.024) was pointed out. The multiple abnormalities index was also higher after fasting rising from 1.81 to 1.89 (p= 0.046).

Comparing semen abnormalities, we have shown that the percentage of asthenozoospermia decreased from 74.5% to 90.2% (p= 0.006) and the percentage of hypospermiaincreased from 9.8% to 11.8% (p< 0.001).

**Conclusion:** Our study treats a non-explored aspect of fasting which obviously impacts male fertility. Contrary to the beneficial effect of fasting in many diseases, we have shown through the current study that fasting may impair semen quality, impacting vitality, motility, and morphology.

**Keywords:** Male infertility; semen parameters, fasting.

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**P141** | The effect of fasting on semen parameters of hypofertile men

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**Background:** Sex chromosome aneuploidies are defined by the loss or gain of one or more sex chromosomes. 48,XXYY syndrome is a very rare syndrome of males (up to 1:20,000 live births), phenotypically similar to Klinefelter Syndrome, but with a more severe clinic. Clinically, it is characterized by tall stature, testicular dysfunction associated with infertility and reduced testosterone production, cognitive, affective and social interaction deficits, global development delay and increased risk of congenital defects.

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Case report: a 36-years-old man presented to our andrological outpatient clinic for secondary infertility. Patient reported a history of orchidopexy for right cryptorchidism at 6 years old and former thrombophlebitis. Upon right physical examination our patient’s height was 184 cm and he weighed 120 kg (Body Mass Index = 35.5 kg/m²); external genitalia were normal, except for small bilateral testes. At scrotal ultrasound we found testicular hypotrophy with right dysmetry (right volume 6.2 mL, left volume 9 mL). Both testes were characterized by markedly and diffusely inhomogeneous echotexture due to the presence of different echogenicity areas, with increased vascularization. Semen analysis revealed oligospermia (total sperm count: 5 x 10⁶), reduced motility (15% progressive and 5% dyskinetic), teratozoospermia (atypical 98%). Hormonal assessment revealed a low testosterone (9.6 nmol/L), with elevated FSH (15.15 mU/mL) and high LH (9.45 mU/mL). Thyroid assessment and prolactin levels were within the normal range. A molecular study to detect Y chromosome microdeletion was performed using the Multiplex Oligo-Azoospermia Kit1 and the result showed no deletion in any of the Y chromosomes. Therefore, a karyotype was performed and showed a 48,XXYY male karyotype due to the presence of a duplicated X chromosome and a small acrocentric chromosome, identified as part of the short arm of the Y chromosome: 48,XXY + mar.arr [GRCh38] Yp11.31p11.2(311030_6212.305)x2.

Conclusion: Major aneuploidies were initially considered a variant of Klinefelter syndrome but nowadays it are widely accepted as distinct clinical and genetic syndromes. A review of the literature showed that all 48,XXYY patients are usually azoospermic. To the best of our knowledge, this is the first case of a 48,XXYY patient with spermatogenesis, probably due to the extra part of the short arm of the Y chromosome.

CLINICAL SCIENCE - OTHER

P107 | The effect of FSH on glucose metabolism in young healthy males

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Introduction: Follicle-stimulating hormone (FSH) has important effects on gonadal function, but it has been suggested that FSH also can have extra-gonadal effects with high levels impacting impairment of glucose metabolism.

Materials and Methods: Sixty-three healthy men aged 19-32 years had fasting blood samples taken at three-time points/visits (V1, V2, and V3). Gonadotropin releasing hormone (GnRH) antagonist was administered to all subjects at V1. One group (n = 16) was randomized to 300 IU recombinant FSH (rFSH) subcutaneously 3 times/week for five weeks. The rest (n = 47) served as controls not receiving rFSH. The V2 samples were obtained three weeks after V1, and at the same time point, 1000 mg testosterone undecanoate was administered intramuscularly. V3 samples were collected two weeks thereafter. Thus, men who received rFSH had increased FSH levels at V2 and V3, whereas controls had close to zero. Both groups had testosterone at castration level at V2, but slightly increased concentration of this hormone at V3. At all-time points, serum levels of lipids, glucose, and insulin were also measured. Moreover, the homeostatic model assessment of insulin resistance (HOMA-IR) and the triglyceride-glucose index (TyG) used for the prediction of type 2 diabetes mellitus and metabolic syndrome were calculated. TyG was calculated as ln (fasting triglyceride [mg/dL] x fasting glucose [mg/dL]/2). V2 and V3 levels were expressed as percentages of V1, and, using the Mann-Whitney test, the metabolic markers were compared between the two groups.

Results: At V2, the group receiving rFSH had statistically significantly higher glucose (medians: 104% vs. 98%, p = 0.003), and higher TyG levels (medians: 103% vs. 99%, p = 0.011) compared to controls. For all other comparisons, no statistically significant differences were found.

Conclusion: Increased FSH levels in the low testosterone milieu were associated with increased glucose levels and TyG. FSH may hence have a negative effect on glucose metabolism.

P108 | Cardiometabolic indices predict hypogonadism in male patients with type 2 diabetes

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Objective: To evaluate, in men with uncomplicated type 2 diabetes, the association of cardiometabolic indices [Visceral Adiposity Index (VAI), Triglyceride Glucose Index (TyG), and Lipid Accumulation Product (LAP)] with testosterone (T) plasma levels, and to assess their predictive cut-off values in identifying hypogonadism.

Research Design and Methods: Two hundred and sixty-five consecutive men aged 40-70 years with type 2 diabetes performed a clinical andrological evaluation, including IIEF (International Index of Erectile Function), IPSS (International Prostatic Symptoms Score), and AMSS (Aging Male Symptoms Scale) questionnaires. Metabolic parameters, total T (TT), and luteinizing hormone were determined. The association between the different variables was performed by multivariate analysis, and ROC (Receiver Operating Characteristic) curves were used to identify cut-off values of cardiometabolic indices in predicting low testosterone (TT < 12 nmol/l).
ABSTRACT

The effect of testosterone treatment on bone mineral density in Klinefelter syndrome

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Background: Although Klinefelter syndrome (KS) is the most frequent sex-hormone disorder, there is ongoing uncertainty about the often-associated sex-hormone deficiency, its impact on common comorbidities, and therefore about adequate prevention and treatment. In this study, we focus on bone loss, reported to occur in over 40% of KS patients.

Objectives: This single-center retrospective cohort study in a tertiary hospital assessed the effect of treatment with or without testosterone replacement therapy (TRT) on evolution of bone mineral density (BMD) in KS patients.

Methods: A total of 52 KS patients were included and divided into 3 groups, according to TRT use. BMD was measured by dual-energy X-ray absorptiometry (DXA) and expressed as T-scores.

Results: We observed significant gain in BMD T-score at the lumbar spine (0.58 ± 0.60, p = 0.003; mean gain of 0.62% areal BMD per year) and total femur T-score (0.24 ± 0.39, p = 0.041; mean gain of 0.25% areal BMD per year) after start of TRT. Compared to untreated patients, a significant difference in evolution was seen at the lumbar level (+0.58 ± 0.60 vs. -0.14 ± 0.42, p = 0.007).

In untreated patients without measured hypogonadism, a loss of BMD (-0.27 ± 0.37, p = 0.029; mean loss of 0.49% areal BMD per year) at the femoral neck was measured. This decline was equal to the predicted loss, seen in the general male population, and can thus be seen as clinically irrelevant.

Conclusion: TRT results in BMD gain, especially at the lumbar spine (0.62% per year), in patients with KS with clear hypogonadism before start of treatment. However, the effect remains limited, and patients who were not treated with TRT because of sufficient endogenous testosterone levels, did not suffer from substantial bone loss during follow-up. The need for TRT in maintaining bone health in KS may not be universal and should be evaluated on an individual basis according to the grade of sex steroid deficiency.

P110 | Introducing Cavernosal Banding: a new intraoperative strategy for significant weakening/loss of albuginea following multiple penile prosthesis surgeries

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We present an original technique to reinforce penile tunica albuginea during prosthetic surgery, that we deem appropriate in cases of significant weakening and/or loss of albuginea, particularly following multiple penile prosthesis surgeries. We named this technique “cavernosal banding”.

Case report: A 52 y.o. white male presented with a malfunctioning inflatable penile implant: upon activation no erection developed, while the scrotum enlarged. He had ad an overall of four former surgeries with a penoscrotal approach: a first inflatable implant and three replacements. MRI disclosed a bilateral extrusion of the cylinders at the likely area of former corporotomies for cylinders insertion, with aneurysm in the right side, and kinking in the left one.

Replacement surgery: we used a penoscrotal incision. In the extrusion area there was bilaterally a wide loss of albuginea, with weakened tissue in the adjacent areas.

CAVERNOSAL BANDING technique: We performed in the involved area a complete circumferential isolation of the albuginea of both corpora, i.e. isolating the corpora: dorsally from Buck’s fascia, inclusive of the neurovascular bundle, and ventrally from urethra. We also managed to develop some tissue planes to cover the albuginea loss in the area of the former corporotomies. All the components of the old device were removed, Henry’s stepwise antibiotic protocol was applied, and a new device (AMS 700CX 24 cm with bilaterally 5 cm rear tips) inserted.

The following were measured: circumference of isolated corpora in tumescence state (12 cm), and distance between the limits of the two isolated circumferences -proximal and distal- (5 cm). A 12x5 cm rectangle of Tutopatch® (scaffold of bovine pericardium) was then fashioned. When closing the albuginea losses of the former corporotomies, care was taken to proximally incise the tissue to reach healthy albuginea, to let the device connecting tubes exit there. Cavernosal banding was achieved transferring the fashioned Tutopatch® rectangle around the isolated albuginea of the two corpora cavernosa, with its smooth surface against the albuginea. The two short sides were sutured together and to the ventral albuginea, beneath the urethra. Following, anchoring stitches were applied to fix the patch to the albuginea, to hold the patch in site, flat. Final steps: closure in layers, suction drain kept overnight, device left deflated.
P111  |  miR-30b as a biomarker for male pubertal onset

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ABSTRACT

Background: Puberty marks the transition from childhood to adulthood, where full reproductive capacity is attained. For boys, a testicular volume of ≥ 4 mL is considered a hallmark of pubertal onset. Essential for pubertal onset is the activation and release of gonadotropin-releasing hormone (GnRH) from the hypothalamic neurons. In the rodent hypothalamus, the expression of Makorin ring finger protein 3 (MKRN3) decreases before the activation of the Hypothalamo-pituitary-gonadal (HPG) axis. In children, circulating levels of MKRN3 decrease during the peripubertal transition, although with high inter- and intra-individual variation. microRNAs have been shown to be involved in the reactivation of hypothalamic GnRH production and thus regulate pubertal timing in rodents. Specifically, miR-30b seems to directly influence MKRN3 expression and in boys, circulating levels of miR-30b increase during induced puberty. In this study, we measured the levels of miR-30b in serum from boys during pubertal development to reveal its potential as a novel biomarker for pubertal onset.

Materials and Methods: Forty-six boys from the longitudinal part of the Copenhagen Puberty Study were included. All boys underwent multiple clinical examinations including estimation of testis size by palpation. Age at pubertal onset was defined as the age between the last examination with a testis volume < 4 mL and the first examination with a testis volume ≥ 4 mL. The boys were between the age of 7.6 and 11.8 years when the first blood samples were collected. miR-30b was measured in serum by RT-qPCR. Thirty-nine boys had miR-30b levels measured in three consecutive samples (pre-, peri-, and post-pubertal onset) in serum. miR-30b was also measured in ten consecutive samples (4-5 pre-, 1 peri-, and 4-5 post-pubertal onset).

Results: There was a significant increase in circulating miR-30b levels when the post-pubertal samples were compared with the pre- and peri-pubertal samples; median pre: 6.77x10^-9, peri: 7.18x10^-9, and post: 1.58x10^-8, p = 1.21x10^-5 and p = 6.0x10^-4, respectively. There was great inter-individual variation in miR-30b levels during pubertal development (CV: 1.1) and we were unable to define a specific threshold of miR-30b levels for pubertal onset. The circulating concentrations of miR-30b and MKRN3 were not correlated (R2: 0.03).

Conclusion: Serum miR-30b increases during the pubertal transition supporting its role as a key activator of the HPG axis but is unlikely to serve as a novel clinical biomarker for pubertal onset.

P112  |  Comparison of triglyceride-glucose (TyG) index and homeostatic model assessment of insulin resistance (HOMA-IR) index in prediction of hypogonadism

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Introduction: Insulin resistance (IR) plays an important pathologic role in metabolic syndrome (MetS), which is associated with male hypogonadism. Homeostatic model assessment of insulin resistance (HOMA-IR) is a well-established marker of decreased insulin sensitivity. Recently, a new marker, the triglyceride-glucose index (TyG), calculated as ln (fasting triglyceride [mg/dL] x fasting glucose [mg/dL]/2), was suggested to be a cheaper and a reliable surrogate marker to detect IR. Our aim was to compare the TyG index with HOMA-IR in the prediction of male hypogonadism.

Material and Methods: The data on 192 subfertile patients (18-50 years; sperm concentration <20 x 106/ml) and population-based matched controls (n = 199) collected during the years 2009-2012 were evaluated retrospectively. Sperm concentration of 20 x 106/mL was used as cut off since, at the start of the study, this level was considered as lowest level of normozoospermia by World Health Organization. Half of these subjects (72 subfertile men and 122 controls) were re-investigated 5 to 10 years later. The patients receiving any hormonal therapy were excluded. Primarily, using ROC analysis, we defined cut off values of HOMA-IR and TyG for prediction of MetS at re-examination. Using these cut-off values, in a logistic regression model, we tested the association between normal/abnormal HOMA-IR or TyG and +/− hypogonadism. Hypogonadism was defined as fasting, morning serum testosterone below 12 nmol/L.

Results: In ROC curve analysis, for prediction of future incident MetS, HOMA-IR had slightly higher AUC values than TyG (AUC: 0.886, p < 0.001 vs. 0.816, p < 0.001). The optimal diagnostic cut-off values for HOMA-IR and TyG were 2.68 and 8.60, respectively. Moreover, in binary logistic regression analysis the ORs for having hypogonadism were 4.93 (95% CI: 2.77 – 8.76; p < 0.001) for high values of HOMA-IR and 2.19 (95% CI: 1.25 - 3.824; p = 0.006) for high TyG, respectively.

Conclusion: Both high HOMA-IR and high TyG are significantly associated with the risk of hypogonadism. This association seems to be stronger for HOMA-IR than for TyG. However, being not user-friendly and expensive to calculate limits HOMA-IR usage in clinical practice.
ABSTRACT

Education degree as a predictor of major adverse cardiovascular events in men suffering from erectile dysfunction

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Background: The level of education has been recognized as a CV risk factor; nevertheless, it is often neglected in CV risk stratification.

Aim: To evaluate, in men seeking medical care for ED, whether the level of education is associated with cardio-metabolic risk factors, sexual and psychological features and if it could predict the occurrence of forthcoming major adverse CV events (MACE).

Methods: A consecutive series of 3733 men (aged 49.8 ± 13.7 years) attending an andrology outpatient clinic for ED was studied. A subset of 956 patients was included in a longitudinal retrospective study with a mean follow-up 3.9 ± 2.4 years. Patients were categorized according to the education level (higher, upper secondary, lower secondary, and primary).

Outcomes: Several clinical, biochemical and instrumental (penile color Doppler ultrasound; PCDU) parameters were evaluated. In the longitudinal study, incidence of MACE was assessed.

Results: As compared with men with university degree, those with lower education had an increased frequency of moderate-severe ED (OR = 1.21 [0.99;1.48], 1.41 [1.14;1.73], 1.70 [1.26;2.30] for upper secondary, lower secondary and primary school, respectively) and reduced flaccid peak systolic velocity at PCDU. Men with a lower level of education had a worse metabolic profile, including higher waist circumference, glucose and glycosylated hemoglobin. Accordingly, patients with a lower level of education had a greater probability of suffering from metabolic syndrome (OR = 1.38 [1.06;1.79], 1.73 [1.34;2.24], 1.72 [1.24;2.37] for upper secondary, lower secondary and primary school, respectively) and were more likely to have personal history of previous CV events. Subjects with primary education had a higher estimated 10-year CV risk according to Progetto CUORE score (mean difference from university degree group = 1.94 [1.01;2.87]%). When considering patients included in the longitudinal evaluations, those with higher level of education had a significantly lower incidence of MACEs. The protective role of higher education was confirmed even after adjusting for confounders (HR = 2.14 [1.24;3.69]).

Conclusion: In subjects consulting for ED, lower level of education is associated with a more severe ED of atherogenic pathogenesis and with a worse cardio-metabolic profile. In addition, a lower level of education predicts forthcoming MACEs. Education level should be considered as a costless but valuable information in the assessment of CV risk in patients suffering from ED.
P115 | Biochemical predictors of structural hypothalamic-pituitary abnormalities detected by magnetic resonance imaging in men with secondary hypogonadism

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Purpose: Organic conditions underling secondary hypogonadism (SH) may be ascertained by magnetic resonance imaging (MRI) of the hypothalamic-pituitary region that could not be systematically proposed to each patient. Based on limited evidence, the Endocrine Society (ES) guidelines suggest total testosterone (T) < 5.2 nmol/L to identify patients eligible for MRI. The study aims to identify markers and their best threshold value predicting pathological MRI findings in men with SH.

Methods: A consecutive series of 609 men seeking medical care for sexual dysfunction and with SH (total T < 10.5 nmol/L and LH ≤ 9.4 U/L) was retrospectively evaluated. An independent cohort of 50 men with SH was used as validation sample. 126 men in the exploratory sample and the whole validation sample underwent MRI.

Results: In the exploratory sample, patients with pathological MRI findings (n = 46) had significantly lower total T, luteinizing hormone (LH), follicle stimulating hormone (FSH) and prostate specific antigen (PSA) than men with normal MRI (n = 80). Receiver Operating Characteristics analysis showed that total T, LH, FSH and PSA are accurate in identifying men with pathologic MRI (accuracy: 0.62-0.68, all p < 0.05). The Youden index was used to detect the value with the best performance, corresponding to total T 6.1 nmol/L, LH 1.9 U/L, FSH 4.2 U/L and PSA 0.58 ng/mL. In the validation cohort, only total T ≤ 6.1 nmol/L and LH ≤ 1.9 U/L were confirmed as significant predictors of pathologic MRI.

Conclusion: In men with SH, total T ≤ 6.1 nmol/L or LH ≤ 1.9 U/L should arise the suspect of hypothalamus/pituitary structural abnormalities, deserving MRI evaluation.

P116 | Clomiphene citrate for male hypogonadism

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Background: Hypogonadism is a worldwide problem among men causing sexual, physical and mental problems. Testosterone therapy is the first-choice treatment for male hypogonadism, with several side-effect i.e. subfertility. Clomiphene citrate (CC) is an alternative off-label therapy for hypogonadal men especially for those with an active or future child wish. There is scarce literature in usage of CC for men with hypogonadism. The aim of this retrospective study was to evaluate the effectiveness and safety of CC for hypogonadal males.

Methods: In this single-centre study, men treated with CC for hypogonadism were evaluated retrospectively. Primary outcome was hormonal evaluation including total testosterone (TT), free testosterone (FT), luteinizing hormone (LH) and follicle stimulating hormone (FSH). Secondary outcomes were hypogonadal symptoms, metabolic and lipid parameters, hemoglobin (Hb), hematocrit (Ht), prostate specific antigen (PSA), side-effects, reversed TT response, the effect of a physician-initiated proof stop and potential predictors for biochemical and/or clinical response.

Results: In total, 153 hypogonadal men were treated with CC. Mean TT, FT, LH, and FSH increased during treatment. TT increased from 9 to 16 nmol/L, with a biochemical increase in 89% of the patients. Increased level of TT persisted after eight years of treatment. With CC treatment, 74% of the patients experienced hypogonadal symptom improvement. LH at the lower normal range before CC treatment was predictive for better TT response. During CC therapy, few side-effects were reported and no clinical important changes in PSA, Hb and Ht were found.

Conclusion: CC seems to be an effective therapy on short- and long-term, improving both clinical symptoms and biochemical markers of male hypogonadism with few side-effects and good safety aspects.

Keywords: Clomiphene citrate, male hypogonadism, testosterone deficiency syndrome

P117 | Reproductive Parameters and Bone Mineral Density in Men from infertile couples - first clinical data from ReproUnion Biobank and Infertility Cohort (RUBIC)

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Objective: Male infertility is associated with shorter life expectancy and higher risk of developing various non-communicable diseases. Research has indicated associations between subfertility and testosterone deficiency which, to a certain degree, might serve as a possible mediator between impaired fertility and co-morbidity. Low levels of sex hormones may have a negative effect on bone mineralization. If untreated, testosterone deficiency leads to decreasing bone mineral density (BMD). However, the studies showing the association between total testosterone (T) and BMD often focused on men in their 60s and 70s. Thus, possible preventive measures would be of relatively limited success at this time point. Bone health among younger men is scarcely studied. Previous study has shown decreased BMD to be associated with hypogonadism in a small group of young sub fertile men. We
Pharmacodynamics and safety of human recombinant luteinising hormone (LH) in hypogonadotropic hypogonadal men: a new ongoing multicenter study

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State of the art: In pharmacology, human chorionic gonadotropin (hCG) is considered equivalent to luteinising hormone (LH) since both act on the same receptor. Thus, when testicular function needs to be clinically restored (i.e. in case of hypogonadotropic hypogonadism [HH]), hCG is used instead of LH. However, growing evidences showed LH and hCG activate different molecular pathways and offer different outcomes in women undergoing assisted reproduction. The different action between LH and hCG is still not evaluated in men.

Aim of the study: To assess the pharmacodynamics of recombinant LH in HH men, comparing recombinant LH (Luveris®) to the gold standard approach, i.e. hCG (Gonasi HP®).

Study design: A multicenter, longitudinal, randomized, open label, phase II, ‘non-inferiority’ clinical trial was designed. Endpoints will be testosterone serum levels and drug safety. 32 men with acquired HH will be enrolled and randomized (1:1) to study group treated with Luveris or to control group treated with Gonasi. In both groups, increasing drug doses will be administered (75, 150, 225, 300 IU daily for LH and 500, 1000, 1500 and 2000 IU two times weekly for hCG). Both treatments will be performed for 8 weeks, during which the patient will be evaluated twice weekly, followed by a 4-week washout period. Testosterone and its metabolite will be evaluated at the end of study using the gold standard mass spectrometry methodology.

Expected results: We expect to describe for the first time the pharmacodynamics of both LH and hCG chronically administered in men, creating a dose-response curve for both compounds. Moreover the clinical hCG dosage is still empirical, thus we will recognize the best treatment regimen to restore normal testosterone levels in HH, for both LH and hCG treatment. This study is preliminary to further studies assessing LH in hypogonadal and/or infertile patients.

Current study state: The study started in March 2022 and we currently enrolled 3 HH patients.

An overview on the biochemical assessment of male andrological status using sex hormone-binding globulin and total and free testosterone in European clinical laboratories

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Sex steroid deficiency in men with chronic kidney disease is characterized by low T/LH ratio, suggesting testicular failure

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Introduction: Sex steroid levels have been reported to be decreased in male patients with chronic kidney disease (CKD), parallel to the severity of the kidney dysfunction. These studies, at large, used immunoassays, a method that is inferior to gold standard chromatography measurements and, in addition, failed to give insights into the underlying pathophysiology as they lacked data on estradiol and gonadotropin levels.

Objective: Comprehensive mapping of the gonadal status in male CKD patients not yet on dialysis and age- and BMI-matched controls, using gold standard techniques.

Methods: We performed a case-control study in 121 male CKD patients (age 65.0 ± 8.5 years, BMI 26.8 ± 4.2 kg/m2), matched (1:1) with non-CKD controls for age and BMI. We divided CKD patients into 3 categories based on KDIGO classification: CKD 1-2 (n = 24), CKD 3 (n = 42) and CKD 4-5 (n = 55). We measured total testosterone (T) and estradiol (E2) using liquid chromatography tandem mass-spectrometry. Albumin, sex hormone binding globulin (SHBG), luteinizing hormone (LH), follicle stimulating hormone (FSH) and prolactin levels were measured on the Roche Cobas 8000 platform. Free T levels were calculated via Vermeulen formula.

Results: CKD patients showed lower total T levels (421.0 ng/dL [14.1-1350.0] vs. 541.0 ng/dL [15.5-985.0]; p < 0.0001) and lower free T levels (7.4 ng/dL +/-3.2 vs. 9.3 ng/dL +/-2.7; p < 0.0001) as compared to non-CKD counterparts. SHBG and E2 levels, conversely, were comparable in cases and controls. Prolactin levels were only increased in CKD 4-5 patients as compared to controls. Finally, LH and FSH levels were higher in CKD patients (9.1 IU/L [2.4-102.6] vs. 5.3 IU/L [0.3-25.5]; p < 0.0001 and 7.8 IU/L [1.4-118.0] vs. 5.6 IU/L [1.7-71.6]; p < 0.0001) respectively. Consequently, the T/LH ratio, a marker of testicular function, was markedly depressed in CKD (1.73 [0.03-5.57] vs. 3.98 [0.50-10.51]; p < 0.0001). In the CKD cohort, regression analysis identified eGFR and age as independent determinants of T/LH ratio (R2 0.2988).

Conclusion: Male patients with CKD not yet on dialysis show low total and free T levels, confirming CKD as a risk factor for male hypogonadism. The high levels of gonadotropins and low T/LH ratio point to testicular failure as the underlying pathophysiological mechanism.
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Background: ED is the most common sexual problem in male PLWH, occurring earlier and more frequently in comparison to age-matched HIV-uninfected men. Although several HIV-related factors (e.g. duration of infection, antiretroviral drugs) rather than classical risk factors (sex steroids) have been identified as causative factors for ED in HIV, the impact of each factor has not been well established so far.

Aim: To investigate the contribution of sex steroids and other etiopathogenetic components in ED development in a cohort of young to middle-aged HIV-infected men.

Methods: A prospective, cross-sectional, observational study was conducted enrolling HIV-infected men aged <50 years on receiving antiretroviral therapy (ART). The validated questionnaires International Index of Erectile Function (IIEF-15, IIEF-5) and Structured Interview of Erectile Dysfunction (SIEDY) were used to assess sexual function; particularly, ED was defined as a score ≤25 at the specific domain of IIEF-15 (3), <22 at IIEF-5 (4) and <2 at SIEDY appendix A (question 1a+2) (5). IIEF-15 version specific for homosexual subjects was not used since the translation in Italian is still to be validated. Psychological component of ED was explored using scale 3 of SIEDY survey. Serum total testosterone (TT), estradiol (E2) and dihydrotestosterone (DHT) were measured by liquid chromatography-tandem mass spectrometry (LC-MS/MS), while free testosterone (cFT) was calculated by Vermeulen equation. Lifestyle habits, use of phosphodiesterase type 5 inhibitors (PDE5-i), sexual orientation and morbidity of patients were recorded.

Results: A total of 313 consecutive HIV-infected men were enrolled (median age 47.0 years [25.2:50.5]; median duration of HIV-infection 16.2 years [1.1-35.4]). At bivariate regression analysis, IIEF-15 score was inversely related to years of HIV-infection (p = 0.002), SIEDY scale 1 (p = 0.014) and scale 3 (p = 0.42); no significant relation was found between ED and sex steroids. At multivariate analysis only SIEDY scale 3 was included in the model. A robust linear regression was found between IIEF-15 score and IIEF-5 score (p = 0.0001; R2 0.546) as well as between IIEF-15 and SIEDY Appendix A (p < 0.0001; R2 0.123). According to IIEF-15, 187 patients (59.7%) had ED, with more severe ED degree in the last decade of age (p = 0.003). ED was more prevalent among homosexual than heterosexual patients (p = 0.003); no difference was found considering smoke, BMI, diabetes and cardiovascular diseases. Only 18.7% (n = 35) of patients affected by ED reported the use of i-PDE5.

Conclusions: In PLWH ED seems to be not strongly influenced by patients’ gonadal status and other classical risk factors non-related to HIV. Conversely, the psychogenic sphere, assessed by SIEDY scale 3, is found to be more deeply associated to the onset of ED in PLWH, further highlighting the contribution of peculiar factors related to HIV distress (e.g., fear of virus transmission, changes in body image, and stigma). IIEF-15, IIEF-5 and SIEDY Appendix A scores correlate each other, suggesting that these tools are equally reliable in recognizing ED patients even in PLWH. Despite the high prevalence of ED, only few patients report the use of PDE5-i revealing that sexual issues tend to go unaddressed in the clinical HIV management.

Objective: Expression of follicle-stimulating hormone FSH (FSH) receptor has been reported in many extra-gonadal tissues, raising the question of non-reproductive effects of FSH. Increasing usage of FSH in treatment of male infertility warrants deeper knowledge of possible harmful off-target effects of FSH.

Methods: In total 33 healthy men were included. At the first visit (V1), all received a s.c. injection of gonadotropin-releasing hormone (GnRH) antagonist and subsequently, 16 men were randomized to recombinant FSH (300 IU 3 times/week) for 5 weeks. N = 17 served as controls. Blood samples were taken at V1, after 3 weeks (V2) and after 5 weeks (V3), when the study ended. At V2, all subject received 1000 mg testosterone undecanoate i.m. At V2 and V3 relative levels of a standard set of bio- and inflammatory markers were compared between the groups using Mann-Whitney test adjusted for multiple testing. Additionally, in order to increase statistical power, a sub analysis was performed including 31 men to the control group from a previous study with similar protocol except the administration of FSH.

Levels of following markers were measured at all three time points: P-testosterone, P-sex hormone binding globulin (SHBG), P-follicle stimulating hormone (FSH) and P-luteinizing hormone (LH). Furthermore, P-thyroid-stimulating hormone, P-C-reactive protein (CRP), P-Sodium, P-Potassium, P-Creatinine, P-Albumin, P-Calcium, P-Urea, P-Magnesium, P-Troponin T, P-creatine kinase, P-aspartate aminotransferase (ASAT), P-alanine aminotransferase (ALT), P-alkaline phosphatase (ALP), P-Gamma-Glutamyl Transferase (GT), P-Cholesterol, P-Triglycerides, P-Apolipoprotein A1, Apolipoprotein B incl. ratio, P-Haemoglobin, B-Leukocytes, B-Thrombocytes and Blood Differential Test. These biomarkers were chosen because they mirror the function of different non-reproductive organs.

Results: As compared to controls, the FSH treated men had higher levels of SHBG (p = 0.024) and albumin (p = 0.027) at V2, and lower levels of alanine aminotransferase (p = 0.026) and magnesium (p = 0.028) at V3. However, none of the results remained statistically significant after Bonferroni correction (p > 0.0011). The sub analysis with the extended cohort did not change the results.
Conclusions: When FSH was given in standard therapeutic doses to young men for five weeks, it had no significant effects on selected parameters monitoring the function of non-reproductive organs. Therefore, FSH treatment can be considered safe in otherwise healthy young men, being candidates for infertility treatment with FSH.

P123 | Pathophysiology and molecular insight of testicular germ cell tumor

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Aims: This study aims to address sperm reproductive parameters and testis germ cell pathologies related to testicular germ cell tumour (TGCT) from both experimental and clinical angles. The goal is to deliver novel diagnostic strategies for the evaluation of sperm samples collected from TGCT men prior to surgery and after the therapeutic intervention, to monitor and compare the quality of fertility parameters and recommend the first choice of cryopreserved semen sample for assisted reproduction.

Methods: A combination of Ki67, a marker of cell proliferation was used for TGCT proliferation assessment accompanied by hematoxylin-eosin stain based histopathological assessment. Assessment of tumour progression and sperm quality parameters such as acrosome integrity (PNA lectin), DNA integrity (TUNEL), motility (CASA) and apoptosis (ApoFlowEx) were addressed. Assessment of testicular and sperm mitochondrial activity was evaluated by oroboros O2k based on oxygen consumption by complex I and complex II and complex IV and OXPHOS by Seahorse. The 2-photon FLIM was performed to assess NADH lifetime in mitochondria of sperm samples and TGCT tissue. Gene regulation was assessed by qPCR to understand the genomic expression patterns and roles. Ultrastructure of sperm was analysed by Cryo-SEM and Cryo-TEM to assess the sperm morphology in TGCT patients for the first time. Epigenetic profiling and analysis of sperm and testicular germ cell epigenome was addressed through monitoring of H3K9ac, H3K36me3 and H3K27me3 patterns.

Results: Sperm analyses of acrosome integrity and sperm motility provide a detailed analysis of impaired sperm function which correlates with elevated DNA fragmentation. Sperm parameters correlate with the severity of the TGCT tumour assessed by Ki67 marker. Sperm morphology and motility is highly affected. Mitochondrial respiration and ATP generation in TGCT sperm are lower than for normospermic sperm. Oxygen consumption by mitochondrial complex I, complex II, and complex IV was estimated and revealed that CI CII and CIV oxygen consumption is higher in tumour compared to normal tissue. High heterogeneity in the TGCT tissue for NAD(P)H is discovered, and patients’ specific unique lifetimes ranging between 0-5 ns, indicate differences in respiration metabolism. Various important genes related with spermatogenesis and germ cell development are down-regulated. The epigenetic profile of sperm is severely altered and a multi-parametric correlation of TGCT sperm pathologies is needed.

Conclusions: A ki67 proliferation marker is a promising tool for an assessment of the severity of TGCT. Histone modification assessment in testicular tissue and sperm shows differences between tumour and non-tumour tissue. Total and progressive sperm motility by CASA points to the best sperm quality in the sample prior to 1st and 2nd chemotherapy. Assessment of acrosome damage is crucial for the prediction of optimal IVF method and correlates with DNA fragmentation. The altered mitochondrial activity in TGCT patients’ sperm and testicular tissue compared to sperm of healthy donors and non-tumour tissue points to the potential use of mitochondrial markers as a diagnostic tool for reproductive parameters of TGCT affected men.

P124 | Impact of the cancer treatment received and the disease itself on the quality of the human (pre)pubertal testicular tissue prior to testicular tissue freezing

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Background: Testicular tissue freezing (TTF) is proposed for fertility preservation in (pre)pubertal boys with cancer before highly gonadotoxic treatment. Studies accurately comparing human (pre)pubertal testicular tissue quality before freezing and after thawing are exceptional. No study has reported this approach in a systematic manner and routine care.

Objectives: To assess the impact of a control slow freezing (CSF) protocol on testicular tissue architecture and integrity of (pre)pubertal boys after thawing.

Materials and Methods: (Pre)pubertal boys (n = 87) with cancer from 8 Reproductive Biology Laboratories of the French CECOS network benefited from TTF before hematopoietic stem cell transplantation. Seminiferous tubule cryodamage was determined histologically by scoring morphological alterations and by quantifying intratubular spermatogonia and the expression of DNA replication and repair marker in frozen-thawed testicular fragments.

Results: A significant increase in nuclear and epithelial score alterations was observed after thawing (p < 0.0001). The global lesional score remained lower than 1.5 and comparable to fresh testicular tissue. The number of intratubular spermatogonia and the expression of DNA replication and repair marker in spermatogonia and Sertoli cells did not vary significantly after thawing. These data showed the good preservation of the seminiferous tubule integrity and architecture after thawing, as previously reported in our studies performed in prepubertal mice and rats.

Discussion: The current study reports, for the first time, the development of a semi-quantitative analysis of cryodamage in human (pre)pubertal testicular tissue, using a rapid and useful tool that can be proposed in routine care to develop an internal and external quality control for TTF. This tool can also be used when changing one or several parameters of the freezing-thawing procedure.

Conclusion: CSF protocol without seeding maintains the seminiferous tubule architecture and integrity, the concentration of spermatogonia and the expression of DNA replication and repair marker in spermatogonia and Sertoli cells after thawing.

Introduction: Apo A-I Leu75Pro amyloidosis is a rare form of systemic hereditary amyloidosis that finds a peculiar prevalence in the province of Brescia (northern Italy). The hallmark and earliest involvement of this disease is the testicular impairment, characterized by hypogonadism and macrorchidism; renal and hepatic late onset involvement is the other characteristics.

Objective: To evaluate for the first time the prevalence of osteopenia, osteoporosis and vertebral fractures in a large cohort of men with hypogonadism due to Apo A-I Leu75Pro amyloidosis under long term testosterone replacement therapy (TRT).

Methods: Cross-sectional retrospective study of 50 men >50 years (median age 64.5 years) with hypogonadism under TRT due to this amyloidosis form, who performed andrological examination, gonadal and bone biochemical assay, and a lumbar and femoral Dual-Energy X-ray Absorptiometry (DXA) exam.

Results: At a median of 7.5 years from the testicular impairment and start of TRT, lumbar or femoral osteopenia and osteoporosis were found in 54.0% and 10.0% of patients, respectively. Of the 34 men who performed a morphometric assay, 14.7% (n = 5) had a vertebral fracture. With respect to patients with normal Bone Mineral Density (BMD), men with osteopenia or osteoporosis were significantly older, had a lower Body Mass Index (BMI), higher sex hormone binding globulinine (SHBG) before the start of TRT, and had a more frequent renal involvement. Osteopenia-osteoporosis was significantly more frequent in men with renal (22/29, 75.9%), hepatic (16/20, 80.0%) or renal and hepatic involvement (15/19, 78.9%) and in patients with macrorchidism (16/23, 69.6%, p = 0.008). In addition, increasing the organ disease involvement, without different TRT dosage, serum levels of total testosterone (TT), calculated free testosterone (cFT) and hematocrit were significantly lower, while luteinizing hormone (LH) was higher.

The presence of osteopenia-osteoporosis was correlated with age (r = 0.29, p = 0.042), BMI (r = -0.34, p = 0.013), renal involvement (r = 0.29, p = 0.041) and SHBG levels before TRT start (0.46, p = 0.005). Among 5 men with lumbar fracture, 3 had osteopenia, 1 osteoporosis, and 1 normal BMD. All patients with lumbar fracture showed (in addition to testicular impairment) renal and hepatic involvement, and 3 had macrorchidism.

Conclusion: Among men with hypogonadism due to Apo A-I Leu75Pro amyloidosis, at a median follow up of 7.5 years of TRT we found a prevalence of osteopenia, osteoporosis and vertebral fractures of 54%, 10% and ~ 15%, respectively. Osteopenia-osteoporosis was mainly found in older patients with systemic disease involvement. In the latter, it has been identified biochemical signs of inadequate TRT compensation, which may further impaired bone health.

P125 | Osteoporosis in men with hypogonadism due to Apo A-I Leu75Pro amyloidosis under long-term testosterone replacement therapy

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P126 | The strength of the pelvic floor muscles improves female sexual function and reduces sexual distress

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Patients who need psychological support during andrological investigations

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Introduction: Performing andrological investigations and especially semen analysis could be perceived as the cause of lower self-esteem feelings in patients dealing with infertility issues. Literature data on the topic are scarce in the Tunisian population.

Objective: This study aimed to assess levels of anxiety and depression among men undergoing andrological investigations and to identify their associated factors.

Methods: We conducted a cross-sectional study in the Laboratory of Cytogenetics and Reproductive Biology of Fattouma Bourguiba University Teaching Hospital (Monastir, Tunisia) between August 30th, 2020, and March 16th, 2021. Were included in the current study patients addressed to the laboratory for semen quality assessment as well as infertility management. All patients who had a previously diagnosed psychological disorder, those who presented severe depression and/or anxiety symptoms or had a stressful life event were excluded. Anxiety and depression levels were assessed using the valid Arab version of the HAD (Hospital Anxiety and Depression) scale. Semen parameters were analyzed and interpreted according to 2021 WHO (World Health Organization) guidelines.

Results: A total of 282 patients were included in the current study. The mean HAD-D (depression) and HAD-A (anxiety) scores were of 6.56 ± 3.07 (IIQ [4-8]) and 7.94 ± 3.73 (IIQ [5-10]) respectively. Univariate analysis showed that patients having two or more comorbidities were nearly five times more likely to be anxious than those without or with only one comorbidity (ORc = 4.71; p = 0.007). Furthermore, single patients were about four times more anxious than those in couple having primary or secondary infertility (ORc = 3.85; p = 0.027). With regards to semen parameters, patients having hypospermia were more than two times more anxious compared with those with normal semen volume (ORc = 2.33; p = 0.034). As for depression, we observed that patients with an infertility history lasting for a year or more have a nine times greater risk of depression (ORc = 9.848; p = 0.007). With regards to semen parameters, patients exhibiting two or more semen abnormalities were two times and half more depressed (ORc = 2.478; p = 0.036).

The multivariate analysis has shown that anxiety level was associated with comorbidities (ORa = 3.74; p = 0.046) and that depression symptoms were associated to single marital status (ORa = 7.20, p = 0.035), professional exposure (ORa = 2.35; p = 0.044) as well as urogenital history (ORa = 4.9; p = 0.023). A positive correlation between...
HAD-A and HAD-D scores was shown (R = 0.44; p < 0.001). HAD-A was also correlated to the patient age (R = -0.14, p = 0.015).

Conclusion: We pointed out through the current study the associated factors with anxiety and depression in patients performing andrological investigations to precociously identify those who need psychological counseling and hence to better manage infertility issues.

P128 Total Osteocalcin Levels are Independently Associated with Worse Testicular Function and a Higher Degree of HPG Axis Activation in Klinefelter Syndrome

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Context: Osteocalcin (OCN) is an osteoblast-produced polypeptide, emerging as the core element of the bone-testicular axis together with its undercarboxylated form (uOCN), proposed to increase testosterone (Te) levels in healthy men, by binding the Gprcr6a receptor on Leydig cells and modulating the GnRH pulse frequency and amplitude. However little is known with regards to its role in pubertal development and male hypogonadism.

Objective and Design: We investigated OCN concentrations in 47,XXY men affected by Klinefelter syndrome (KS), a model of adult hypergonadotropic hypogonadism, in a retrospective longitudinal study between 2007 and 2021 at an academic referral center.

Patients and Methods: 254 KS subjects, divided into the following groups: 1) pre-pubertal (n = 48, from 1 year of age until Tanner stage II), 2) pubertal (n = 46, Tanner stages II through V, <18 years), and 3) adults (n = 160, Tanner stage V, ≥18 years). All (pre-)pubertal patients were Te-naïve. Adult patients were categorized as: 1) eugonadal (total Te > 10.4 nmol/L; n = 47), 2) hypogonadal (Te < 10.4 nmol/L; n = 39), and 3) receiving testosterone replacement therapy (TRT) (n = 74). Data are presented as means ± SD, were tested with Brown-Forsythe and Welch ANOVA tests for unequal variances, corrected for multiple comparisons (Dunnett T3), and with partial correlations, after bootstrapping on 2000 samples.

Main outcomes: Total serum OCN (tOCN), hypothalamic-pituitary-gonadal (HPG) axis hormones (LH, FSH, total Te, 11β-estradiol, SHBG), and derived indexes.

Results: tOCN levels varied throughout the life span, with a mean of 85.9 ± 30.4 ng/mL in pre-pubertal infants, peaking at 130.0 ± 77.2 ng/mL in pubertal children (p = 0.243 vs. pre-pubertal) and then declining to 22.9 ± 9.0 ng/mL in adults (p < 0.001 vs. pre-pubertal and pubertal). In (pre-)pubertal boys no correlation with HPG axis hormones was found. When comparing adult KS, tOCN values were highest in eugonadal (26.5 ± 10.4 ng/mL), slightly lower in hypogonadal (24.5 ± 8.1 ng/mL, p = 0.268 vs eugonadal) and significantly lower in TRT subjects (20.4 ± 8.0 ng/mL, p = 0.008 vs. eugonadal and = 0.013 vs. hypogonadal). In adults, tOCN correlated with both LH (r = 0.23, p = 0.017) and FSH levels (r = 0.28, p = 0.004). These significances were maintained after the exclusion of subjects on TRT. Surprisingly, adjusting for age and BMI confirmed previous findings and revealed significant inverse correlations with total Te (r = -0.44, p = 0.004), calculated free Te (r = -0.37, p = 0.016), the Te/LH (r = -0.40, p = 0.010) and the calculated free Te/LH ratios (r = -0.33, p = 0.031).

Conclusions: In an experimental model of hypergonadotropic hypogonadism, tOCN showed no association with gonadal function during normal pre-puberty and pubertal development. In adults, tOCN levels were unexpectedly associated with worse testicular function and a higher degree of HPG stimulation.

P129 The dose-effect of supernumerary X chromosomes on clinical, metabolic and cardiac outcomes of male subjects

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Introduction: High grade aneuploidies of sexual chromosomes with a male phenotype (HGAs) are exceedingly rare conditions. For long HGAs were considered as complex variants of Klinefelter syndrome (KS), until recent evidence prompted to reconsider the two as distinct conditions. We first illustrated the endocrine and metabolic differences between the two clinical conditions demonstrating the more premature gonadal failure of HGAs compared to KS and their worse metabolic profile. Previous works highlight how HGAs patients exhibit a poorer outcome as the number of X increase, but comprehensive studies are lacking, and little is known of the mechanism involved.

Objective of the study: to investigate the impact of the number of supernumerary X chromosomes on clinical, hormonal, metabolic and echocardiographic features of male subjects with HGAs.

Subjects and Methods: We compared 23 HGAs and 46 KS subjects according to the number of supernumerary X chromosomes: 56 subjects with two supernumerary X (47,XXY and 48,XXYY), 5 with three supernumerary X (48,XXXY and 49,XXXXY) and 8 with four supernumerary X (49,XXXXXY). Clinical, hormonal, metabolic and echocardiographic parameters were analysed.

Results: The increase in the number of extra-Xs was associated with a progressive decrease of ultrasonographic bitesticular volume (p < 0.001); total testosterone (p = 0.01); DHEAS (p < 0.001); Δ4-androstenedione (p = 0.001); fT4 (p = 0.01). Conversely, a progressive linear increase in ACTH levels was found (p = 0.02). OGTT 2-hour insulin levels and HOMA-index were also higher in patients with ≥2 supernumerary X chromosomes, without a progressive trend (respectively p = 0.047 and p = 0.01). Right and left ventricular end-diastolic diameters were decreased (p = 0.016 and p = 0.001, respectively). Cardiac ejection fraction was also found associated with the extra-X, but without an apparent “dose-dependent” trend (p < 0.001): we found out that the addition of more than one extra X chromosome does not result in further worsening of cardiac output.
Conclusions: The increase in supernumerary X chromosomes is associated with a "dose-dependent" progressive impairment in steroidogenic function, insulin resistance and worse cardiac performance, namely the higher the number of X the higher the impairment of clinical, metabolic and cardiac outcomes.

P130 | A difficult case of Burned-out testicular tumors: can orchiectomy be avoided?

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Background: A "burned-out" tumor (BOT) is a rare clinical entity which describes the presence of distant metastases with the complete regression of the primary testicular lesion. We report the complex management of a case of BOT in a young male.

Case report: A 22-year-old male presented to ER with acute low back pain, severe vomiting and sweating. In past medical history: bilateral orchidopexy for cryptorchidism. Abdominal CT scan displayed two retroperitoneal masses of 35 and 20 mm. The patient was discharged with suspicion of paraganglioma and referred to our Department only 6 months after. In the suspicion of testicular tumor metastasis, a scrotal US was performed, showing multiple calcifications within the left testis. Serum tumor markers (STM) were increased: β-hCG 663 mIU/ml (<1.5), αFP 8 ng/ml (<5). A new CT scan showed a dimensional doubling of retroperitoneal masses. BOT was therefore suspected, and the case was handled in a multidisciplinary unit (Testis Unit). Retroperitoneal biopsy confirmed the diagnosis of metastatic embryonal testicular carcinoma. The patient underwent first-line chemotherapy (BEP protocol, three cycles). Post-treatment 18F-FDG-PET confirmed hypermetabolic activity in the left para-aortic site lesion and showed an unexpected additional uptake in the right, healthy testis (SUV max 3.7). STM were negative and a new US showed no focal lesions on both testicles. Retroperitoneal lymph node dissection was performed and a single left para-aortic lymph node (3.5 x 2.9 cm), site of focal residual neoplastic repetitions, was found. Post-surgery 18F-FDG PET confirmed a diffuse right testicular uptake (SUV max 3.4). Again, no intratesticular mass was visible. Because of the SUV reduction, the absence of suspicious lesions within the right testicle and negative STM, the board scheduled a strict testicular US follow-up together with repetition of STM and a PET re-evaluation after 6 months. US and STM remained stable, but 18F-FDG PET showed an increase in the intensity of uptake of the tracer at the right testicle (SUV max 5.6).

Considering the non-concordance of PET and US, a false positive testicular uptake, due to testicular cellular hyperactivity, was hypothesized. Therefore, the multidisciplinary board decided to temporarily suppress pituitary-gonadal axis with injectable testosterone undecanoate, subject to the patient’s informed consent. After three injections (performed at 0, 6 and 8 weeks), the 18F-FDG PET showed no areas of pathological uptake in both testes. Follow-up was continued with regular testicular US along with STM (every 3 months) and six-monthly CT, which remained negative. Hormonal assessment showed biochemical eugonadism with a progressive increase of gonadotropins within the normal range after discontinuation of Testosterone.

Conclusion: Scrotal US is an essential part of the work-up of male retroperitoneal mass. In this difficult case functional imaging would probably have led to a not necessarily bilateral orchiectomy. The suppression of pituitary-gonadal axis avoided this option in our patients, and opens the debate on whether orchiectomy is actually necessary in these conditions.

P131 | The bidirectional relationship between testosterone and metabolic disorders: testosterone deficiency as an early marker of cardiovascular risk in young men

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In the last years, an increasing incidence of cardiovascular diseases (CVD) has been reported in young adults (18-45 years), probably accounted by the significant increase in CV risk (CVR) factors. Observational and interventional studies, mainly focused on middle-aged and elderly men, demonstrated that metabolic CVR (mCVR) factors and CVD manifestations are common in hypogonadal men and, conversely, testosterone deficiency is highly prevalent in metabolic disorders; the lack of similar robust studies in younger adults requires focused investigation. The current single centre, observational, cross-sectional study aimed at better defining the mutual relationship between androgenic status and the prevalence of mCVR factors in a large cohort of 720 young adult (18-35 yrs) men, subjected to physical examination and fasting morning venous blood sampling for the assessment of anthropometric, metabolic and hormonal parameters. Body weight, BMI and waist circumference (WC) significantly decreased across total testosterone (TT) (< 0.0001), SHBG (< 0.01; p < 0.01; p < 0.0001) and calculated free testosterone (cFT) (< 0.05) tertiles, whereas systolic blood pressure (SBP) and triglycerides (TG) levels significantly decreased across TT (< 0.05; p < 0.01) and SHBG (< 0.05; p < 0.0001) tertiles, and diastolic blood pressure (DBP) across SHBG (< 0.05) tertiles. Spearman correlation analysis revealed a negative association of TT, SHBG and cFT with BMI (r = -0.204; p < 0.001)(r = -0.165; p < 0.05)(r = -0.132; p < 0.05), and of TT and SHBG with WC (r = -0.234; p < 0.001)(r = -0.225; p < 0.01), SBP (r = -0.112; p < 0.05)(r = -0.142; p < 0.05) and TG (r = -0.107; p < 0.01)(r = -0.204; p < 0.001), whereas a positive association of TT and SHBG with...
HDL-cholesterol ($r = 0.167; p < 0.01$) ($r = 0.251; p < 0.001$) was demonstrated. In multiple linear regression analysis in models adjusted for age, BMI and WC, TT and SHBG were strong independent predictors of serum HDL-cholesterol levels ($\beta = 0.151; p < 0.01$) ($\beta = 0.186; p < 0.01$), and SHBG was an independent predictor of SBP and DBP ($\beta = -0.177; p < 0.05$) ($p = 0.008; \beta = -0.204$). Lastly, in the subgroup of men with hypotestosteronemia (TT $\leq 12.1$ nM), the prevalence of normal weight was significantly lower ($p < 0.05$) and that of obesity ($p = 0.0003$), visceral obesity (WC $> 102$ cm) ($p = 0.059$), hypertension ($p = 0.173$) and metabolic syndrome ($p = 0.457$) was significantly higher, compared to normal-testosterone subgroup. Consistently, in the subgroup of overweight/obese men, serum TT levels were significantly lower ($p < 0.01$) and the prevalence of hypotestosteronemia was significantly higher ($p < 0.01$), compared to normal-weight subgroup. The current study demonstrated that in young adult men a bidirectional relationship between testosterone deficiency and metabolic disorders exists, and that a worse androgenic status is associated to a worse cardiometabolic profile, and might represent a strong early predictor of mCVR factors, potentially associated to the onset of future CVD.

**P132** | Cardiovascular risk and metabolic profile of transgender people: a retrospective, observational study

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Cardiovascular (CV) and metabolic wellbeing of transgender (T*) people assuming hormonal transitional therapy or undergone gender-affirming surgery has been recently a matter of debate. The aim of the current study was to retrospectively evaluate the CV risk and the metabolic profile of a cohort of T* people, at their last available follow-up visit. 155 T*people (54 T*women, 101 T*men, mean age: 29.3 ± 9.3 yrs) were enrolled, having assumed hormonal transitional therapy for at least six months, in particular 13.5% had also undergone major gender-affirming surgery (i.e. gonadectomy), whereas 86.5% exclusively performed hormonal transitional therapy or hormonal transitional therapy and secondary gender-affirming surgery (breast augmentation and aestheticsurgery for T* women, mastectomy for T*men). Considering T*women, 81.5% assumed estradiol gel (mean dose: 1.1 ± 0.4 mg/day), whereas the remaining 18.5% assumed oral estradiol (mean dose: 2.2 ± 0.6 mg/day); moreover, 77.8% assumed cyproterone acetate (mean dose: 54.2 ± 37.4 mg/day). Considering T*men, 58.4% assumed transdermal testosterone (mean dose: 24.4 ± 7.9 mg/day), whereas 41.6% assumed injective testosterone esters (mean dose: 9 ± 2 mg/day). The PROCAM score was calculated to assess 10-year CV risk for each subject. Data regarding metabolic assessments, including body mass index (BMI), systolic (SBP) and diastolic (DBP) blood pressure, total, LDL, and HDL cholesterol, triglycerides, and glycemia; hormonal profile (FSH, LH, 17b-estradiol, testosterone, prolactin levels); smoke, family history for CV events, current hormonal treatment, duration of treatment, and eventual gender-affirming surgery were collected. Overall, the mean PROCAM score for T* people was 18.7, accounting for a CV risk $< 1%$. In T*men, PROCAM scores ($p = 0.002$) and the prevalence of CV risk 1-2% ($p = 0.008$) were significantly higher, while the prevalence of CV risk $< 1%$ significantly lower compared with T*women ($p < 0.001$). Moreover, PROCAM score was positively correlated with testosterone gel dosage in T*men ($r = 0.304p = 0.02$). T* men had significantly higher SBP ($p = 0.001$), DBP ($p = 0.001$), total ($p = 0.001$), LDL ($p < 0.001$), and HDL ($p = 0.016$) cholesterol, and triglycerides levels ($p = 0.001$), as well as a significantly higher prevalence of arterial hypertension ($p = 0.048$), hypercholesterolemia ($p = 0.005$) and hypertriglyceridemia ($p = 0.05$) compared with T*women. Moreover, T*people undergone gender-affirming surgery had significantly higher BMI ($p = 0.033$), DBP ($p = 0.004$), total ($p = 0.001$) and HDL cholesterol ($p = 0.012$), triglycerides ($p < 0.001$) and glycemia levels ($p = 0.015$) compared with those who did not. In the overall population, age was positively correlated with BMI ($r = 0.224p = 0.005$), SBP ($r = 0.232p = 0.004$) DBP ($r = 0.277p < 0.001$) and with total ($r = 0.512p < 0.001$), LDL ($r = 0.444p < 0.001$), HDL ($r = 0.192p = 0.018$) cholesterol, triglycerides ($r = 0.298p < 0.001$) and glycemia ($r = 0.309p < 0.001$); hormonal treatment duration was positively correlated with LDL ($r = 0.227p = 0.005$) and total ($r = 0.320p < 0.001$) cholesterol and triglycerides ($r = 0.231p = 0.004$). In T*men, gel testosterone dosage was positively correlated with triglycerides ($r = 0.334p = 0.01$), injective testosterone dosage was negatively correlated with BMI ($r = -0.380p = 0.013$) and 17b-estradiol levels were negatively correlated with total cholesterol ($r = -0.263p = 0.008$) and triglycerides levels ($r = -0.257p = 0.011$). The current study demonstrated a low CV risk and a normal metabolic profile in T* people under hormonal replacement therapy for at least six months, although T* men experienced a higher CV risk and a worse metabolic profile compared to T* women, as T* men assuming higher dosages of transdermal testosterone.

**P133** | Covid-19 pandemic and two-month mass quarantine in Italy: impact on psychological distress in men with Klinefelter syndrome

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ABSTRACT

Since December 2019, worldwide population has been facing the rapid spread of Severe Acute Respiratory Syndrome-Coronavirus2, causing the highly transmissible Covid-19 global pandemic, requiring restrictive measures to reduce contagion, including extensive mass quarantine, lasting in Italy for two months (March 8th-May 16th). A severe psychological burden of Covid-19 pandemic and mass-quarantine has been reported in the general population, likely determined by forced confinement, social deprivation, freedom limitation and uncertain future. Men with Klinefelter Syndrome (KS) display an increased risk of psychological/psychiatric disorders compared to the general population, possibly representing the underlying cause of social anxiety and impulsivity, often resulting in social challenging interactions/withdrawal. It has been argued as to whether psychological disorders are associated with alterations of brain regions deputed to emotional control, to genetics, or to hormonal cues (i.e. hypogonadism); conflicting data exist on the effects of testosterone replacement therapy (TRT). The current multicentre case–control study aimed at evaluating the occurrence and severity of Covid-19 pandemic– and two-months mass quarantine-related psychological distress in 148 KS aged 40.3 ± 12.9 years compared to 148 age-matched non-KS from the general population, through questionnaires anonymously and telematically administered during the last three weeks of Italian mass quarantine for the evaluation of perceived stress (PSS), anxiety (GAD-7), depression (PHQ-9), ego-resiliency (ER89-R) and psychological wellbeing (PWB). KS and non-KS did not differ for age, marital, cohabitation and working status, and going out from home frequency during the Covid-19 mass quarantine. KS had significantly higher mean scores in PSS (p = 0.003), GAD-7 (p = 0.000) and PHQ-9 (p = 0.001) scales. Moreover, in KS: a significantly lower prevalence of low (p = 0.0261) and higher prevalence of moderate (p = 0.0357) perceived stress severity; a significantly lower prevalence of minimal (p < 0.0001) and higher prevalence of moderate (p = 0.0008) anxiety severity; a significantly lower prevalence of none-minimal (p = 0.0270) and higher prevalence of moderately severe (p = 0.0180) depression. Assessed outcomes did not correlate with age, TRT or age at TRT initiation in KS. KS diagnosed before age 18 years (30.41%) had significantly lower mean scores in PWB scale, compared to KS diagnosed later in life (p = 0.049). Noteworthy, KS diagnosed before age 18 years vs after age 40 years, displayed a significantly lower ability to adapt and react to traumatic or stressful experiences (optimal regulation ER89-R subscale: p = 0.014), a lower independence and self-determination (autonomy PWB subscale: p = 0.014), and a lower ability to have a satisfying relationship with others (positive relations with others PWB subscale: p = 0.047). KS with one or more (67.57%) vs without concurrent disorders/comorbidities (cryptorchidism, gynecomastia, obesity, diabetes, osteopenia, osteoporosis, cardiovascular and neurological complications) had significantly lower mean scores in optimal regulation (p = 0.041). The current multicentre case–control study demonstrated for the first time that KS experienced significantly higher and more severe levels of Covid-19- and mass quarantine-related perceived stress, anxiety and depressive symptoms, and a significantly lower positive attitude toward the self and self-past life, vs non-KS. A lower age-related maturity at diagnosis, a longer awareness of the syndrome, and concurrent disorders/comorbidities might all contribute to psychological burden and therefore influence the personal attitude in challenging situations.

P134  I The effect of the new 75 mg orodispensible film of Sildenafil on erection and sexual quality of life: insights from an observational study

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Introduction: The newly devised orodispersible film (ODF) of Sildenafil is the first phosphodiesterase type 5 inhibitor (PDE5i) in a 75 mg dosage. This intermediate dosage and the particular properties of the ODF formulation can improve the clinical management of erectile
dysfunction (ED) patients. We investigated the effects of the sildenafil ODF 75 mg dose on both sexual quality of life and erectile function based on the results from an observational study in daily practice in Italy.

Materials and Methods: This was a post-hoc analysis of results from an observational study carried out in six centers in Italy and performed on a real-life population of men coming for a complaint of ED and treated in accordance with the individual investigators’ normal clinical practice. All subjects were asked to take the prescribed dosage of Sildenafil ODF at inclusion (Visit 1) and to return for a control visit (Visit 2) to confirm or adapt the prescribed dose after a minimum of 4 weeks. An End of study Control visit (Visit 3) was performed after additional 4 weeks. Patients were encouraged to attempt sexual intercourse using the drug on at least 8 occasions during the period between visits. Erectile function was assessed by the International Index of Erectile Function – Erectile Function Domain (IIEF-EF); sexual quality of life was measured using the sexual QoL instrument for men (SQoL-M).

Results: Among the 36 subjects initially recruited at visit 1 for the 75 mg dose, three patients dropped out of the study at visit 2 and two dropped out at visit 3. None of the subjects withdrew due to treatment inefficacy or serious adverse events. At visit 2, the mean IIEF-EF scores significantly increased (Δ = 7.97 ± 4.71, p < 0.0001) in the overall population; likewise, SQoL-M also increased significantly (Δ = 10.76 ± 10.46, p < 0.0001). At visit 3, IIEF-EF and SQoL-M scores were still significantly improved compared to baseline (Δ = 10.64 ± 7.01, p < 0.0001 and Δ = 18.15 ± 12.32, p < 0.0001, respectively). By using ANCOVA, we found no significant effect for age, BMI, history of previous use of PDE5i, presence of metabolic comorbidities, and smoking habits on IIEF-EF and SQoL-M scores at both visits 2 and 3.

Conclusion: The new 75 mg ODF Sildenafil formulation is a safe and effective treatment for erectile dysfunction, significantly improving both erectile function and sexual quality of life in patients undergoing treatment. Such improvement is not transient and limited to the first weeks of treatment, but rather more stable and reliable over time. Additionally, factors known to affect sexual health, including age, BMI, smoking status, metabolic comorbidities, and symptoms duration, resulted in significantly different outcomes for treatment efficacy, suggesting that the treatment with this intermediate dose is equally viable for all patients on average. The use of the ODF formulation for sildenafil represents a novel opportunity to take care of ED patients.

P137  |  Nocturnal penile tumescence test in patients with erectile dysfunction associates with penile and systemic vascular disease

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Background: Erectile dysfunction (ED) is the most frequent male sexual dysfunction along with premature ejaculation. Moreover, its prevalence is increasing worldwide due to ageing of the population and spreading of risk factors like obesity, diabetes, hypercholesterolemia, hypertension, etc. Also, it represents an important cardiovascular risk marker because of its demonstrated tight association with cardiovascular disease (CVD). We dispose of many diagnostic tools to assess ED and a proper workup includes the anamnesis, a physical exploration, blood tests for hormonal and metabolic evaluation, and ED grading through validated questionnaires such as the International Index of Erectile Function (IIEF).

One of the instrumental tests we may indicate is the nocturnal penile tumescence (NPT) test or “Rigiscan”. This test measures the spontaneous erections overnight and helps discriminating between organic and psychological ED. However, this is a highly specific but low sensitive test since a positive (normal) result can roll out an organic ED and suggest a psychological etiology while a negative result cannot confirm or exclude an organic condition.

Aim: In this context the aim of this study is to investigate the predictive role of Rigiscan in discriminating patients with a organic ED and the presence of vascular disease in patients with ED.

Methods: We conducted a prospective study in patient 25 patient who underwent a Rigiscan test as part of ED assessment. Each patient had blood tests for hormonal and metabolic analysis, a measurement of the carotid intima-media thickness (cIMT), a brachial flow-mediated vasodilation test (FMD), and a penile color doppler ultrasound (PCDU) to assess penile hemodynamics.

Results: Our patients had a mean age of 47.9 ± 13.7 years, with a mean IIEF score of 9.0 ± 6.5. Among them 13 (54.2%) were active smokers, 5 (20.8%) were diabetics, 8 (32%) had dyslipidemia, and 4 (16.7%) had hypertension. Eleven patients (44%) had a normal Rigiscan while 14(56%) had a pathological result.

We found that patient with a pathological Rigiscan presented with a cluster of impaired vascular function at different levels. In particular, they had statistically lower peak systolic velocity (PSV) in the PCDU (52.5 vs 80.2 cm/s p = 0.014) and a higher prevalence of arterial insufficiency (42.9% vs 0.0% p = 0.020). Moreover, in patients with negative Rigiscan, there was a higher cIMT (0.9 vs 0.7 mm) and lower response to FMD (7.7% vs 9.6%) with a more frequent diagnose of impaired FMD (63.6% vs 46.2%) but without reaching a statistically significance.

Conclusions: We can conclude that a negative Rigiscan is indicative of altered penile hemodynamics and may suggests the presence of systemic vascular disease. Therefore, while a positive Rigiscan excludes organic ED, a negative Rigiscan should be considered a cardiovascular risk marker and induce the clinician to address the cardiovascular risk in that patient. More studies with larger population are warranted to confirm and further investigated these findings.