

Journal für

Reproduktionsmedizin und Endokrinologie

– Journal of Reproductive Medicine and Endocrinology –

Andrologie • Embryologie & Biologie • Endokrinologie • Ethik & Recht • Genetik
Gynäkologie • Kontrazeption • Psychosomatik • Reproduktionsmedizin • Urologie

**Andrology 2020 12th International/11th European/ 32nd
German Congress of Andrology 5–9 December 2020 DIGITAL**

Abstracts

*J. Reproduktionsmed. Endokrinol 2020; 17 (Supplementum
1), 5-85*

www.kup.at/repromedizin

Online-Datenbank mit Autoren- und Stichwortsuche

Offizielles Organ: AGRBM, BRZ, DVR, DGA, DGGEF, DGRM, D-I-R, EFA, OEGRM, SRBM/DGE

Indexed in EMBASE/Excerpta Medica/Scopus

Krause & Pachernegg GmbH, Verlag für Medizin und Wirtschaft, A-3003 Gablitz



ENDO FERTI FORUM

ENDOKRINOLOGIE & FERTILITÄT
FÜR KLINIK & PRAXIS

20.-21. März 2026

Universitätsmedizin Mainz

Einladung zu unserer wissenschaftlichen Veranstaltung Endo-Ferti-Forum

Brücke(n) zwischen Unikliniken und Praxen an Rhein und Main(z)

– die aus dem bisherigen Format „Ferti Forum“ ab 2026 hervorgeht –



Freuen Sie sich auf spannende Vorträge und den lebendigen Austausch mit Kolleg:innen und Expert:innen aus Klinik und Praxis. Freitagabend laden wir Sie herzlich zu einem entspannten Empfang ein – eine perfekte Gelegenheit, Kontakte zu knüpfen und den Tag genussvoll ausklingen zu lassen.

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Andrology 2020

12th International/11th European/ 32nd German Congress of Andrology

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DIGITAL

Abstracts*

Vorträge geladener Redner

Saturday, 5 December 2020

10:00–10:45 h

Kryokonservierung von Spermien – Relevantes und Neues

Cryopreservation of germ cells in German law and guidelines – the right to preserve fertility?

S. Kliesch

Center of Reproductive Medicine and Andrology (CeRA), Department of Clinical and Surgical Andrology, WHO collaboration center for research in male reproduction, Training Center of the European Academy of Andrology (EAA), University Clinic Münster (UKM), Germany

Fertility preservation in males and females facing a potentially gonadotoxic treatment is highly relevant with a high impact on patients' quality of life. In adolescence after spermatarche and adults in their reproductive age, fertility preservation should be routinely performed by cryopreservation of ejaculated or testicular sperm (after surgical sperm extraction, TESE, in case of azoospermia or anejaculation). In Germany, the costs of these procedures have to be paid by the patients themselves. However, in May 2019 a new law was set up and cryopreservation will be covered by the health insurances from now on. The detailed regulations for refunding of fertility preservation measures are nearly set up by now, but still under discussion. In its essentials, all patients with diseases and treatments leading to the loss of gonads or to a potential failure of gonadal function will have access to the refunding of fertility preservation by their health insurances. This is not restricted to oncological diseases only. However, prior to cryopreservation a consultation by the specialized doctor treating the disease and an expert in the reproductive field (for females a gynecologist trained in reproductive medicine and for males a doctor trained in andrology) is mandatory and a kind of

check-list has to be answered to fulfill the criteria for payment. There are still some major deficits in the present regulations, e.g. how to deal with the prepubertal boys with only experimental treatment options available: in prepubertal boys, immature testicular tissue samples offer the chance for cryopreservation of spermatogonial stem cells. The later use of these cells may include methods such as grafting or in-vitro maturation of spermatozoa. Therefore, several networking European Centers offer this kind of fertility preservation to boys by now. In Germany the network Androprotect cares for the boy, while the girls get help by the network FertiProtekt.

Although further discussion of unresolved questions is needed, the present regulatory guideline offers an important progress and advantage for patients suffering from an illness which by itself or by the necessary treatment will threaten their reproductive capacity.

16:00–16:30 h

Therapie der Infertilität – Endpunkt Schwangerschaft

Medikamentöse Therapieoptionen und „life style“ Veränderungen – Wie kann man den Patienten beraten?

F.-M. Köhn

Andrologikum Munich, Germany

Andrological therapy should improve male fertility and – if necessary – ability for sexual intercourse.

The first intention is pregnancy achieved by natural way; however, optimization of semen quality may also be worthwhile in order to perform less invasive assisted reproductive technologies (intrauterine insemination instead of in vitro fertilization).

Basis of the andrological workup is the correct diagnosis and the estimation of the success of therapeutical strategies.

Some andrological diseases can be treated causally such as hypogonadotropic hypogonadism, hyperprolactinemia, infections or ejaculatory disorders.

It seems to be logical, to treat male immunological infertility with glucocorticosteroids; however, doubtless proof of evidence is still missing.

In some cases the cause of male infertility is known (non-obstructive azoospermia due to AZF gene deletions) without causal therapy available.

A significant percentage of male infertility is still considered idiopathic and a variety of empiric therapies has been used in these cases, even if active principle and effectiveness are not proven.

Antiestrogens such as tamoxifen and clomifen have an exceptional position in andrological therapy. Although treatment of infertile males is an off label use, their pharmacological effects are known (inhibition of hypothalamic and pituitary estrogen receptors and stimulation of testosterone production and spermatogenesis by increase of LH and FSH).

Apart from drugs or factors related to life-style such as alcohol and tobacco smoke, various environmental and occupational agents, both chemical and physical, may impair male reproductive functions. Reproductive toxicity may evolve at the hypothalamic-pituitary, testicular, or post-testicular level; endpoints comprise deterioration of spermatogenesis and sperm function as well as endocrine disorders and sexual dysfunction. With regard to the complex regulation of the male reproductive system, the available information concerning single exogenous factors and their mechanisms of action in humans is limited. This is also due to the fact, that extrapolation of results obtained from experimental animal or in-vitro studies remains difficult.

Considering these aspects history-taking is an important basis of andrological diagnosis.

*Begutachtet und zusammengestellt vom wissenschaftlichen Komitee. Ein Verzeichnis der präsentierenden Autoren finden Sie auf Seite 86 f, alphabetisch geordnet nach Erstautoren. Mit Genehmigung von Wiley Doppelpublikation der Abstracts in „Andrology“ und „JRE“

16:30–17:00 h

Therapie der Infertilität – Endpunkt Schwangerschaft**Surgical sperm collection – gold standard microTESE – is conventional testicular biopsy out?**

I. Hoffmann

Center of Reproductive Medicine and Andrology (CeRA), Department of Clinical and Surgical Andrology, University Clinic Münster (UKM), Germany

Surgical extraction of spermatozoa from testicular tissue can be carried out using the following surgical procedures:

- Microscopic Testicular Sperm Extraction (mTESE)
- Conventional Testicular Sperm Extraction (cTESE)
- Microscopic epididymal sperm aspiration (MESA)

The principle of testicular sperm extraction is based on the fact that the testicular spermatogenesis a certain threshold beyond, in order for spermatozoa can be found in the ejaculate [Silver 1999].

While testicular and epididymal spermatozoa can be extracted with a high degree of probability in obstructive azoospermia, surgical testicular sperm extraction remains a challenge in non-obstructive azoospermia. In the microscopic surgical technique, the seminiferous tubules are visualized and assessed using a surgical microscope at a magnification of up to 20 times. A relationship between the diameter of a tubule and the probability of finding sperm is known [Schlegel 1999]. Through the targeted collection, the mTESE increases the probability of identifying the spermatogenesis islands obtained in non-obstructive azoospermia and of extracting sperm for ICSI therapy. Many studies have shown a significantly higher sperm extraction rate of the mTESE compared to the cTESE [Schlegel 1999, Bernie et al., 2015 etc.]. This also applies to certain groups of patients, such as patients with a AZFc deletion [Black et al., 2015], patients with Klinefelter's syndrome [Sabbaghian et al., 2011], patients after already unsuccessfully performed cTESE [Kalsi et al., 2014].

A recent meta-analysis has shown that sperm extraction rate at the cTESE and mTESE are equal [Corona et al., 2019]. Interpreting the study results the heterogeneity of patient populations and study designs should be considered, particularly the significantly different risk profile between the groups of patients, inclusion of randomized and non-randomized trials and studies without information on histological findings.

Sunday, 6 December 2020

10:06–10:15 h

Der Patient mit Azoospermie – praxisrelevante Empfehlungen zur Diagnostik und Therapie**Was macht eine gute Hodenhistologie aus und warum?**

D. Fietz

Institute for Veterinary Anatomy, Histology and Embryology, Justus-Liebig-University Giessen, Germany

Following EAU guidelines, testis biopsies are indicated in case of azoospermia (obstructive or non-obstructive), Klinefelter syndrome, in suspicion of testicular germ cell tumours (TGCTs) and adult cryptorchism. Multiple testicular biopsies from each testis enhance the chances to detect a) elongated spermatids for assisted reproduction (in combination with testicular sperm extraction, TESE) and/or b) TGCTs. Testicular histology is essential for the evaluation of biopsies – but only if done correctly.

Sampling and fixation of testis tissue is of crucial importance: Squeezing artefacts need to be avoided and a fixation with formalin (as performed commonly in pathologies) is not recommended. The latter leads to shrinking artefacts and a decreased histological quality in regard to structural preservation and nuclear morphology. Therefore, use of Bouin's or Stieve's solution is recommended. As standard staining, hematoxylin and eosin or Periodic Acid Schiff staining are sufficient as both enable qualitative and quantitative evaluation of germ cell development (normal or reduced spermatogenesis, maturation arrests, germ cell aplasia and TGCTs) and somatic cells (Sertoli and Leydig cells, immune cells). Additionally, further analyses as e.g. immunohistochemistry as indicated for TGCT diagnosis is possible with Bouin-fixed material. The last step in histological evaluation is the use of an objective score count analysis to predict the chances for a positive TESE outcome for the clinic.

Only if all these aspects are taken under careful consideration, testicular histology is significant and with this an essential part of testicular biopsy and andrological examination.

13:30–14:00 h

Pre Congress Course, clinical**Arguments for using fresh or cryopreserved sperm when offering TESE in NOA patients**

H. Lin, H. Zhang, J. Mao, H. Jiang

Department of Urology, Peking University Third Hospital, Peking, China

Using fresh or cryopreserved sperm when offering TESE in NOA patients is controversial. We performed a study to evaluate the clinical outcomes of microdissection testicular sperm extraction-intracytoplasmic sperm injection

(micro-TESE-ICSI) treatment that used fresh or cryopreserved sperm in patients with nonobstructive azoospermia (NOA). A total of 338 NOA patients with 344 consecutive cycles received treatment in the reproductive medicine center of Peking University Third Hospital in Beijing, China, from January 2014 to December 2017. Fresh oocytes and fresh sperm were used in 222 patients with 234 cycles (Group A). Fresh oocytes and cryopreserved sperm were used in 116 patients with 110 cycles (Group B). We compared patient characteristics, embryonic development, and pregnancy outcomes between Groups A and B. There was no statistical difference in the patient characteristics, and no differences were observed with fertilization or quality embryo rates between Groups A and B. The rates of clinical pregnancy and live birth were both higher for Group A than those for Group B (both $P < 0.05$). In conclusion, fresh testicular sperm appears to produce better ICSI outcomes than cryopreserved testicular sperm in patients with NOA.

14:00–14:30 h

Clinical use of FSH in male infertility

H. M. Behre

Center for Reproductive Medicine and Andrology, University Hospital Halle (Saale), Martin Luther University Halle-Wittenberg, Germany

For several decades, the established clinical use of FSH in male infertility is the treatment of male patients with hypogonadotropic hypogonadism. Successful therapy will be stimulation of spermatogenesis in the testis and appearance of viable spermatozoa in the ejaculate - and finally the induction of a clinical pregnancy in the female partner and the birth of a healthy child.

Several clinical studies with various FSH preparations in combination with hCG have demonstrated the high treatment efficacy regarding these clinical endpoints. Shortcomings of this treatment are the long duration of therapy – that might last longer than 2 years in some patients – and the inconvenience of FSH injections every second or third day. Relevant improvements of FSH therapy in male patients can be expected with modern FSH treatment options already available for assisted reproduction treatment in the female patient.

In most countries, FSH treatment of patients with normogonadotropic idiopathic infertility and oligozoospermia is still considered experimental. Recent meta-analyses have shown that FSH can significantly increase pregnancy rates in the female partners of these patients, but the effect-size is relatively low. Therefore, predictive factors for treatment success have to be identified, including FSH pharmacogenetics, to select the right normogonadotropic patients with idiopathic infertility for FSH therapy.

15:30–16:00 h

Systematic diagnosis of the infertile male

S. Kliesch

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The diagnostic work-up in the infertile male aims to identify underlying reasons for infertility and may lead either to a causative treatment or at least to a better understanding of the disease. Both will facilitate adequate counselling of the couple and influence the final treatment decision in patients with couple infertility. The diagnostic workflow follows a systematic approach to elucidate previous factors influencing fertility and the present status. Medical history, preferably taken in the presence of the female partner, clinical examination with focus on gonadal phenotype and function as well as laboratory testing of hormones (including gonadotropins and androgens) and semen analysis according to the World Health Organization (WHO) standards form the diagnostic basis. The final results may trigger further investigations such as genetic testing or sperm function tests. Apart from sperm count, motility and morphology, additional tests have been developed in recent years to improve the understanding of sperm functional capability. DNA fragmentation analysis has gained evidence and tests on sperm motility by computer-assisted analysis have been introduced in the diagnostic work up recently. Finally, the differential diagnosis of hypothalamic–pituitary, testicular malfunction or congenital reproductive failure will finally determine the possible treatment options prior to the use of assisted reproductive techniques (ART). After systematic diagnostic work-up, 30% of patients will be identified with distinct diagnosis which are worthwhile to be treated prior to ART. Endocrine, surgical, or empirical therapeutic options may be performed as stand alone treatment or in combination with ART after interdisciplinary consultation of both partners, male and female, by the andrologist and a specialized gynecologist. This effort may finally result in an optimized patient-centered treatment approach for couple infertility.

16:00–16:30 h

The importance of histological analysis in diagnosing male infertility

D. Ježek

Dept. Histology and Embryology, University of Zagreb, School of Medicine, Dept. of Transfusion Medicine and Transplantation Biology, University Hospital Zagreb, Croatia

Histological analysis in andrology/male infertility is mostly applicable for cases of azoospermia, where there is a need to analyse testicular biopsies. Azoospermia is a difficult

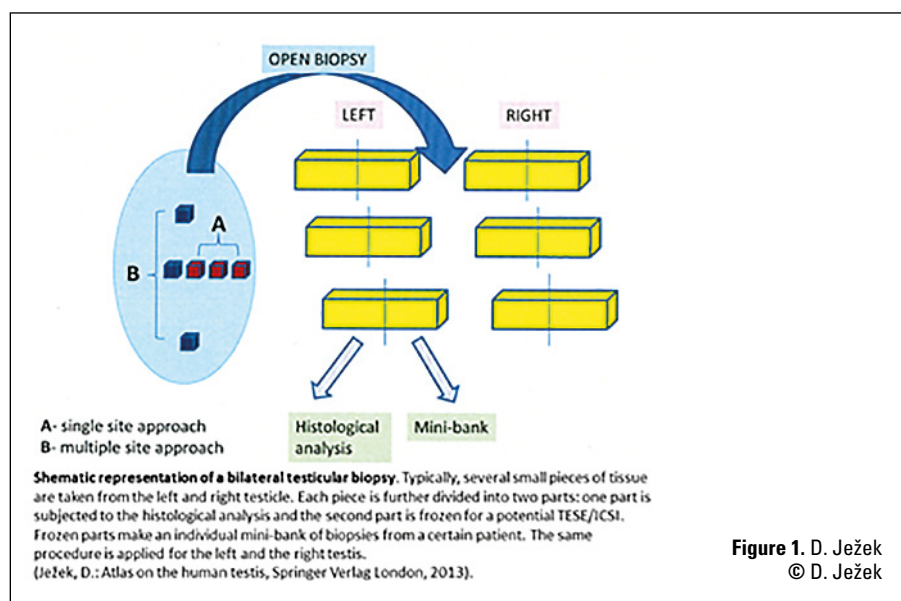


Figure 1. D. Ježek
© D. Ježek

form of male infertility that affects 8–20% of infertile patients. It has two forms, i.e. obstructive and non-obstructive form (OA, obstructive azoospermia, NOA, non-obstructive azoospermia). In the case of OA, the vast majority of testicular parenchyma is preserved. A significant number of seminiferous tubules have full spermatogenesis. In contrast to OA, NOA is much more severe type of azoospermia, with extensive changes in the structure of the male sex gland and impaired spermatogenesis. However, in 60–70% of patients with NOA, there are foci of spermatogenesis with a maintained production of mature spermatids and/or spermatozoa (“mixed atrophy of seminiferous tubules”). These cells can be used for ICSI after the application of microsurgical procedure - open biopsy of the testis with cryopreservation. This procedure is both a diagnostic and a therapeutic procedure during which several pieces of the tissue (usually from both testicles) are taken for histological analysis and cryopreservation. Due to the cryopreservation, a mini-bank of testicular biopsies for a given patient is formed. Testicular sperm extraction (TESE) and ICSI procedure may be repeated several times, using frozen biopsy material. Thus, the patient with azoospermia, as a rule, is subjected to a single surgical procedure. A detailed histological analysis involves determining the degree of preservation of spermatogenesis and routine detection of possible germ cell neoplasia *in situ* (GCNIS) using immunohistochemical methods. In our setting, after excision, each testicular biopsy is processed immediately in the operation theatre. The excised tissue is immersed in the transport medium that preserves gametes from further degradation. The biopsies are brought to the sterile cabinet inside the operation room. Within the cabinet, each biopsy is divided into two parts: one part is plunged into the freezing medium whereas the other part is fixed for the histological analysis. Thus, “paired” or “matched” biopsies are formed. After fixation and paraffin embedding, an extensive cutting of the paraffin block is necessary to provide a detailed insight into the histological structure

of the testis parenchyma. Apart from routine hemalaun-eosin staining, immunohistochemistry (IHC) is mandatory in order to check for GCNIS. Most commonly used IHC markers include placental alkaline phosphatase (PLAP) and octamer-binding transcription factor 4 (OCT-4). Using our technique of tissue handling, detailed histological analysis and freezing-thawing, we were able to isolate spermatozoa in more than 68% of cases with azoospermia (Fig. 1).

References:

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16:30–17:00 h

Genetic causes of spermatogenic defect – Novel diagnostics in the clinical workup

F. Tüttelmann

Institute of Reproductive Genetics, University of Münster, Germany

Impaired spermatogenesis is the most common cause for male infertility. Quantitative defects – namely oligo-/azoospermia – can go hand in hand with qualitative defects of sperm motility and morphology, while these can also occur in cases with normal sperm numbers. In both quantitative and qualitative spermatogenic failure, genetic causes increase with severity of the phenotype. For example, azoospermia and especially the subtype of meiotic arrest is clearly a genetic disorder. Fittingly, a steadily increasing number of associated genes has been described. Likewise, the genetic cause can be identified in men with severely and specifically impaired motility and/or morphology. The former is genetically overlapping with primary ciliary

dyskinesia (PCD), while the latter is recognised as the phenotypic entity of MMAF – Multiple Morphologically Abnormalities of the Sperm Flagellum.

For many years, chromosome and AZF deletion analyses were the only frequently applied genetic tests. However, these provide only ~20% of diagnoses in azoospermic men and ~4% in all men in infertile couples. In fact, male infertility genetics has been lagging behind almost all other fields. Fortunately, the increased application of large-scale genetic analyses triggered by the development of next-generation sequencing (NGS) has now also arrived to Andrology. As such, exome sequencing has lately led to the continuous discovery of genes associated with impaired spermatogenesis. Now, these findings together with NGS technology (exome or gene panel sequencing) need to be translated into the diagnostic setting. Since 2017, we have pioneered prospective exome sequencing in azoo-/cryptozoospermic men as well as in men with specific motility/morphology defects. This has significantly increased the diagnostic yield, which is not only beneficial in itself for affected men but also increasingly therapeutically relevant, e.g., to assess success for testicular sperm extraction (TESE) prior to the biopsy.

17:30–18:00 h

Sleep and Andrological Health

P. Y. Liu

Lundquist Institute at Harbor-ULCA Medical Center, David Geffen School of Medicine University of California Los Angeles, CA, United States

It has long been recognized that the environment impacts reproduction and reproductive health in all mammalian species through seasonal, infradian, diurnal, and ultradian processes. Sleep is one tightly regulated diurnal process which, when disordered, is recognized to cause hypersomnolence and neuropsychological deficits, alter inflammatory pathways, and ultimately lead to cardiometabolic ill health. Disordered sleep may be insufficient as occurs with the modern 24/7 lifestyle, misaligned to the environment due to shift work or jet lag, or disrupted from obstructive sleep apnea. Epidemiological and interventional data are accumulating to show that insufficient, misaligned and disrupted sleep in men may lead to hypogonadism, erectile dysfunction, and infertility; although more research is needed to determine the exact mechanisms by which the hypothalamo-pituitary testicular axis in young and older men is impacted. This symposium talk will highlight the clinical research methodologies utilized to interrogate these processes, in order to contextualize and summarize the available epidemiological and interventional studies performed. Intricate chronobiological study designs and mathematical deconvolution of hormone time series to determine hormone secretion, pulsatility and circadian influences will be outlined where relevant. In summary, available studies show that sleep restriction decreases 24 hour testosterone in

a time of day dependent manner, and that treatment of obstructive sleep apnea by continuous positive airway pressure probably increases overnight testosterone concentrations. The impact of circadian misalignment on testosterone has been inadequately studied, but does not appear to influence mean testosterone levels.

Monday, 7 December 2020

Plenary Lecture 1

08:30–09:15 h

The Healthy Male – evolution of Australia's men's health programme

R. McLachlan

Medical Director, Healthy Male [Andrology Australia], Director of Andrology Services, Hudson Institute of Medical Research, Dept of Endocrinology, Monash Health, Adjunct Professor of Andrology, Dept. of Obstetrics & Gynaecology, Monash University. Consultant Andrologist, Monash IVF Group, Australia

Healthy Male [formerly Andrology Australia] was created in 2000 to improve community and professional awareness and education in male reproductive health [MRH]. Supported by the Federal Government, this national 'virtual centre' brings together experts to implement a collaborative strategy. Initially focussing on MRH issues [androgen use/abuse, infertility, ED, testis cancer, prostate disease] HM recognised the linkages to chronic disease [cardiovascular disease, diabetes, mental health] and expanded its focus.

The overall approach has been to first understand consumer needs, then assess the evidence, educate and upskill health professionals and finally 'close the loop' through community education of what and how help can be accessed. Although not resourced as a research organisation the longitudinal 'Men in Australia telephone survey' [MATEs, Lancet 2006] identified knowledge gaps for future attention.

Core material were generated by expert advisory groups and collaborative programs developed with primary health practitioners and disadvantaged groups, such as indigenous people. Strong demand from grassroots organisations for evidence-based information has occurred for local and national use. Re-branding to 'Healthy Male' in 2018, a new website and social media engagement expanded its profile.

Most effort has been given to the primary care workforce, but specialist issues are being addressed e.g. IVF gynaecologists in male fertility care, the Endocrine Society re androgen guidelines, and HM trainee fellowships. A close relationship has been forged with the Federal Health Department with HM as a trusted resource for policy development, notably overseeing National Men's Health Strategy 2020–2030. Recognizing the need to better support young men from pre-conception though their first year of fatherhood, the

'Plus Paternal Project' is currently developing a roadmap for action.

HM is now well-recognized as a trusted resource in all sectors: its innovative, responsive and flexible approach is a model of independent evidence-based men's health advice and training targeted to the needs of consumers and health professionals.

Symposium 1 – Genetics in Male Reproduction

09:30–09:50 h

The identification and validation of genetic causes of male infertility

M. O'Bryan

Brendan Houston and the International Male Infertility Genomics Consortium, The School of Bio-Sciences, The University of Melbourne, Australia

As explored earlier in the congress, the aetiology of human male infertility is frequently thought to be genetic. Unfortunately, proving this is the case is often extremely difficult. This difficulty arises for several reasons, including that infertility, by definition, will limit family size, thus making traditional linkage studies extremely difficult. Further, the number of genes expressed during spermatogenesis (>19,000 in humans, including >2,000 that are testis-enriched) means that offspring infertility is likely a common consequence of mutagenesis within the germ line. At a practical level this means that the frequency of male infertility within a population will be relatively high, but individual gene-specific causes of male infertility will be low. Thus, while identifying a potential genetic cause of infertility is now technically feasible, finding the second and third infertile patient containing mutations in the same gene will be very laborious. To meet this challenge, the International Male Infertility Genomics Consortium is working collectively to assemble DNA samples from large numbers of clinically well described infertile men so as to allow cross-referencing of sequencing data across countries. In a parallel test of causality, and as a step towards defining the mechanism of gene action, the role of individual genes in male infertility is being tested using a range of model systems ranging from cell lines, to flies, to zebrafish, to mice. Each model system has its advantages and disadvantages. Within this presentation we will explore the analytical pipeline used to prioritize male infertility candidate genes identified via whole exome/genome sequencing, the production of models and their phenotyping as a means to validate the genetic causes of male infertility.

09:50–10:10 h

Translational aspects of novel gene mutations affecting human male fertility

M. Laan

Institute of Biomedicine and Translational Medicine, University of Tartu, Estonia

Infertility affects ~7% of men, caused by congenital or acquired spermatogenic impairment due to either testicular damage or defective gametogenesis per se. Today, up to >50% of cases remain unexplained. Current andrological workup includes limited genetic tests with clear value in patient management – standard karyotyping, analysis of *AZF* deletions and *CFTR* gene mutations. Insufficient knowledge of mechanisms underlying infertility limits primary management and patient-centred clinical care. Active research has been ongoing to identify novel genetic factors in male factor infertility, uncovering far more diverse genetic heterogeneity than initially thought. Even for the extreme phenotypes, such as azoospermia, only a small number of genes have been identified with recurrent mutations explaining the lack of sperms. Leadership in translating the findings of monogenic causes for the immediate patient benefit concerns rare conditions, congenital hypogonadotropic hypogonadism and disorders of sex development with the diagnostic yield ~50%. For oligozoospermia, representing the majority of infertility cases, current known single gene defects mostly do not apply. Extensive research is urgently needed to capture the ‘genetic landscape’ of reduced sperm counts, e.g. possibly explained by e.g. combined effect of several low-penetrant variants or uncovered structural variants that destabilize chromosomal stability in spermatogenesis. Data indicates that targeted analysis of single or few genes would not serve as an efficient approach to majorly improve the diagnostics in male infertility. Instead, a favored long-term goal could be setting up a clinical quality pipeline for genome-wide approaches, e.g. exome sequencing analysis. Improved molecular diagnostics would not only impact patients’ clinical management and reproductive decision making, but also assessment of accompanying health-related issues and better estimates for potential congenital health risks of the offspring.

10:10–10:30 h

Impact of healthy ageing on male germ cells

J. Gromoll
Centre of Reproductive Medicine and Andrology,
Münster, Germany

Male germ cell ageing is an important, but grossly understudied question. Life-long sperm production led to the assumption that male fertility remains unchanged throughout life. However, there are indications that advanced age has profound consequences to male fertility and offspring health, such as increased time to pregnancy and miscarriage rates, and higher incidence of certain disorders in the offspring. The fact that some common age-associated somatic diseases affect male fertility hinders the assessment of the true effects of ageing on male reproduction. Therefore, we aim to assess effects of “pure” ageing on germ cells by studying healthy men at different ages. We noticed that while normal classical male reproductive parameters, e.g. hormones and sperm parameters,

are maintained, an intrinsic ageing process continues which is specific to germ cells, interfering with DNA integrity and different from somatic ageing. Moreover, healthy ageing enables full spermatogenesis during lifetime and this seems to be an intrinsic determinant. However, to maintain normal sperm output compensatory mechanisms such as an increased proliferation of type-A spermatogonia, the reserve testicular stem cells, are taking place. Overall, healthy ageing effects male germ cell integrity at different levels and could be responsible for the observed reduced fecundity and poorer health prognosis for the offspring of elderly fathers.

Grants: Supported by the German Research Foundation and internal CeRA grants.

Symposium 4 – Microbiome and Male Health

13:51–14:09 h

Identification of new protein markers of biological androgen activity in humans

A. Giwercman¹, I. Pla¹, K. B. Sahlin¹, K. Pawłowski², C. Fehniger¹, Y. Lundberg Giwercman¹, I. Leijonhufvud¹, R. Appelqvist¹, G. Marko-Varga¹, A. Sanchez¹, J. Malm¹

¹Lund University, Sweden, and ²Warsaw University of Life Sciences SGGW, Poland

Introduction The testosterone concentration in plasma does not reflect the biological androgenic activity (BAA). Hypogonadism is a predictor of infertility but also several other diseases, e.g. metabolic syndrome, diabetes, cardio-vascular disease, and osteoporosis, as well as premature mortality. Understanding the biology of androgen action may therefore contribute to clarifying pathogenetic mechanisms linking androgen deficiency to these diseases.

Patients and Methods 30 healthy men (19–32 years) were castrated with an GnRH-antagonist and three weeks later given testosterone. Blood samples were collected at baseline, and three and five weeks after baseline, and used for proteomic studies.

Testosterone dependent proteins were analyzed in serum samples from 75 males (37.8 ± 5.5 years) recruited among men from infertile couples. Comorbidities in this cohort were insulin resistance (IR), cardiovascular risk, diabetes (DM) and low bone density (LBD).

Samples were analyzed using mass spectrometry and results using various statistical and bioinformatic methods.

Results In total, the levels of expression of forty-six of 676 proteins varied statistically significantly with testosterone concentration. Levels of thirty-seven proteins were positively associated, whereas, the remaining nine markers were negatively associated. Eighty percent of the proteins (37 out of 46) significantly distinguished the low testosterone group from the normal ones. Three of the proteins were selected as potential candidate markers for biological androgen activity and

showed statistically significant differences between the three groups and also discriminated low testosterone values in the cohort of patients analyzed. The power of the candidate proteins for discriminating IR, CVR and MetS were in general similar or better than testosterone.

Conclusion New testosterone dependent proteins were identified which could form the basis for more accurately measuring the biological androgen activity.

14:09–14:27 h

Fertility awareness – Time to focus on the male partner

M. Bodin
Centre for Sexology and Sexuality Studies, Malmö University, Sweden

Measures to improve reproductive health through health promotion usually focus on women and their behaviors and responsibilities. Although men’s fertility health is equally important, men have become the second sex in reproduction. Consequently, many men have poor knowledge about, and feel distanced from, their reproductive functions and bodies. By interviewing young and adult men, I found that most men were unaccustomed to talk about their fertility, especially with friends. In cases of reproductive decision-making, only women’s health and age were of concern. Many men did, however, appreciate talking about their reproductive health when given the opportunity, which implies that men’s fertility awareness can easily be raised merely by including them in the discussion. I also found that there is great uncertainty among men about what truly affects fertility, pointing to a need for more studies on the relationship between lifestyle factors and male fertility, but also public information that separates myths from facts. My conclusion is that fertility education is something that could start already in school, but we also need to find a format for education/counseling that appeals to young and adult men.

Symposium 5 – Male Ageing

15:05–15:25 h

What happens to the DNA of sperm as men age?

S. Laurentino
Center of Reproductive Medicine and Andrology (CeRA), Department of Clinical and Surgical Andrology, University Clinic Münster (UKM), Germany

Although males are capable of producing sperm throughout their entire adult lives, male fertility decreases with increasing age. In addition, advanced paternal age is associated with increased miscarriage rates and the offspring of older men has a higher incidence of so-called paternal age effect disorders and poor perinatal health. The reasons behind these adverse outcomes may lie within the sperm DNA. While sperm itself is approximately 74 days old, the male gamete is the result of spermatogonial stem

cells that remain in the gonad from early embryonic development and support continuous spermatogenesis for decades from puberty onwards. These cells can be negatively affected by advancing age both directly (e.g. due to repeated cell divisions) and indirectly (e.g. through the action of an altered somatic environment). Ageing ultimately results in a plethora of male germline-specific molecular effects which include a high *de novo* mutation rate, increased genomic instability and DNA damage, elongating telomeres, and profound changes in sperm DNA methylation. These molecular changes occur even in healthy men with normal sperm and hormonal parameters and they may contribute to the poorer reproductive and offspring health outcomes experienced by older men.

Symposium 6 – Sperm Production and Function

CatSper & Co: How ion channels modulate sperm function

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Out of millions of ejaculated sperm, only a few reach the fertilization site in mammals. Flagellar Ca²⁺ signaling nanodomains, organized by multi-subunit CatSper calcium channel complexes, are pivotal for sperm migration in the female tract, implicating CatSper-dependent mechanisms in sperm selection. Here, using biochemical and pharmacological studies, we demonstrate that CatSper1 is an O-linked glycosylated protein, undergoing capacitation-induced processing dependent on Ca²⁺ and phosphorylation cascades. CatSper1 processing correlates with protein tyrosine phosphorylation (pY) development in sperm cells capacitated in vitro and in vivo. Using 3D in situ molecular imaging and ANN-based automatic detection of sperm distributed along the cleared female tract, we demonstrate that all spermatozoa past the UTJ possess intact CatSper1 signals. Together, we reveal that fertilizing mouse spermatozoa in situ are characterized by intact CatSper channel, lack of pY, and reacted acrosomes. These findings provide molecular insight into sperm selection for successful fertilization in the female reproductive tract.

Symposium 8 – Male contraception

17:45–18:00 h

Population growth – Will male contraception create a game change?

E. Nieschlag

Center for Reproductive Medicine and Andrology, University of Münster, Germany

Life expectancy is increasing worldwide, as are birth rates, especially in Sub-Saharan Africa and South Asia, resulting in ever increasing world population growth. Since 1950 the world population has quadrupled and the current 7.7 billion inhabitants are predicted to reach 11 billion by 2100. Overpopulation and increasing standards of living for all threaten the existence of mankind by contributing to climate change and damaging the environment. Would new methods of male contraception be a game changer in population growth? Before the “pill” and other modern female methods, men were fully in charge of contraception by using condoms, withdrawal and vasectomy and even today 25% of contraceptive use consists of these male methods. From these facts it can be concluded that men would be ready to use new male contraceptives if they would be reversible, free of serious side effects and affordable. Several opinion polls conducted on men participating in clinical trials for male contraception have shown high approval rates. A study performed in 9 countries extrapolated that 44 million men in these countries would use the tested male hormonal contraceptive. New male methods would also help to reduce the unplanned and undesired conceptions, estimated to amount to about half of the 1 million daily conceptions worldwide. However, these methods are not yet available and intensive research by the pharma industry would be necessary. Existential economic and social threats to livelihood and family life caused by the current pandemic may increase pressure for new male methods. The green activists have to recognize the link between overpopulation and climatic effects. Governmental financing might be necessary. Education and family planning programs should not only address women, but include males, especially adolescents. Finally, male contraception needs politicians and celebrities as protagonists. Altogether, this might bring about the desired game change.

18:00–18:15 h

The Male Hormonal Contraceptive Pipeline

S. T. Page

University of Washington School of Medicine, WA, United States

Nearly 40% of global pregnancies are unplanned, a reflection of significant unmet contraceptive needs. Men play a significant role in effective family planning, accounting for nearly one-quarter of all contraceptive use worldwide; thus, the development of novel male contraceptive methods that are efficacious, reliable, safe and reversible could make a significant impact on the health of both men and women. Exogenous androgens form the basis of male hormonal contraceptives (MHC), but combinations of testosterone plus progestins are more effective. While a novel contraceptive gel is currently under Phase 2 efficacy evaluation, efforts are ongoing towards expanding hormonal contraceptive options for men. Novel

compounds with androgenic or androgenic-progestogenic properties, dimethandrolone undecanoate (DMAU) and 11 β -methyl-19-nortestosterone 17 β -dodecylcarbonate (11- β MNTDC), show promise in early human trials. These steroids, derivatives of 19-nortestosterone, are both well-tolerated when administered orally to men, without liver toxicity, and with pharmacokinetics and pharmacodynamics suggesting effectiveness with once daily dosing. These characteristics are desirable for an effective “male pill”, a method many men prefer. In parallel studies, DMAU administered intramuscularly shows similar promise, with a goal towards developing a reversible male contraceptive administered every 3–6 months. Early clinical studies of both DMAU and 11- β MNTDC suggest side effects are mild and may include acne, modest weight gain and changes in serum cholesterol. Longer term studies are needed to evaluate the safety and efficacy of these prototype products, including evaluation of potential impacts on mood, libido and sexual function, with a goal towards expanding the method mix and uptake of male contraceptives.

18:15–18:30 h

Progress in Efficacy and Safety Study with NES-T gel

C. Wang

Clinical and Translational Science Institute, The Lundquist Institute; Division of Endocrinology, Harbor-UCLA Medical Center, CA, United States

On behalf of the Investigators of “Clinical Evaluation of Daily Application of Nestorone® (NES) and Testosterone (T) Combination Gel for Male Contraception (CCN017)

The Contraceptive Development Program, Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, United States together with the Population Council initiated planning of the study on the efficacy of daily application of Nestorone (NES)® and Testosterone (T) gel in 2016 through the Contraceptive Clinical Trial Network Male Centers. This is the first user controlled male hormonal contraceptive study where prevention of pregnancy in the female is the primary endpoint of the study. All other hormonal male contraceptive efficacy studies utilized injections or implants. This late phase 2 hormonal male contraceptive study is based on a phase 1 study that determined the optimal dose of NES and an early phase 2 study that demonstrated the combination of NES+T applied daily on the skin will suppresses sperm output to levels that are compatible with preventing pregnancy in the female partner. NES/T gel was manufactured containing NES 8 mg and T62 mg applied daily to the skin and recruitment started in the US centers (Los Angeles and Seattle) at the end of 2018 and in Santiago (Chile), Edinburgh, Manchester (UK), Stockholm (Sweden) and Nairobi (Kenya) in the spring of 2019. In the summer of 2020, four additional centers (Portland, David, Norfolk, and Philadelphia)

in the US were recruited to participate in the center. Currently the study has enrolled 169 couples with 94 that are in efficacy phase where the couple relies on NE/T gel as their sole method of contraception. There are very few adverse events. The study incorporates a detailed assessment of both male and female partners' behavior and attitude to male contraception and the study. Our goal is to recruit 420 couples, the enrollment is anticipated to complete by 2021 and end in 2023. The plan is to plan for a phase 3 study 2022–23 to meet the regulatory agencies guidelines for an acceptable contraception if NES/T gel is safe, well tolerated, efficacious and acceptable to the couple.

Tuesday, 8 December 2020

Plenary Lecture 4

09:30–10:15 h

Neuroendocrine regulation of male puberty: Certainties, assumptions and open questions

M. M. Tena-Sempere

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Puberty, as key maturational event in the lifetime of any individual, is under the precise control of complex neuroendocrine regulatory networks, which impinge onto the upper levels of the so-called hypothalamic-pituitary-gonadal (HPG) axis. These pathways transmit the regulatory effects of a large number of endogenous and environmental signals, including metabolic and nutritional cues, that allow to timely and precisely modulate the full activation of the secretory activity of GnRH (gonadotropin-releasing hormone) neurons in the basal forebrain, as major driving force for pubertal progression. While notable chronological and phenotypic differences exist between male and female puberty, there are also important commonalities, including the central role of GnRH neurons, and some of their major upstream regulators, in the neuroendocrine control of puberty. The latter include kisspeptins, the products of Kiss1 neurons, which have been recently recognized as major gatekeepers and indispensable regulators of puberty onset in both sexes, by virtue of their capacity to activate GnRH neuro-secretion. Admittedly, however, most of our current understanding on the ultimate mechanisms whereby puberty is centrally controlled stems from preclinical studies conducted mainly in females; key aspects of the neurohormonal basis of male puberty being mostly inferred from female data. This is especially relevant when addressing the control of puberty by nutritional cues, where direct inference of is hampered by the intrinsic differences in the sensitivity

of female vs. male puberty to metabolic cues. In this presentation, I will recapitulate what is known about the basic neuroendocrine mechanisms for pubertal control in males, with particular emphasis of current gaps of knowledge and open questions regarding the brain control of male puberty. Recognition of such limitations should illuminate new areas of research that may help to better manage pubertal disorders, also in boys.

Plenary Lecture 5

13:00–13:45 h

Fertility Preservation – translation into the clinic

E. Goossens

Biology of the Testis lab, Vrije Universiteit Brussel, Belgium

Introduction Infertility is an important side effect of treatments used for cancer and other non-malignant conditions in males. This may be due to the loss of spermatogonial stem cells (SSCs) and/or altered functionality of testicular somatic cells (e.g. Sertoli cells, Leydig cells). Whereas sperm cryopreservation is the first-line procedure to preserve fertility in post-pubertal males, this option does not exist for pre-pubertal boys. For patients unable to produce sperm and at high risk of losing their fertility, testicular tissue freezing is now proposed as an alternative experimental option to safeguard their fertility.

This presentation will provide an update on clinical practices and experimental methods, as well as on patient management inclusion strategies used to preserve and restore the fertility of pre-pubertal boys at high risk of fertility loss.

Methods Based on the expertise of European and North American centres offering fertility preservation programs and a literature search of the progress in clinical practices, patient management strategies and experimental methods used to preserve and restore the fertility of pre-pubertal boys at high risk of fertility loss were identified.

Results Since the first publication on murine SSC transplantation in 1994, remarkable progress has been made towards clinical application: cryopreservation protocols for testicular tissue have been developed in animal models, and are now offered to patients in clinics, however, still as an experimental procedure. The implementation of testicular tissue cryopreservation as a means to preserve the fertility of pre-pubertal boys is increasing. In 2019, more than 1033 young patients (up to the age of 18 years) had already undergone testicular tissue retrieval and storage for fertility preservation.

At the same time, important scientific progress has been made concerning fertility restoration methods. The technique of SSC transplantation has been translated to human cadaver testis, proof-of-concept has been obtained for testicular tissue grafting in non-human primates, and important steps have been taken in establishing spermatogen-

esis in vitro. However, important practical, medical and ethical issues must be resolved before fertility restoration can be applied in the clinic.

Conclusions Considering the progress that has been made and the barriers that still have to be overcome, the first patients eligible for autologous transplantation of testicular tissue or cell suspensions are likely to be those treated for solid non-metastatic tumours or non-malignant diseases, as for these patients there is no known risk of re-introducing malignant cells into the testis. However, until successful clinical trials demonstrating safety and efficacy of fertility restoration have been conducted, testicular tissue cryopreservation for fertility preservation should remain experimental.

Symposium 11 – Imaging and Surgery in Andrology

14:20–14:40 h

Diagnostic value of Dual-Energy CT angiography in the diagnosis of arteriogenic erectile dysfunction

M. Wang¹, X. Zhang²

¹Clinical Medical College, Anhui Medical University, Hefei, China; ²Department of Urology, The First Affiliated Hospital of Anhui Medical University, Hefei, China

The purpose of this study was to investigate the diagnostic value of Dual-Energy CT angiography (DE-CTA) in patients with arterial erectile dysfunction.

Methods A total of 49 patients with suspected arteriogenic ED were enrolled. Colour duplex Doppler ultrasonography (CDDU) was taken to evaluate arterial insufficiency of penile. After induction of an erection with prostaglandin E₁, DE-CTA performed with the latest generation 256-slice CT scanner was used to obtain angiograms of the pelvis and penis. The arterial system supplying the penis (internal pudendal artery, common penile artery, penile dorsal artery, and cavernous artery) was evaluated with Multiple maximum intensity projection (MIP) and volume rendering (VR).

Result Of the 49 patients, 47 (95.9%) had pelvic images of sufficient quality for evaluation. A total of 376 segments were finally obtained and 163 segments were considered as abnormal. The distribution was 29 (17.8%) in internal pudendal artery segment, 31 (19.0%) in common penile artery segment, 42 (25.8%) in penile dorsal artery segment and 61 (37.4%) in cavernous artery segment. The diagnostic validity of DE-CTA with CDDU as gold standard was sensitivity = 85.4% and specificity = 83.3%.

Conclusion DE-CTA can detect macroangiopathy and delineate small vessels clearly, and thus can provide additional anatomic evaluation of artery compared with CDDU. DE-CTA has the potential to become a reliable method in the diagnosis of arteriogenic erectile dysfunction.

Symposium 12 – WHO semen analysis manual

14:20–14:40 h

When will sperm DNA testing become mandatory?

E. Baldi, S. Marchiani, L. Tamburrino, M. Muratori
Depts. of Experimental and Clinical Medicine and of Biomedical and Clinical Sciences "Mario Serio", University of Florence, Italy

Routine evaluation of semen has been demonstrated to be insufficient to assess accurately male fertility status. Sperm DNA fragmentation (sDF) is one of the most frequent among defects found in DNA of spermatozoa of sub-fertile men. Several meta-analyses demonstrated a clear association between sDF levels and outcomes of natural and assisted reproduction and frequency of miscarriage. sDF is moderately associated with semen quality, thus prospecting as a good parameter to be added to routine semen analysis in the diagnosis of male infertility. sDF may originate in the testis, due to defective spermatogenesis and testicular apoptosis, and because of elevated oxidative stress, the latter likely damaging sperm after spermiation. In addition, there is evidence that sperm manipulation during assisted reproduction techniques, may increase the damage to DNA. Despite the progress in research on sDF, its introduction in clinical setting remains highly debated, mostly because of lack of standardized methods and of effective therapies to reduce sDF levels. Presently, sDF can be measured with several assays, among which, the most popular are TUNEL (terminal transferase-mediated dUTP end labelling), SCSA (sperm chromatin structure analysis), Comet or SCGE (single-cell gel electrophoresis) and SCD (sperm chromatin dispersion). These assays show different cut-off values, and, with the exception of SCSA, are not or only poorly standardized. In addition, they detect different types of nuclear damage making difficult the comparison among the several studies present in the literature. In such a situation, the only possibility for laboratories evaluating sDF is to set their own cut-off values with the established method by comparing fertile and sub-fertile men. Clearly, efforts should be done to establish the gold standard technique to evaluate sDF, to find effective therapies to decrease DNA damage in vivo and to establish correct procedure to limit the damage during in vitro manipulation.

ESAU Symposium 2 – Surgery in Sexual Medicine

15:30–15:38 h

Preparing the patient for phallo-endoprosthesis

O. Ivanovic Apolikhin
N.A. Lopatkin Research Institute of Urology and Interventional Radiology, Moscow, Russian Federation

Penile prosthesis is a highly effective and safe method of final-line treatment of patients with

erectile dysfunction (ED), and is also characterized by a high level of their psychosexual satisfaction, sexual and social rehabilitation.

Knowledge of modern models of penile prosthesis, as well as individual choice of the optimal device, is crucial for both the patient and surgeon.

In addition, the urologist should have sufficient information about possible complications and difficulties that are inherent in prosthetic surgery, have a complete understanding of the patient's preparation, as well as about the features of management in the postoperative period.

Preoperative preparation of the patient should include:

- somatic pathology examination;
- a urine culture with definition of sensitivity to antibiotics;
- implementation of antibiotic prophylaxis;
- wash and shave the operating field on the day of the operation.

Special attention should be paid to patients with diabetes, since the sugar level above the maximum permissible may cause rejection of the implant and become a direct contraindication to surgery. Another serious factor that also needs to be considered is varicose veins of the lower extremities, which is associated with a high risk of developing thromboembolism.

When preparing a patient for genital surgery it is most preferable to remove the scrotal hair with a safety razor on the day of the operation. So, urologists from the University of Toronto studied the frequency of skin damage and the degree of scrotal hair removal when shaving this area of the body before surgery on the male genitals, concluded that any violations of the integrity of the skin before intervention on the external genitals, as well as the presence of hair can increase the likelihood of infectious and inflammatory complications [Grober ED, Domes T, Fanipour M, Copp JE. Preoperative Hair Removal on the Male Genitalia: Clippers vs. Razors. *J Sex Med* 2013; 10: 589-9].

Performing surgery for a penile prosthesis requires both all members of the operating team and the operating nurse to have sufficient experience in penile implantation surgery, as well as knowledge of a variety of special operational and technical techniques.

It is worth considering that to perform a penile prosthesis requires a special surgical instruments:

- ring Scott's retractor;
- diver and ensembler hooks;
- bougienage of the distal and proximal parts of the cavernous channels is performed by Brooks bougie;
- in the presence of cavernous fibrosis, Rosello cavernotomes are used;
- the length of corpus cavernosum is measured using a Furlow ruler.

All surgical instruments necessary for performing prosthetic surgery are laid out by the

operating nurse by type, as well as by stages of surgical intervention.

In addition, the main tasks of an operating nurse at the intraoperative stage include:

- strict adherence to the rules of asepsis and antiseptics throughout the entire operation
- timely warning of the surgeon about any violation of asepsis
- restriction of movement in the operating room of persons who are not involved in the operation (students, residents)
- the presence of double gloves for the surgeon and his assistant, as well as monitoring their change at certain stages of the operation
- frequent irrigation of the operating wound with an antiseptic solution (NaCl 0.9% + rifampicin/gentamicin) throughout the course of surgery.

It is worth noting that careful treatment of skin areas close to the operating field is mandatory, as well as treatment of the operating field itself with an antiseptic solution. This takes into account the exposure time of the skin antiseptic, which should be at least 10–15 minutes. The use of an alcoholic solution of chlorhexidine reduces the risk of wound infection by 40% [Darouiche R. et al., 2010].

After installing the urethral catheter, surgical gloves must be replaced. Just as before implantation of the components of the falloprosthesis, it is mandatory to replace the surgical gloves of each member of the operating team.

It is important to note that before implantation, the components of the penile prosthesis are abundantly irrigated with an antiseptic solution (rifampicin 10 mg/ml and gentamicin 1 mg/ml) with an exposure of at least three minutes. And after dilation of the cavernous bodies, an antiseptic solution (gentamicin/rifampicin) is also introduced into them in order to sanitize the cavernous channels

Postoperative management should include antibiotic prophylaxis, removal of the urethral catheter on the first postoperative day, as well as daily dressings with cleanliness of the postoperative wound, exclusion of postoperative hematomas with immediate evacuation if present, and, finally, sexual rest for one month (in patients with somatic burden, this period can be extended to one and a half months).

It is also worth noting the importance of an individual approach to each case, since to date there are no specific clinical recommendations on the timing of antibiotic prophylaxis and the drugs used.

Symposium 13 – Fertility Preservation

15:50–16:10 h

Who are the patients? A need for fertility preservation beyond oncological diseases

K. Jahnukainen
Children's Hospital, Helsinki University Hospital and University of Helsinki, Finland

Introduction Allogeneic hematopoietic stem cell transplantation (HSCT) is an established therapeutic procedure for severe hematological disorders including leukemia, lymphoma, and benign hematological diseases like aplastic anemia, sickle cell disease, thalassemia and bone marrow failure syndromes. Improvements in treatment modalities and supportive care have led to a growing population of long-term survivors and knowledge on late effects is increasingly needed. Infertility is of major concern following pediatric HSCT with high impact on quality of life. Total body irradiation and high doses of alkylating chemotherapy are acknowledged risk factors for gonadal dysfunction after HSCT. Host genetic background may also influence the acute and long term toxicity after HSCT and could at least partially explain the inter-individual variability in outcome.

Patients and Methods Cross-sectional follow-up study enrolled 2 national cohorts of adult males (≥ 18 years) treated with myeloablative allogeneic pediatric HSCT before age 17 between 1980–2010 in Denmark and Finland. Gonadal function at long-term follow-up was studied.

Spermatogonial numbers were evaluated in cryopreserved immature testicular tissue from patients with non-malignant diseases in the frame of the fertility preservation programs Androprotect and Nordfertil.

Results Late spermatogenic recovery is possible 10–30 years following myeloablative HSCT but depends on cumulative doses of alkylating agents and irradiation. Pre-pubertal status at HSCT increases the risk for later testosterone substitution. Patients with sickle cell disease have a decreased spermatogonial numbers in cryopreserved testicular tissue prior to HSCT.

Conclusion The risk of male gonadal dysfunction after pediatric HSCT is high and depends primarily on the cumulative testicular irradiation dose and pubertal stage at transplant. Our findings pinpoint the need of fertility preservation before HSCT as well as prolonged follow-up of pediatric HSCT patients into adulthood. Severe hematological disorders can have direct effects on spermatogonial quantity and options for fertility preservation.

16:10–16:30 h

In vitro approaches to obtain spermatozoa: Experimental approaches with a clinical perspective

T. Ogawa
Yokohama City University, Japan

If human in vitro spermatogenesis is possible, it would be a valuable technique for studying human spermatogenesis in detail. In addition, in vitro spermatogenesis would serve for producing sperm from cryopreserved immature testis. Thus, it actually serves for preserving fertility of boys with malignant diseases such as leukemia. However, human in vitro spermatogenesis is still unsuccessful, even today.

I have been working on in vitro spermatogenesis since 2007, mainly using mice. In 2011, our group succeeded in generating functional mouse sperm from spermatogonial stem cells using conventional organ culture method. The key to this success was commercially available serum replacements, KSR or AlbuMAX, supplemented to the culture medium. At the time, I thought that the method could be applied to animals other than mice, even to human. However, it did not work in other animals and not sufficiently effective even in rats. To make matters worse, the serum replacements, which is effective for in vitro spermatogenesis in mice, contained various unknown substances, and we were unable to improve the medium because we had no idea what components were actually effective for in vitro spermatogenesis. Therefore, we embarked on a project to identify these important factors, substances, and chemicals and formulated a chemically defined medium (CDM). The CDM would enable us to improve the culture medium and optimize it for each species. In this presentation, I would like to present advances in the composition of media for in vitro spermatogenesis that could be an important step towards human in vitro spermatogenesis.

EAA/ESAU Symposium 3 – Management of NOA: Current Practice and Thoughts for The Future

17:12–17:24 h

Pre-TESE prognostic genetic tests: where we are now?

C. Krausz
Department of Experimental and Clinical Biomedical Sciences "Mario Serio", University of Florence, Italy

Over the last 20 years genetic screening became part of the routine diagnostic work-up of non obstructive azoospermia (NOA) patients. Numerical chromosomal alterations, such as 47, XXY and structural alterations, such as the Y chromosome-linked AZF deletions show a cause-effect relationship with NOA. With the advent of Next Generation Sequencing (NGS) monogenic causes started to be discovered, allowing to define the genetic etiology of an increasing proportion of idiopathic NOA. Apart from their diagnostic value, some genetic factors have also a prognostic value in terms of testicular sperm retrieval. For instance, carriers of a complete AZFa or AZFb deletion have poor TESE prognosis with virtually zero chance of testicular sperm retrieval. For an appropriate prediction of TESE outcome in a suspected complete AZFb deletion, it is fundamental to test for a specific distal marker (sY1192), since the presence of this STS is compatible with positive sperm retrieval. An X chromosome-linked gene, *TEX11*, is susceptible to intragenic deletions which lead to spermatocytic arrest. While *TEX11* deletions have a negative pre-TESE prognostic value, the clinical interpretation of the non-loss of function (LoF) mutations is more complex.

Besides sex chromosome defects, an increasing number of recessive mutations in autosomal genes have been reported in NOA. Some of these autosomal genes play a crucial role in meiosis, therefore LoF mutations cause TESE negative NOA. From a diagnostic point of view, *TEX11* is already classified as a definitive disease-causing gene, whereas several autosomal genes have reached strong or moderate clinical evidence opening a new era towards the development of pre-TESE gene panel.

17:36–17:48 h

Sperm recovery and ICSI outcomes in men with NOA

S. Kliesch
Center of Reproductive Medicine and Andrology (CeRA), Department of Clinical and Surgical Andrology, WHO collaboration center for research in male reproduction, Training Center of the European Academy of Andrology (EAA), University Clinic Münster (UKM), Germany

Patients with non-obstructive azoospermia (NOA) are a challenge for diagnosis and treatment. The differential diagnose of NOA comprises primary testicular failures, endocrine failures and genetic disorders. While hypothalamic-pituitary deficits result in testicular failure of spermatogenesis and will be successfully treated by gonadotropins, AZF a or AZF b deletions of the Y-chromosome render successful sperm retrieval (nearly) impossible. Careful diagnostic assessment has to rule out access to successful causative treatment options in azoospermic patients and to differentiate those who should get primary access to (microsurgical) techniques of testicular sperm retrieval (TESE). Obstructive azoospermia (OA) has to be carefully elucidated, as reconstructive surgery could be a valuable option and 1 out of 6 azoospermic man may be identified with OA instead of NOA. However, differentiation may be difficult with normal clinical phenotype (normal testis volume, normal endocrine profile, normal seminal volume). Only genetic analysis of the newly described genes (as *TEX 11*, *STAG 3* or *TEX 14*) may hint at meiotic arrest of spermatogenesis and finally make TESE unnecessary [Tüttelmann et al., Andrology 2019, Hum Reprod 2019; van der Bijl et al. Hum Reprod 2019]. Even more individualised prognostic aspects may gain relevance, as polymorphisms of the FSH gene may influence the TESE outcome [Busch et al. JCEM 2019]. Moreover, hypogonadism is observed in 1/3 of NOA patients and potential pretreatment options to increase endogenous testosterone levels to at least 8 nmol/l may influence TESE outcome [Ramassamy et al. 2009, Rohayem et al. 2015].

In NOA, sperm recovery is recommended to be performed by TESE either as a multifocal conventional or a microsurgical (microscopic assisted) approach. Especially the most severe patient groups with significantly reduced testicular volume, high FSH levels and severe testicular damage may benefit most from the microTESE approach as it facilitates to iden-

tify focal spots of spermatogenesis in seminiferous tubules by magnification. However, a very recent systematic review and meta-analysis of the ESAU group revealed the shortcomings of published data. Altogether 56 studies on conventional, 43 studies on micro-TESE and another 18 studies with combined surgical approaches in heterogeneous patient cohorts failed to clarify the hypothesized advantage of micro-TESE, as the studies were not comparable, had uncontrolled and mostly retrospective study designs. Nevertheless, the overall sperm retrieval rate in NOA patients is 47% in 117 studies with more than 21,000 patients analysed. The TESE-ICSI procedures finally resulted in a cumulative pregnancy rate of 29% per cycle (reported in 42/117 studies), with 1096 biochemical pregnancies and 569 reported life births (cumulative life birth rate of 24%) with no significant differences between conventional and microsurgical TESE [Corona et al. HRU 2019].

17:48–18:00 h

How close are we to spermatogonia stem cell autotransplantation for the treatment of subpopulations of non-obstructed azoospermic men?

N. Sofikitis

Department of Urology, Ioannina University School of Medicine, Greece

Previous research efforts have indicated that syngeneic or xenogeneic spermatogonia stem cell (SSCs) transplantation efforts into the seminiferous tubuli of immunodeficient animals may result in the development of donor spermatogenesis within the recipient testis. It has been suggested that the recovered donor spermatozoa from the recipient testis can be used for assisted reproductive techniques for the treatment of non-obstructive azoospermia [NOA; Human Reproduction Update, 2003: 9, 291]. However ethical barriers, genetic barriers, and virus-related transfer risks may impede the utilization of donor human spermatozoa generated in a host syngeneic or xenogeneic testis for subsequent ICSI techniques for the treatment of NOA.

On the other hand, employing similar microsurgical procedures, frozen/thawed human testicular germ cells or SSCs can be autotransplanted back to the patient's testis without using a recipient animal testis as a surrogate organ. Candidates may be men with NOA and unilateral testicular cancer who are about to undergo unilateral radical orchiectomy. At the time of such surgery, germ cells can be isolated from the contralateral healthy testis. In vitro culture techniques may increase the subpopulation of recovered SSCs [Hum Reprod Update, 2005: 11, 229]. In addition, it is important that biopsies from the contralateral testis show an absence of neoplasia; fractions of isolated/cultured testicular germ cells or SSCs from the healthy testis will be then frozen. Several months after completion of chemotherapy in men with advanced testicular cancer, frozen/thawed germ cells or SSCs can be transplanted back to the rete testis or

the seminiferous tubuli of the contralateral testis to the neoplastic testis. At several months after autotransplantation, semen samples should be evaluated for the presence of spermatozoa. Autotransplantation of testicular frozen/ thawed germ cells post-chemotherapy may represent a means of colonizing the human testis with its own cells, having as an overall target the appearance of spermatozoa in the ejaculate. On the other hand, the autotransplanted SSCs may undergo cellular divisions within the recipient patient's testis. Thus the number of the non-exposed to chemotherapy- SSCs may increase in vivo. Even if the final semen samples are negative for spermatozoa, the recipient testis may be positive for spermatozoa that have been derived by the autotransplanted SSC cellular subpopulation. The latter spermatozoa may be used for ICSI techniques.

Symposium 14 – Sexually transmitted diseases

17:20–17:40 h

Zika, Covid and beyond – Do we need to be concerned?

N. Dejucq-Rainsford

Inserm, Ecole des hautes études en santé publique (EHESP), Institut de recherche en santé, environnement et travail (Irset), Université de Rennes, France

While several viruses such as HIV or HBV are well-known vectors of sexually transmitted chronic diseases, lately, emerging viruses triggering acute infections were unexpectedly associated with long-term shedding in semen and sexual transmission despite systemic clearance. The sexual transmission of the deadly Ebola virus by survivors over 1 year after recovery led to the resurgence of epidemic foci, while that of the teratogenic mosquito-transmitted Zika virus led to its dissemination in 14 countries outside the mosquito vector area¹. In addition to diseases dissemination, viral infections of the male genital tract (MGT) can cause male infertility, hormonal disturbances and viral transmission to the embryo, affecting fetal development and pregnancy outcome¹. Worryingly, reports of seminal shedding and sexual transmission of other emerging viruses, including lethal ones, are accumulating [1]. The tropism for the MGT of SARS-CoV-2, the virus responsible for the Covid19 pandemic, is currently unknown. A single study reported SARS-CoV-2 in semen from infected men in the acute or recovery stage, whereas others failed to detect seminal excretion [2]. The interplay between SARS-CoV-2 and male hormones is an important question. Indeed, testosterone is suspected to participate to the increased disease severity in men over women by boosting virus entry into the cells. Deciphering the mechanisms for persistence in the MGT of emerging viruses and their interactions with MGT functions is therefore of prime importance.

We recently demonstrated that ZIKV infects the human testis ex vivo [3] and that persistently infected human testicular germ cells are released in semen up to 160 days post-

symptoms onset [4]. Why some viruses such as ZIKV escape from immune surveillance and persists in human testis? In response to viral stimuli, the production by human testis/germ cells of IFN-I, a key cytokine for antiviral defense, is very low and transient, which may prevent germ cells apoptosis and sterility induced by IFN-I [5]. However, in the absence of a robust antiviral response, germ cells may represent an ideal shelter for viruses, since they are naturally segregated from immune cells and antibodies. In this talk, I will summarize the state of the art on the mechanisms of viral persistence in the testis and on SARS-CoV-2 interactions with the MGT.

References:

1. Le Tortorec et al, Physiological Reviews 2020.
2. Entezami et al, EClinical Medicine 2020.
3. Matusali et al, Journal of Clinical Investigation 2018.
4. Mahé et al, Lancet Infectious Diseases 2020.
5. Satie et al, Journal of Biological Chemistry 2011.

17:40–18:00 h

Viruses and testis cancer

A. Garolla

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Testicular cancer represents the more frequent solid tumor affecting males aged 15–35 years. In the last decades, its incidence showed a progressive increased probably due to genetic and environmental factors. Despite exposure to some viruses such as HIV, HCV, EBV, and HPV is frequently related to cancer development, there are no studies aimed to evaluate the possible implication of viral infections in the pathogenesis of testicular cancer. In this study, we analyzed sperm parameters and prevalence of HPV on sperm in 155 testicular cancer patients at diagnosis (T-1), after orchiectomy (T0) and after 12 months from surgery or from the end of adjuvant treatments (T12). All patients showed a significantly higher prevalence of semen infection than controls (9.5% and 2.4% respectively) and altered sperm parameters both at T-1 and T0. Considering sperm parameters, at T-1 we observed a reduction of progressive motility, and after orchiectomy patients showed a reduction of sperm concentration and count and a further worsening of motility. Thereafter, patients were assigned to three groups on the basis of medical option after surgery: S = surveillance, R = radiotherapy, and C = chemotherapy +/- radiotherapy. At T12, untreated patients had an improvement of sperm parameters while R group and even more C group had a strong decrease of sperm number ($p < 0.01$ both vs. T0 and S group). Moreover, patients who received radio and/or chemotherapy had a very high prevalence of HPV semen infection (S = 7.7%, R = 30.8%, and C = 61.5%). In conclusion, patients with testicular cancer had frequently altered sperm parameters and higher prevalence of HPV semen infection that were worsened after radio and chemotherapy. Because HPV infection is a risk factor for cancer development and it may further reduce fertility, we suggest

screening for HPV in testicular cancer patients at diagnosis and particularly after adjuvant treatments.

Wednesday, 9 December 2020

Symposium 15 – Emerging Technologies

08:00–08:20 h

Shedding light on signaling in sperm flagella

D. Wachten

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Introduction Mammalian oocytes are enveloped by the *zona pellucida* (ZP), an extracellular matrix of glycoproteins. To fertilize the oocyte, sperm have to penetrate the ZP. To this end, interaction with ZP proteins evokes certain sperm behavioral responses. However, the mechanisms underlying ZP action in sperm are only ill-defined.

Results Here, we delineate the sequence of ZP-signaling events in mouse sperm. We show that ZP proteins evoke a rapid intracellular pH increase that rests predominantly on Na⁺/H⁺ exchange by NHA1 and requires cAMP synthesis by the soluble adenylyl cyclase sAC as well as a hyperpolarized membrane potential set by the sperm-specific K⁺ channel Slo3. The alkaline-activated CatSper channel translates the ZP-induced pH increase into a Ca²⁺ response.

Conclusion Our findings reveal the molecular components underlying ZP action on mouse sperm, opening up new avenues for understanding the basic principles of sperm function and, thereby, mammalian fertilization.

08:20–08:40 h

AKT Signaling Inhibition Favors Culture of Human Undifferentiated Spermatogonia

M. F. Wilkinson

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Spermatogonial stem cells (SSCs) are self-renewing undifferentiated spermatogonia (uSG). SSCs are essential for the continuous production of sperm and have potential therapeutic value for treating male infertility, which afflicts > 100 million men world-wide. There are several bottlenecks in developing SSC therapy, including identifying human SSC-specific markers and developing methods for human SSCs long-term culture. Here, we report the identification of a selective human SSC marker that allowed us to define the transcriptome of highly enriched SSCs, which, in turn, led us to identify signaling pathways that influence the fate of uSG and thereby develop a method for their short-term culture. To screen for SSC marker genes, we employed single-cell RNA sequencing (scRNAseq) to analyze adult human testicular cells. Genes exhibiting enriched expression

in the primitive uSG cell cluster defined by scRNAseq were candidates to specifically label SSCs. Immunofluorescence and immunohistochemical analysis revealed several of these genes encode proteins selectively expressed in uSG. We chose one of these – PLPPR3 – to purify uSG by FACS. Xenograft germ-cell transplantation analysis demonstrated that these cell-surface PLPPR3+ human testicular cells are 38-fold enriched for SSCs, demonstrating that PLPPR3 is a highly selective human SSC marker. Comparative RNAseq analysis of these PLPPR3+ highly enriched SSCs with differentiating (d) SPG (KIT+ cells) revealed the full complement of genes that shift expression during the uSG-to-dSG developmental transition. Among these dynamically regulated genes were genes encoding key components in the AKT, GDNF, JAK-STAT, and TGFβ signaling pathways. Manipulating these signaling pathways in cultured human SPG revealed that GDNF and BMP8B broadly support human SPG culture, while Activin A selectively supports more advanced human SPG. One condition – AKT pathway inhibition – had the unique ability to selectively support the culture of primitive human uSPG with the characteristics of SSCs. This raises the possibility that supplementation with an AKT inhibitor could be used to culture human SSCs *in vitro* for therapeutic applications.

08:40–09:00 h

Testicular organoids – Valid expectation for clinical andrology?

J.-B. Stukenborg

NORDFERTIL Research Lab Stockholm; Childhood Cancer Research Unit; Department of Women's and Children's Health; Karolinska Institutet, and Karolinska University Hospital, Stockholm, Sweden

Infertility is a multifactorial and complex problem. It affects approximately one out of seven couples in Europe [1] and at least 7.5 % of men between 15 and 60 years of age [2].

The lack of detailed knowledge regarding the biological processes important for human gametogenesis, and therefore the lack of proper treatment options for these patients, highlights the need of novel treatment methodologies, including *ex vivo* approaches, to differentiate mature gametes from immature germ cells or even pluripotent stem cells.

Strategies employed to study male fertility, and thereby establish treatment strategies for sub- and infertile men, will therefore require both, existing biobank material as well as registry data to identify factors related to the disease, and *ex vivo* approaches to differentiate mature gametes from immature germ cells or even pluripotent stem cells.

The successful production of murine sperm *in vitro* using testicular explant culture conditions, reported for the first time in 2013 [3], can be considered as a real breakthrough study. However, this culture condition lacks requirements enabling controlled monitoring of endo- and paracrine pathways needed to create a robust model to study specific aspects crucial to the spermatogenic process

(e.g. spermatogonial stem cell (SSC) renewal, SSC niche formation, and blood-testis-barrier formation).

Therefore, the focus of testicular cell culture-based research has been the development of novel three-dimensional culture conditions. By transferring knowledge obtained from novel cell-culture methodologies established recently in other fields of medical research, as for example organoids, new strategies have been designed to provide new tools for more defined research approaches regarding gametogenesis and its failures [4]. Organoids are small cell aggregates similar to organs found *in vivo*, but generated from single cell suspension containing stem cells. These organ-like structures are also defined by their functionality, which makes them a valuable research model and most likely a useful clinical tool in future.

References:

1. Krausz. Best Pract Res Clin Endocrinol Metab 2011; 25: 271–85.
2. Agarwal, et al. Reprod Biol Endocrinol 2015; 26: 37.
3. Sato, et al. Nature 2011; 471: 504–7.
4. Alves-Lopes, Stukenborg. Hum Reprod Update 2018; 24: 176–91.

Symposium 16 – Failure of Testis Development

08:00–08:20 h

Trends in human semen quality after nearly a half century of literature

B. Jegou

Inserm researcher emeritus, director of research at EHESP, University of Rennes, France

The theme of this presentation is the results of a critical appraisal and analysis that we have performed based on a stringent focus on the study designs applied in the literature on trends in semen quality. This was based on a Medline search of the relevant article from 1974 until April 2020, which was carried out using a set of cross-keywords followed by selecting the sole publications relating to spatial and temporal variations in semen characteristics.

This critical analysis of the literature was based on a careful distinction within the corpus of available studies between those corresponding to (i) multicenter investigations categorized as studies based on mean values or estimates; (ii) studies based on retrospective data from individuals in a given area, as well as (iii) studies covering the paradigm of geographical differences on semen quality.

We conclude that plausible decreasing trends have occurred in some demarcated urban areas. Furthermore, geographical contrasts have been strongly established. However, studies based on aggregating multiple data from countries and / or continents cannot establish a global conclusion that Human semen quality has been deteriorating in Western countries. To move way forward on this sensitive topic, prospective studies that minimize the impact of the key well-described factors

of bias should be combined with the assessment of men's exposomes to improve our understanding of the relations between Human semen quality and the environment.

08:40–09:00 h

Health behavior and environmental exposure during pregnancy and reproductive function in the adult son (FEPOS Cohort)

Sa. S. Tøttenborg¹, K. Keglberg Hærvig^{1,2}, K. Sørig Hougaard^{2,3}, C. Høst Ramlau-Hansen⁴, G. Tøft⁵, C. Lindh⁶, J. P. Ellekilde Bonde^{1, 2}

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Introduction The hypothesis that disruption of the fetal programming of the testicles may result in reduced sperm count and infertility in adulthood has since the 1980s shifted focus from exposures in adulthood to those in prenatal life. With few exceptions, however, it has been impossible to put the hypothesis to a critical test in epidemiological studies as appropriate prospective data on time-specific exposures combined with clinical follow-up of sons when they reach adulthood have been lacking. A new male-offspring cohort – the Fetal Programming of Semen Quality (FEPOS) cohort [1] – created within the auspices of ReproUnion serves an unprecedented chance for clarification.

Materials and Methods Young men ≈19 years of age born to women in the Danish National Birth Cohort for who two interviews and a blood sample obtained during pregnancy were available, were enrolled 2017–2019. Participants answered a comprehensive questionnaire, underwent a physical examination, and provided biospecimens. Blood from mothers and sons were analyzed for biomarkers of per- and polyfluorinated substances (PFAS), several phthalate metabolites, triclosan, acetaminophen, and cotinine. Reproductive biomarkers included semen volume, concentration, total count, morphology, motility, DNA fragmentation index, and reproductive hormones.

Results Of 21,623 eligible sons 5,697 were invited of whom 1,058 participated (19%). All xenobiotic chemicals except triclosan and acetaminophen were detected in quantifiable concentrations > 90% of women. The contrast was large, exemplified by PFOA with 10th to 90th percentiles 2.5–7.2 ng/ml (median 4.6 ng/ml). Other first-trimester exposures of interest were also prevalent e.g. 23% reported tobacco smoking (19% light and 4% heavy smokers) and 42% to be burdened by life or emotional stress.

Conclusion FEPOS is the world's largest population-based male-offspring cohort specifically designed to investigate prenatal determinants of male fertility. Extensive data on maternal exposures combined with biological fertility markers in their sons allow for answers to long sought questions.

References:

Keglberg Hærvig K, Bonde JP, Ramlau-Hansen CH, et al. Fetal programming of semen quality (FEPOS) cohort – A DNBC male-offspring cohort. Clin Epidemiol 2020; 12: 757–70.

Symposium 17 – Guidelines EAA

10:30–10:50 h

Management of oligo-astheno-teratozoospermia

S. Francavilla

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Oligo-astheno-teratozoospermia (OAT) is frequently reported in men from infertile couples. Its etiology remains, in the majority of cases, unknown with a variety of factors to contribute to its pathogenesis. The aim of this presentation is to present the European Academy of Andrology (EAA) guideline to discuss available management options.

PubMed was searched for papers in English for articles with search terms: male infertility and oligo-astheno-teratozoospermia. For evidence-based recommendations, the GRADE system was applied.

For men with OAT, the EAA recommends:

- A general physical examination to assess signs of hypogonadism.
- A scrotal physical examination to assess i) the testes and epididymes for volume and consistency, ii) deferent ducts for total or partial absence and iii) occurrence of varicocele.
- Performing two (2) semen analyses, according to World Health Organization (WHO) guidelines to define an OAT.
- An endocrine evaluation.
- A scrotal ultrasound (US) as part of routine investigation.
- Karyotype analysis and assessment of Yq microdeletions in infertile men with a sperm concentration $\leq 5 \times 10^6$ /mL.
- Cystic fibrosis transmembrane conductance regulator (CFTR) gene evaluation in case of suspicion for incomplete congenital obstruction of the genital tract.
- Against quitting physical activity in order to improve the chance of achieving pregnancy.
- Against androgen replacement therapy in order to improve the chance of achieving pregnancy.
- Assisted Reproduction Techniques (ART) in order to improve the chance of achieving pregnancy, in case other treatment options are not available or not efficient.

– Androgen replacement therapy in patients with biochemical and clinical signs of hypogonadism, after completion of the fertility treatment.

Symposium 18 – Young Andrologists

10:30–10:50 h

Comparative investigations of the sperm metabolome in fertility patients and healthy subjects

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²Institute for Medical Physics and Biophysics, Leipzig University, Germany; ³Department of Molecular Systems Biology, Helmholtz Centre for Environmental Research, Germany

Because of a high number of idiopathic infertilities among couples observed in fertility clinics, the common spermogram parameters do not seem to be sufficient for the indication of male fertility. Thus, the search for biomarkers to predict semen quality with methods allowing deeper investigations of the ejaculate, such as “omics” methods is highly relevant. Analyzing the molecular composition of a cell by metabolomics gives more detailed information than a superficial investigation by common spermogram analysis and could provide reasons for an otherwise undetected fertility disorder.

In a pilot study the concentrations of 180 metabolites – among them amino acids, biogenic amines, acyl carnitines, lipids and hexoses – of sperm and seminal plasma from 10 smoking and 10 non-smoking subjects have been elucidated by a targeted LC-MS/MS-based metabolomics approach to exclude a possible confounder, namely smoking. Furthermore, conventional spermogram analyses and flow cytometric analyses of caspase-3 activity (apoptosis), CD46 (acrosome reaction) and TUNEL (DNA fragmentation) have been performed. The results show that the conventional spermogram is not altered in smokers compared to non-smokers. However, the deeper investigation reveals an activated caspase cascade as well as an activated nitrogen oxide synthase in sperm of smoking subjects. Both are signs for oxidative stress and are also detectable in the sperm metabolome. These molecular foot prints together with an inconspicuous spermogram show that the standard spermogram might lead to rash conclusions regarding the putative fertility of an ejaculate while the metabolome is more differentiated and could provide causes for an otherwise undetected fertility disorder.

In a subsequent study sperm and seminal plasma of idiopathic and oligoastheno-zoospermic patients will be investigated in comparison to healthy subjects by the above named methods.

11:10–11:30 h

Metabolic cooperation between testicular cells is essential for spermatogenesis

M. G. Alves

Department of Microscopy, Laboratory of Cell Biology, Institute of Biomedical Sciences Abel Salazar (ICBAS), University of Porto, Portugal; Unit for Multidisciplinary Research in Biomedicine (UMIB), University of Porto, Portugal

Male fertility issues have been overlooked for many years. Although statistics from the World Health Organization (WHO) clearly show that sperm quality is decreasing worldwide to dangerous levels that can promise natural conception, the molecular mechanisms that control spermatogenesis remain as a technically challenging topic. Spermatogenesis is a complex and highly regulated process orchestrated by testicular cells. In the 19th century, Enrico Sertoli described for the first time irregularly shaped somatic cells in the testis and suggested that they did not produce spermatozoa but support their production. Indeed, those cells form the blood testis barrier. This way, the Sertoli cells (SCs) separate the interstitial fluid from the intratubular fluid. Later it was shown that in addition to controlling the passage of substances and metabolites for the compartment where germ cells develop, SCs also establish a strict metabolic cooperation with developing germ cells. The distinct energetic needs during the different stages of spermatogenesis are controlled by the SCs and they also produce the lactate, used as main substrate energy source by developing germ cells. This topic has been overlooked even though there is an increase on the prevalence of metabolic diseases. Most studies aim to correlate the incidence of metabolic diseases with sperm quality and there is not yet a consensus. Recently, several authors showed the impact of hormonal dysfunction and metabolite shifting caused by those diseases in the testis, particularly on the metabolic cooperation established between testicular cells. Thus, it is crucial to understand how those mechanisms are sensitive to energy homeostasis regulating hormones and dietary habits. Overall, the study of SCs is an emerging field for researchers interested in the understanding of the molecular mechanisms responsible for male (in)fertility. Ultimately, those studies may highlight new therapeutic targets for the control of male fertility.

Symposium 19 – Public Awareness

12:00–12:20 h

Preventive approach to male reproductive potential in Russian Federation

O. Ivanovich Apolikhin

N. Lopatkin Research Institute of Urology & Interventional Radiology, Moscow, Russian Federation

Realization of reproductive potential is important not only for the reproduction of the population, but also, for guarantees of national and social stability and security of the state itself.

At reproductive age, women have marked regressive reproductive behavior (a large number of abortions), which depends on the behavior of men, since the potentiating force in the birth of children both in marriage and outside of it is the man. In the case of an out-of-wedlock pregnancy, 90% of decisions to keep the child depend on the man. The decision to start a family also depends on the man.

According to experts, behavioral risk factors have a huge impact on reproductive potential. Smoking increases the risk of infertility by 340%, and alcohol increases the risk of prematurity by 300%. Therefore, further actions to preserve reproductive health should be largely aimed at combating risk factors that reduce the likelihood of conception and safe birth of a child, especially the second and the third.

In addition, in the CIS countries, there is a high mortality rate among the working-age population, especially at the age of 40–65 years, and the majority of these deaths are male. Therefore, the position of health authorities should be particularly active in relation to men aged 40–65 years, most of whom are fathers and breadwinners of the family.

Since men aged 40–65 years are experienced and valuable employees at any enterprise, there is no doubt that large employers are interested in maintaining the social activity of their employees. Therefore, we consider it appropriate to involve them in preventive programs.

Since the main potentiating role in realization of the nation's reproductive potential and in maintaining reproductive activity is played by men, it is men who should be the main focus of efforts. One of the aims should be creation of preventive environment and development of principles of medical examinations in the system of male reproductive health care – not only the use of expensive high-tech medical technologies.

The proposed prevention paradigm should also be family oriented.

Protection of the population's reproductive health should take place only through the formation of natural reproductive behavior. Here, preventive measures, prevention of reproductive losses and preservation of reproductive potential are the key factors. This is particularly important when the system of reproductive health protection is implemented in the CIS countries in the mass.

The demographic situation in the country is such that the window of opportunity for decisive action is 3–5 years. For the full realization and preservation of reproductive potential, as well as active social longevity, a separate concept of “Male reproductive health” is necessary, which should be based on the following principles:

- creating a preventive environment in the field of reproductive health with responsibility of the doctor and the patient as “subjects of law”
- support for natural reproductive behavior in collaboration with traditional religious denominations
- active involvement of employers as “subjects of law” in the protection of family reproductive health
- recognition of men's health as an integral factor in protecting the reproductive health of the population of the CIS countries.

12:20–12:40 h

Male Reproductive Health Initiative – MRHI in global perspective

C. J. De Jonge

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Introduction A spotlight is shining more brightly on male reproductive health because of concerns about globally declining sperm counts and, more recently, emerging data showing an association between somatic and reproductive health. While there is argument regarding the certainty of sperm count decline the opposite is true for the link between reproductive and somatic health. Adding to concern is emerging data that the reproductive health of the father can have an intergenerational impact on the wellness of future offspring. Perhaps more basic is the seeming diminished status of the semen analysis for assessing male fertility as evidenced by globally increased use of ICSI for non-male factor cases. Given the breadth and complexity of all the aforementioned, which includes basic, clinical and social science research evidence, discussion amongst opinion leaders initiated about what can and needs to be done globally to address these issues and to ultimately benefit patients and their families.

Materials and Methods Topical materials in the scientific literature database and popular media were scoured.

Results A review of the evidence demonstrated that male reproductive health is globally in crisis and worsening in the virtual absence of effective healthcare policies, research funding, societal awareness and education. Evidence of a significant gender gap in healthcare policies and initiatives demonstrates the urgent and critical need for global gender-inclusive healthcare strategies.

Conclusions The Male Reproductive Health Initiative (MRHI) was established with a mission of advancing the science and practice of male reproductive medicine. The MRHI aims to accomplish its mission by promoting and contributing to high quality research in both basic and clinical science, forging collaborative relationships with professional societies to contribute to a mission of education, and through advocacy of MRHI to all stakeholders including patient support groups, funding agencies, non-for-profit organizations, industry, and the public.

Post Congress Course Basic Science

14:15–14:45 h

Viral delivery systems for making rapid somatic cell transgenics in the testis

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Introduction The production of transgenic mice has permitted the functional investigation of thousands of genes. However, these methods are costly in time, money and animals required to obtain a consistent pedigree. In addition, developmental impacts often preclude analysis at later ages.

Materials and Methods Development of tools capable of gene manipulation at any given time of life obviate developmental issues and permit rapid production of genetic models from wild-type stock. We have undertaken an empirical evaluation of different viral vector delivery systems to identify the pros and cons of each system for manipulation of somatic cells and testis function *in vivo*.

Results Methodologies to target Sertoli cells for robust, long term delivered transgene expression are now well established, and we have utilised this efficacy to demonstrate lineage tracing, knockout, knock-down and rescue of gene function *in vivo*. Leydig cells have also been targeted with different viral vectors. However, until now, no one study has demonstrated robust and long-term transgene expression. Here, we also characterise and compare three commonly used and readily available viral vectors and define their efficacy for delivery of transgenes to Leydig cells. Together these tool kits provide options for rapid generation of somatic cell transgenics in the testis.

Conclusion The wide availability of viral vectors now available and ease of delivery to the intra-tubular and interstitial compartment of the testis, means that rapid generation of transgenic mice now takes just days from initial design to final analysis. The approach circumvents developmental phenotypes whilst simultaneously reducing cost, time, and numbers of animals required. These technologies will change the pace of genetic research in testis function and have potential as bespoke therapeutics for the treatment of male reproductive disorders.

15:15–15:45 h

In vitro organogenesis of the testis

S. Schlatt

Centre of Reproductive Medicine and Andrology, Münster, Germany

In recent years many breakthroughs in cellular research have opened fascinating scenarios for the *ex vivo* generation of germ cells from pluripotent stem cells as well as estab-

lishment of organoids from gonadal somatic cells. For rodent models standardized protocols already exist to derive various stages of primordial germ cells and precursors of male and female germ cells. It is relevant to expose the cells *in vitro* to sequentially changing cocktails of specific growth factors. These conditions mimic the *in vivo* induction of primordial germ cells in specialized regions of the early embryo. In parallel, various cell culture systems have been developed for a number of organs to derive highly organized multicellular structures resembling many aspects of normal physiological function. Gonadal organoids can also be created in many experimental settings like three dimensional culture systems, hanging drops, microfluidic chips and rotating flasks or wells. Primary cells from fetal gonads show the desired ability to undergo spontaneous organ development. Bringing together these technological advances germ cells established from pluripotent precursors developed into mature gametes. As yet mouse eggs and sperm derived in such systems were used to generate mouse offspring. The importance of the somatic tissues to guide germ cells into the correct pathway is one of the important lessons learned. In this talk the field will be reviewed and the state of the art is described. Future directions and the transfer to human applications will be discussed.

16:15–16:45 h

Epigenetic changes and their roles in male germ cells

J. Trasler, D. Chan¹

Research Institute of the McGill University Health Centre and Departments of Pediatrics, Human Genetics and Pharmacology & Therapeutics, McGill University, Canada

Introduction Sperm-DNA methylation patterns are unique and play a key role in the health of the next generation. Both folate in the diet and enzymes such as 5,10-methylenetetrahydrofolate reductase (MTHFR) impact the delivery of methyl groups for DNA methylation. Our objectives were to assess which areas of the human sperm DNA methylome are most susceptible to altered folate and MTHFR levels.

Methods Whole genome bisulfite sequencing (WGBS) was performed on a sperm pool from 30 men to identify regions of variable sperm DNA methylation. The WGBS results were used to create a customized human sperm methylC-capture panel to probe sperm from individual *MTHFR* 677CC or *MTHFR* 677TT (50% decrease MTHFR activity) men and test the impact of high dose folic acid supplements (5 mg/day for 6 months) on the sperm DNA methylome.

Results With WGBS we discovered 1 million sperm CpGs characterized as having intermediate methylation levels (20–80%); these sites were significantly more variable in sperm than regions of < 20% or > 80% methylation and were termed dynamic sites. These dynamic sites, along with 2 million commonly targeted CpGs, were used to create the

customized human sperm methylC-capture panel. With the panel, *MTHFR* 677TT men as compared to *MTHFR* 677CC men, showed both hyper- and hypomethylation in their sperm. Sperm hypomethylation in *MTHFR* 677TT men was increased by high dose folic acid supplements. For both the *MTHFR* genotype and folic acid supplement effects, > 80% of the alterations in DNA methylation occurred in the dynamic CpGs targeted uniquely by our panel.

Conclusions Dynamic sites in the human sperm DNA methylome appear to be particularly susceptible to alterations in folate metabolism and high dose folic acid supplements. Approaches such as the use of the customized human sperm methylC-capture panel described here will likely improve our ability to explore the effects of additional environmental exposures on the sperm DNA methylome. (Supported by CIHR).

16:45–17:15 h

Single cell RNA seq to study spermatogonial (dys-) function

N. Neuhaus

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Spermatogonial stem cells (SSCs) form the basis of human spermatogenesis. These are the least differentiated germ cells in the adult human testis and are a subpopulation of the diploid spermatogonia. SSCs are defined based on their functional properties, specifically their ability to self-renew and to give rise to differentiating germ cells, which ultimately form sperm. Due to the complexity of human testicular tissues and the lack of marker genes to isolate specific spermatogonial subpopulations, their molecular properties have largely remained unknown. The advent of high-throughput single cell RNA sequencing strategies (scRNA-Seq) has facilitated the analysis of testicular cell suspensions, providing hitherto unequalled insights with regard to the cell-type-specific expression profiles, including those of the spermatogonial subpopulations. Moreover, by comparison with scRNA-Seq datasets obtained from testicular cells suspensions from infertile men, crucial insights have been gained regarding the transcriptional changes of testicular cell types associated with infertility. In this talk, current scRNA-Seq datasets obtained from human testicular tissues will be reviewed with a particular focus on the novel insights regarding the spermatogonial compartment in situations of spermatogonial (dys)function.

18:15–18:45 h

Oxidative stress and the structure and function of human spermatozoa

J. R. Drevet

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The integrity of the paternal and maternal genetic material and their respective epigenetic

information is the guarantee of reproductive success, the health of the offspring and the sustainability of the species. The oxidative metabolism that sustains the life of all aerobic cells is at the crossroads of responses to the various environmental stimuli to which organisms are exposed, whatever their nature, whether physical, chemical or biological. The gametes are no exception to this situation and, due to its characteristics, the spermatozoa is particularly exposed to oxidative alterations. To date, the links between oxidative damage to the spermatozoa and functional impacts (motility, gamete interaction, capacitation) have been clearly established. However, it is necessary to consider another level of oxidative alteration of the male gamete which concerns the paternal nucleus. In this talk, combining fundamental research carried out on mouse models and clinical investigations, a presentation of oxidative damage on the sperm nucleus will be made with its possible consequences at the genetic and epigenetic

levels. Diagnostic and therapeutic aspects will also be briefly discussed.

18:45–19:15 h

The immune system of the testis and epididymis: mastering paradoxical tasks

A. Meinhardt

Institute of Anatomy and Cell Biology, Justus-Liebig University of Giessen, Germany

Testicular macrophages (TM) are heterogeneous and represent the largest immune cell population in the male gonad. TM are located in the testicular interstitial space and play an essential role in maintaining normal organ functions, i.e. steroidogenesis and spermatogenesis. TM contribute to the establishment and maintenance of the testicular immune privilege by displaying an immunoregulatory

M2 macrophage phenotype. Epididymal macrophages (EM) are less well studied, but also comprise the largest cohort of resident leukocytes in this organ. They can be found in the epididymal interstitium, but also intraepithelially. In the epididymis, which consists of a single duct, the opposing ends face different immunological challenges. Tolerance against the neoantigens on spermatozoa is the predominant task at the proximal end, whilst an active immune defense is required at the distal end to combat ascending pathogens. The different local microenvironments lead to a varied immunopathological response in acute bacterial epididymo-orchitis with permanent damage occurring in the epididymal cauda, little to no obvious pathology in the caput and regeneration of tissue damage in the testis. This talk will describe similarities in the immunopathology in mice and men and the organ specific identity of TM and EM as well as crucial factors necessary for their maintenance.

Freie Vorträge

O 01

Genetic dissection of spermatogenic arrest through exome analysis: clinical implications for the management of azoospermic men

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Introduction Azoospermia affects 1% of men and it can be the consequence of spermatogenic maturation arrest (MA). Although the etiology of MA is likely to be of genetic origin, only 13 genes have been reported as recurrent potential causes of MA.

Patients and Methods Exome sequencing in 147 selected MA patients (discovery cohort and two validation cohorts).

Results We found strong evidence for 5 novel genes likely responsible for MA (*ADAD2*, *TERB1*, *SHOC1*, *MSH4*, and *RAD21L1*), for which mouse knockout (KO) models are concordant with the human phenotype. Four of them were validated in the two independent MA cohorts. In addition, 9 patients carried pathogenic variants in 7

previously reported genes –*TEX14*, *DMRT1*, *TEX11*, *SYCE1*, *MEIOB*, *MEI1* and *STAG3* – allowing to upgrade the clinical significance of these genes for diagnostic purposes. Our meiotic studies provide novel insight into the functional consequences of the variants, supporting their pathogenic role.

Conclusions Diagnosing complete MA based on a genetic test is clinically relevant because affected patients should not undergo invasive testis surgery (TESE). Our findings contribute substantially to the development of a pre-TESE prognostic gene panel. Wider implications include the understanding of potential genetic links between NOA and cancer predisposition, and between NOA and premature ovarian failure.

O 02

Diagnostic interests of a custom designed panel for the analysis of 51 genes involved in non-syndromic human infertility

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Introduction With the advance of genome wide analysis, the genetic of male infertili-

ty is not anymore limited to karyotype or Y chromosome microdeletion. Indeed, an increasing list of human genes involved in infertility is now available. In order to translate this research field to clinical application, we set up a new diagnostic activity offering the analysis of a panel of 51 genes involved in different form of non-syndromic human infertility. The panel encloses 34 genes for male infertility, 15 genes for female infertility and 2 genes causing both male and female infertility. We present here the results of our new diagnostic activity.

Patients and Methods 79 males and 15 females with non-syndromic infertility were recruited. Five patients with known single gene mutations were used as positive controls. Sequencing libraries were prepared using the Agilent SureSelectQXT Target Enrichment system. Multiplex sequencing has been performed on Illumina NextSeq 550 with 2x75bp reads for total 51 genes in a series of 30 samples. Variant analysis has been achieved using our in house bioinformatics pipeline (STARK).

Result With a mean coverage of 457X and 99,8% of target bases successfully sequenced with a depth coverage over 30X, we prove the robustness and the quality of our panel. In total, we identified causative mutations in 8 patients (8.5%), five (6.3%) for the male cohort and three (20%) for the female patients. Such a yield is higher of the one reported so far about sequencing custom panel of genes related to infertility.

Conclusion Our cohort proved the ability of our panel to detect various types of variants including substitution, indel and CNV. The genetic of infertility allow precise diagnosis, which allow to offer a genetic council to the patient and his family and to personalize his treatment.

O 03

Pituitary-gonadal axis in male patients affected by type 1 diabetes mellitus: comparison between continuous subcutaneous insulin infusion vs multiple daily injections: preliminary data

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Introduction It is well known that in type 1 diabetes mellitus (T1DM), continuous subcutaneous insulin injection (CSII) improves metabolic control and reduces the occurrence of hypoglycemia when compared to multiple daily insulin injections (MDIs). HbA_{1c}, body mass index (BMI) and inflammatory parameters represent the most important metabolic outcomes; however, few data have been reported on pituitary-gonadal axis, despite the impact of sexual hormones on metabolic status. Therefore, our aim was to study hypothalamus-pituitary-gonadal axis response in male patients under CSII or MDI, evaluating levels of total (T) and free Testosterone (fT), luteinizing hormone (LH), sexual hormones binding protein (SHBG) in a cohort of T1DM male patients, comparing this two different therapeutic modalities.

Patients and Methods We enrolled 40 T1DM male patients, aged 19–55 ys, 27 treated with MDIs and 13 with CSII. The two groups were matched for age, BMI and years from diagnosis; we evaluated T, fT, LH, SHBG, HbA_{1c}, fasting glucose in all patients. HbA_{1c} were measured by IFCC-NGSP standardized method, hormonal parameters were determined by ECLIA method. fT was calculated by Vermeulen formula.

Results Although similar levels of HbA_{1c} (mean $\pm$ 7.8 $\pm$ 0.1% in MDI, 7.5 $\pm$ 0.1% in CSII), a statistically significant difference was found in LH values between the two groups, with higher levels in CSII vs MDIs (mean $\pm$ SEM 4.68 $\pm$ 0.62 vs 2.55 $\pm$ 0.36 mU/ml, respectively; p < 0.05) and a trend toward high T (9.47 $\pm$ 0.91 vs 7.23 $\pm$ 0.67 ng/ml) SHBG (79.27 $\pm$ 4.07 nmol/l vs 54.49 $\pm$ 4.04 nmol/l) and fT (0.12 $\pm$ 0.01 vs 0.11 $\pm$ 0.01 ng/ml) in the same group.

Conclusions This preliminary data seem to indicate a better pituitary-gonadal axis response in male T1DM patients under CSII compared to the ones under MDIs, with higher LH, T, fT levels in the first group, despite similar glucose control. Further studies to confirm this observation and to evaluate its impact on real clinical practice are needed.

O 04

Sexual function in adrenal insufficiency: data from the DREAM trial cohort

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Context Patients with Adrenal Insufficiency (AI) show an impaired quality of life, metabolic status, immune function and bone metabolism. Data on sexual function in these patients are scarce and often contradictory.

Aim to evaluate sexual dysfunction (SD) in female and male patients with primary and secondary AI and to investigate the effects of once-daily glucocorticoid replacement on sexual function.

Participants and Intervention 89 AI patients and 25 controls were enrolled in the DREAM trial. AI patients taking conventional multiple times a day glucocorticoid therapy were randomly assigned to continue their therapy or to switch to an equivalent dose of once-daily, modified-release hydrocortisone. 63 patients (29 males) completed the questionnaires for sexual function evaluation at baseline and at 24 weeks.

Results SD was increased in female and male AI patients compared to the general population. In the females, sexual health positively correlated with duration of disease (p = 0.005) and estrogen levels (p = 0.007). Questionnaire's items for “arousal” and “desire” negatively correlated with age. In pre-menopausal women, there was no correlation with androgens. In post-menopausal patients there was a positive correlation of sexual function with testosterone (p = 0.008). In males, erectile dysfunction correlated with quality of life (p = 0.020), while there was no correlation with age, androgens and metabolic profile. At 24 weeks there was no detectable difference in sexual function between study groups.

Conclusions Patients with AI show an increased prevalence of sexual dysfunction. Sexual health correlated with estrogen levels and duration of disease in female patients and with quality of life in males. The lack of correlation with androgens in males and pre-menopausal women and the independency from commonly associated factors such as age and metabolic status suggests that sexual dysfunction in AI patients is characterized by different mechanisms and patterns.

O 05

Relationship between natural and intrauterine insemination-assisted live births and the degree of sperm autoimmunisation

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Introduction The WHO recommends a screening for antisperm antibodies as an integral part of semen analysis, with 50% antibody-coated motile sperm as the clinically

relevant threshold. However, the predictive value of the mixed antiglobulin reaction (MAR) test positivity degree above such a cut-off on the occurrence of natural pregnancies remains undetermined. Furthermore, the effectiveness of intrauterine insemination (IUI) in cases of strong sperm autoimmunisation is not yet established.

Patients and Methods This was a retrospective cohort study on 108 men with MAR test + $\geq$ 50%. A post-coital test (PCT) was performed in all couples. Data on subsequent pregnancies were obtained by contacting men by telephone.

Results A total of 84 men (77.8%) were enrolled: 44 men with 100%+ MAR test and 40 with 50%-99%+ MAR test. Couples with 100%+ MAR test showed a lower natural live birth rate per couple (LBR) than that observed with lower positivity degrees (4.5% vs 30%; p = 0.00001). A significant difference between the two groups was observed only for the PCT outcome, which was poor in 100%+ MAR test group. Better PCT outcomes were positively associated with the occurrence of natural live births, for which the sole independent negative predictor was the sperm autoimmunisation degree. IUI was performed as the first-line treatment in 38 out of 44 couples with a 100%+ MAR test, yielding 14 live births (36.8%). In couples with lower MAR test positivity, the LBR after IUI (26.9%) was similar to the natural LBR (30%).

Conclusions The routine use of MAR test in basic fertility workup is justified as it influences decision making. A 100%+ MAR test can represent the sole cause of a couple's infertility, which could be successfully treated with IUI. Nevertheless, a lower degree of positivity may only represent a contributing factor to a couple's infertility, and so the decision to treat or wait also depends on the evaluation of conventional prognostic factors including the PCT outcome.

O 06

Follow-up after testicular tissue biopsy for fertility preservation in Klinefelter boys and adolescents

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Introduction Klinefelter syndrome (KS-47,XXY) is the most common sex-chromosomal aberration found in men affecting 1-2 in 1000 males. Although early testis development appears normal in KS boys, spermatogonial stem cell (SSC) depletion occurs before puberty leading to infertility. Cryopreservation of SSCs before puberty is the only fertility preservation option for KS boys and adolescents without ongoing spermatogenesis. Therefore, KS patients need to undergo testicular tissue biopsy (TTB) at young age. The surgical complications after TTB are rare (1%); however, the long-term

effects are unknown. This retrospective study aimed to investigate the long-term impact of TTB on the pubertal development of KS patients.

Patients and Methods KS patients followed between 2009–2020 at the Universitair Ziekenhuis Brussel and offered fertility preservation were included. Exclusion criteria were mosaic cases, cryptorchidism and testosterone replacement therapy prior to TTB. Retrospectively collected data on testicular volume, reproductive hormones, bone age and density were compared between KS patients who underwent TTB (biopsy group) and those who did not (control group).

Results Of 72 KS patients included, 23 had testicular tissue biopsied (mostly between 12–16 years), while 49 refused fertility preservation. After TTB, no statistically significant difference in testicular volume was found between the biopsied and contralateral non-biopsied testes. No statistically significant differences were found after TTB between the biopsy and control group for the reproductive hormones as well as for bone age and density. Testicular growth arrest/regression and hypergonadotropic hypogonadism became clear after puberty onset in both groups.

Conclusion No differences were found between the biopsy and control group for the different parameters evaluated. These results are reassuring and suggest that TTB has no additional impact on the pubertal development of KS patients on the long term.

O 07

Clinical evaluation of male factor infertility with artificial intelligence integrated Computer Assisted Sperm Analysis (CASA) tools

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The task remains to build an automated semen analysis system that upholds the confidence intervals required by andrology investigation for male factor infertility. This study presents and analyzes the validation of a new automated system using robotics and AI that conform to the WHO guidelines. An automotiv robotic microscope named Mojo was developed for scanning wet semen preparations. This platform uses an AI software based on a unique convolution neural network (CNN) that measures sperm concentration and motility. Mojo sperm counting chambers were used for validation. To rule out sampling error, videos of Hamilton Thorne accubeads+ were captured using the mojo counting chambers and microscope. The concentration of microbeads was manually assessed from videos and compared to their known measures. In this study, we analyzed semen samples from 44 patients and compared standard manual assessment to mojo AI CASA assessment for concentration and motility. The results

were collected and compared to identify the strengths and weaknesses of the current AI system and identify areas for improvement. QC microbead testing validates the microscope and protocol, with no observational error considering the interval of confidence. Correlating the CASA vs. manual concentration results yields a Pearson/Spearman score of 0.96/0.96 with a mean relative error near the WHO LRL of 13%. Deeper inspection of the AI shows false positives classifications leading to over-estimation of concentration and motility. In conclusion, mojo's robotics platform and protocols show promise for clinical use. The AI software is proven capable of measuring sperm concentration with high confidence. During the 2 month study, AI performance increased from a base Spearman of 0.52. Next, the AI will be further clinically trained, reducing false positive incidence of the CNN. Motility classification tuning will be performed to improve progressive and immotile grading.

O 08

Obesity-induced steroidogenesis and spermatogenesis decline: the protective role of *Heterotrigona itama* bee bread

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Background Decline in steroidogenesis and spermatogenesis have been observed in obese men.

Objectives The present study investigated the protective effect of bee bread on obesity-induced decline in steroidogenesis and spermatogenesis.

Methods Thirty-two adult male *Sprague-Dawley* rats weighing between 200–300 g were divided into four groups (n = 8/group), namely; normal control (NC), high-fat diet (HFD), HFD plus bee bread or orlistat administered 6 weeks after induction of obesity (HFD+BB and HFD+O groups, respectively). Bee bread (0.5 g/kg) or Orlistat (10mg/kg) was suspended in distilled water and given by oral gavage daily for 6 weeks after induction of obesity.

Results The HFD group showed significant decreases in follicle stimulating hormone (FSH), luteinising hormone (LH), testosterone and adiponectin levels, and an increase in leptin level compared to NC group. Furthermore, sperm count, viability, motility and normal morphology and epididymal antioxidants decreased as well as increases in

the malondialdehyde (MDA) level and sperm nDNA fragmentation were observed. Also, the levels of testicular mRNA transcript and protein levels of steroidogenesis-related genes [androgen receptor (AR), luteinizing hormone receptor (LHR), steroidogenic acute regulatory protein (StAR), cytochrome P450 enzyme (CYP11A1), 3 β -hydroxysteroid dehydrogenase (HSD) and 17 β -HSD] in the testes decreased significantly. Treatment with bee bread significantly decreased leptin level, increased adiponectin level, upregulated steroidogenesis-related genes and protein levels, attenuated spermatogenesis impairment, and decreased sperm DNA fragmentation in HFD-induced obese rats.

Discussion and Conclusion Bee bread improved spermatogenesis and steroidogenesis decline by upregulating steroidogenic genes. Therefore, bee bread may be regarded as a prospective remedy for subfertility in obese individuals.

O 09

Sperm small non-coding RNA profile obtained by NGS and semen quality among healthy young adults Updates on T-trials

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Introduction For the identification of small non-coding RNA (sncRNA) as prognostic markers of fertility subjects are usually recruited through infertility clinics. We examine the association of semen quality with sncRNA profile in young healthy adults.

Patients and Methods We selected 49 subjects from the prospective cohort Russian Children's Study (n = 516) enrolled at 8–9 years of age. Puberty was evaluated in annual follow-ups and semen samples were collected at 18–20 years. Fresh semen was evaluated for volume, sperm concentration, and motility according to ESHRE manual. Sperm RNA was extracted from 2 layers after centrifugation with 50% and 90% density gradient. Libraries of sncRNA were constructed using NEBNext Kit, n = 34 (NEB) and NEXTFLEX® Kit, n=15 (Perkin-Elmer) and sequenced on NextSeq 500 (Illumina). Reads were mapped to hg38 in a sequential order: ribRNA > miRNA > piRNA > tRNA.

DESeq2 R package was used for the analysis of differentially expressed snRNA.

Result Medians (IQR) for age, semen volume, sperm concentration, total motile sperm and total progressive motile sperm (TPMS) were: 18.3 (18.2–19.1) years, 3.2 (2.5–5) ml, 38.5 (22–66.5) mill/ml, 89.4 (51.0–160.2) mill, and 78.8 (46.4–135.7) mill. Mean number of sequenced reads was 7.97 mill, with 63% alignment to snRNA. 2332 snRNA were identified with ≥ 10 mean counts: 378 miRNA, 1580 piRNA and 374 tRNA. In models adjusted for library kit and sperm layer we identified 431 snRNA significantly differentially expressed by any of 4 semen parameters: 70 miRNA, 278 piRNA and 83 tRNA, FDR < 0.05. piR-7725 and piR-32962 overlapped with all semen parameters. 35 snRNA were significantly associated with TPMS, FDR < 0.05.

Conclusion To our knowledge, this is the first study to examine the association of snRNA profile and semen quality in a cohort of healthy young men. We suggest some candidate markers for evaluating semen quality in young adults.

Funding RSF #18-15-00202; for parent RCS – NIEHS #R01 ES014370.

O 10

Spermatozoal PIWI-LIKE 2 mRNA expression as predictor of fertilization success in ART

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Introduction PIWI-LIKE 1–4 play a pivotal role in stem cell maintenance and transposon repression in the human germline. Therefore, the dysregulation of these genes negatively influence genetic stability of the respective germ cell and subsequent development and maturation. Recently, we demonstrated that a lower PIWI-LIKE 2 mRNA expression in ejaculated spermatozoa is more frequent in men with oligozoospermia [Giebler et al. Asian J Androl 2018; 20: 260–4]. In this study, we aimed to analyse PIWI-LIKE 1–4 mRNA expression in ejaculated spermatozoa to predict ART outcome.

Material and Methods From 160 ART cycles, portions of the swim-up spermatozoa used for fertilization were collected and total RNA was isolated via TRIzol method. After cDNA synthesis, PIWI-LIKE 1–4 mRNA expression was measured by qPCR using TaqMan probes with GAPDH as reference gene. Relative mRNA expression was correlated to ejaculate values and fertilization rate.

Results PIWI-LIKE 1, 2 and 4 mRNA expression was significantly correlated with percentage of motile spermatozoa ($r_S = 0.78$; $p < 0.001$). PIWI-LIKE 4 mRNA expression was inversely correlated to PIWI-LIKE 1 and 2 mRNA expression ($r_S = -0.25$ and $r_S = -0.28$, $p < 0.001$). While PIWI-LIKE 1–4 mRNA expression in motile spermatozoa

was not associated with sperm concentration, motility or morphology in the native ejaculate, a lower PIWI-LIKE 2 mRNA expression was significantly associated with a fertilization rate $\geq 50\%$ ($p = 0.02$, Mann-Whitney-U-Test). Furthermore, a lower PIWI-LIKE 1 mRNA expression was significantly associated with a fertilization rate $\geq 50\%$ ($p = 0.05$, Mann-Whitney-U-Test).

Conclusion The level of spermatozoal PIWI-LIKE 1 and 2 mRNA expression exhibited a significant association to fertilization rate in ART cycles. A detailed characterisation of the mechanisms and regulatory pathways of PIWI-LIKE 1 and 2 during spermatogenesis and of the predictive potential for successful fertilization and implantation is highly needed.

O 11

Discerning the role of different immune cell populations in the pathogenesis of epididymitis

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Previous data have shown that distinct subsets of mononuclear phagocytes reside within the different epididymal regions and could provide a rationale for obvious paradoxical immunological tasks of the epididymis, i.e. maintenance of peripheral immune tolerance within the caput and the preservation of an immune responsive environment within the cauda. Using a murine model of acute bacterial epididymitis (C57BL/6J WT strain, bilateral injection of uropathogenic E. coli (UPEC) into the ligated vas deferens), we analyzed the immune responses within the different epididymal regions in association with the ascension of UPEC (traced by agar plating and immunofluorescence) at days 1, 3, 5, 7 and 10 post infection. While the caput remained mostly unaffected, the cauda underwent dramatic morphological alterations (immune cell infiltration, epithelial detachment, fibrosis, obstruction) as shown by Masson-Goldner/ Azan staining, immunofluorescence and morphometric evaluation ($n=4$ per experimental group). Flow cytometry analyses revealed a massive infiltration of neutrophils (Ly6g⁺) starting at day 3, followed by monocytes (Ly6chi, F4/80lo, CD64lo, MHCint) and monocyte-derived macrophages (Ly6chi, F4/80hi, CD64hi, MHC-IIhi) at day 5 in the cauda, but not in the caput ($n = 4$ per group). In support, levels of mRNA transcripts encoding diverse immunomodulatory factors (e.g. lipocalins, cystatin 11, lactotransferrin)

measured within distinct epididymal regions in untreated mice (by RT-qPCR) and in UPEC-infected mice (by whole transcriptome RNA-Seq analysis at day 10 post infection) exhibited striking region-specific differences. Overall, these data suggest that the cauda is more susceptible to an inflammatory response compared to the caput, indicating that the different immunological milieus might play a critical role in the magnitude of the region-specific immune responses.

O 12

Pathogenic T cell function induced chronic epididymo-orchitis in the T cell transferred Rag1^{-/-} Mice

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Chronic epididymo-orchitis is thought to be an inflammatory autoimmune disease with the testis and epididymis lesions, including germ cells loss, and dilated seminiferous tubules, aggressive and dysregulated structure remodeling, and impaired spermatogenesis. Although sperm antigen-specific T cells have been described in the pathogenesis of autoimmune animal models, the independent role of T cells in the orchitis and epididymitis pathogenesis remains unclearly delineated. Here, we report that T cells transfer can mediate and exacerbate murine testicular and epididymis inflammation and pathogenesis in immunodeficiency mice. Naïve CD4⁺ or CD8⁺T cells transfer reduced the sperm motility and viability, widened seminiferous tubules, and impaired spermatogenesis with severe spontaneous testis and epididymitis histopathology that comparable in Rag1^{-/-} mice with no T cells transfer. T cells transferred testis and epididymitis mice had a lower survival rate of sperm, the less capacity to fertilize, inflammatory cells infiltrated, and severe intestine histopathology with increased permeability. Besides, in the T cells transferred orchitis and epididymitis model, CD25⁺Foxp3-CFP Treg cells with naïve T cells co-transfer ameliorated testis and epididymis pathology and improve the sperm quantity and quality. We further evaluated the role of gut microbiota in the immunopathogenesis of T cells dependent orchitis and epididymitis. The results demonstrated that gut microbiota diversity decreased in naïve T cell transfer mice, with antibiotics treatment alleviated T cell transfer-induced testis and epididymitis. Using this transfer model, we validated that Stat3 deficiency attenuates that T cell-induced testis and epididymis tissue damage and protects the sperm loss and function decrease. Our study reveals that T cells are pathogenic in orchitis and epididymitis, and the transfer model serves as a valuable tool to evaluate pathways modulating T cells in chronic epididymo-orchitis.

O 13

Effects of LPS-induced epididymitis on Wfdc gene expression in mice

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The *Whey-acidic protein four-disulfide core domain* (*Wfdc*) locus on mouse chromosome 2 spans 16 *Wfdc* genes in its centromeric and telomeric subloci. These genes play putative roles in antimicrobial, immune and reproductive homeostasis. Although *Wfdc* genes are highly expressed in the epididymis their contributions to epididymal health and disease remain obscure. Here, we tested the hypothesis that *Wfdc* genes are effectors downstream the inflammatory responses to lipopolysaccharide (LPS) in the epididymis. We induced epididymitis in mice via interstitial or intravascular LPS (50 µg) injection, targeting the initial segment (IS) or cauda epididymidis (CE), respectively. Mice were euthanized 6, 24 and 72 h after LPS injection and tissues were processed for RT-qPCR assays. In the IS, LPS downregulated one centromeric (*Wfdc15b*) and five telomeric (*Wfdc6a*, *Eppin*, *Wfdc8*, *Wfdc11* and *Wfdc16*) *Wfdc* transcripts at 6 h. At later time-points (24 and 72 h) LPS upregulated all centromeric (*Slpi*, *Wfdc5*, *Wfdc12*, *Wfdc15a* and *Wfdc15b*) and five telomeric (*Wfdc2*, *Wfdc3*, *Wfdc6b*, *Wfdc10* and *Wfdc13*) *Wfdc* transcripts. Pretreatment with the NFκB inhibitor PDTC (100 mg/kg, i.p.) prevented LPS-induced upregulation of telomeric but not centromeric *Wfdc* transcripts at 24 h. In the CE, LPS upregulated one centromeric (*Wfdc5*) and one telomeric (*Wfdc2*) *Wfdc* transcripts at 24 h, while downregulated one centromeric (*Wfdc15b*) and three telomeric (*Wfdc6a*, *Wfdc11* and *Wfdc16*) *Wfdc* transcripts at 72 h. Pretreatment with PDTC effectively prevented LPS-induced upregulation of *Wfdc5* and *Wfdc2* transcripts in the CE.

Our findings indicate that members of the *Wfdc* locus are differentially regulated by LPS/TLR4/NFκB signaling pathway in the proximal and distal epididymis, suggesting that *WFDC* family members could play roles in epididymal responses to acute inflammatory stimuli.

Support: FAPESP (2015/08227-0, 2017/20102-3) and CAPES. Ethics approval: 1029-CEUA.

O 14

Testosterone levels are independently associated to sub-clinical atherosclerosis in men with chronic spinal cord injury

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Introduction Men suffering from spinal cord injury (SCI) are at increased risk for cardiovascular (CV) diseases as documented by a higher value of ultrasonographic carotid intima media thickness (cIMT), a surrogate marker of sub-clinical atherosclerosis. Furthermore, when compared to able-bodied general population, SCI men also exhibit a very higher prevalence of androgen deficiency, which can contribute to CV risk. We aimed to verify the possible relationship between testosterone levels and cIMT in men with chronic SCI.

Patients and Methods cIMT of 60 men with chronic (> 1 years) SCI, aged 56.0 (25–75th: 46.0–67.2) years, was evaluated with neck ultrasonography (US). All patients underwent a complete neurological exam, as well as biochemical and hormonal assessment. Comorbidity was scored by Charlson comorbidity index (CCI).

Results The cIMT median value was of 1.3 (1.0–1.8) mm. At the univariate linear regression analyses, cIMT showed a significant positive association with age (beta=0.09, p = 0.008), calculated LDL cholesterol level (beta = 0.09, p = 0.03), and HOMA-index of insulin resistance (beta = 0.03, p = 0.04) and a negative association with total testosterone (TT) levels (beta = -0.04, p = 0.001). At the multivariable linear regression model, including the significant putative predictors identified by univariate analyses, only TT levels showed an independent association with cIMT (beta = -0.03, p = 0.009).

Conclusion In patients with chronic SCI, lower TT levels represent significant and independent predictors of subclinical atherosclerosis, despite the very high prevalence of traditional CV risk factors in this population.

O 15

Differential effects of testosterone treatment on bone density in men with classical vs functional hypogonadism

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Introduction and Objective There are limited data on Testosterone (T) therapy in men with functional hypogonadism (FH) compared to those with classical forms (primary/

secondary hypogonadism, PH/SH) regarding bone density.

Methods Registry data of 2–5 years comprising 189 patients including 67 men with PH (mean age 32.0 ± 10.1 years), 49 with SH (mean age 30.4 ± 9.4 years) and 73 with FH (mean age 44.8 ± 11.6 years) all receiving uniform treatment using intramuscular T undecanoate (1000 mg). All patients received annual assessments of bone density using dual-energy X-ray absorptiometry.

Results Serum T concentrations increased from 6.8 ± 2.7 nmol/L to 18.1 ± 2.9 nmol/L in men with PH/SH and from 7.9 ± 2.5 nmol/L to 17.3 ± 3.2 nmol/L in men with FH.

There was an initial difference in bone density between patient groups (T-Score lumbar spine, PH: -1.8 ± 0.3, SH: -2.3 ± 0.4, FH: -1.2 ± 0.3, all p < 0.001 vs each other) and (T-Score hip, PH: -1.4 ± 0.3, SH: -1.7 ± 0.4, FH: -1.1 ± 0.3, all p < 0.001 vs each other). A significant increase in bone density was observed for all patient groups (lumbar spine and hip, both p < 0.001), albeit the effect was least pronounced in FH and strongest in SH (lumbar spine: p = 0.008 and hip: p = 0.01, *post-hoc* tests). Stepwise multiple Cox regression models could attribute these differences to the different baseline characteristics between groups including age and delta T levels. There was no difference between groups for the overall increase in hematocrit. Changes in PSA levels were more likely to occur in FH (hazard ratio 1.4 [1.2–1.6], p = 0.004).

Conclusions This study provides major new findings regarding effects T therapy in different groups of hypogonadal men. Patients with classical forms of hypogonadism have a more pronounced increase in bone density than patients with FH.

O 16

Low testosterone levels predict clinical adverse outcomes in SARS-CoV-2 pneumonia patients

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Background The pandemic of new severe acute respiratory syndrome (SARS) due to coronavirus (CoV) 2 (SARS-CoV-2) has stressed the importance of effective diagnostic and prognostic biomarkers of clinical worsening and mortality. Epidemiological data showing a differential impact of SARS-CoV-2 infection on women and men has suggested a potential role for testosterone (T) in

determining gender-disparity in the SARS-CoV-2 clinical outcomes.

Objectives To estimate the association between T level and SARS-CoV-2 clinical outcomes (defined as conditions requiring transfer to higher or lower intensity of care or death) in a cohort of patients admitted in the Respiratory Intensive Care Unit (RICU)

Methods A consecutive series of 31 male patients affected by SARS-CoV-2 pneumonia and recovered in the Respiratory Intensive Care Unit (RICU) of the “Carlo Poma” Hospital in Mantua were analyzed. Several biochemical risk factors (i.e., blood count and leucocyte formula, C- Reactive Protein (CRP), procalcitonin (PCT), Lactic Dehydrogenase (LDH), Ferritin, D-Dimer, Fibrinogen, Interleukin 6 (IL-6)) as well as total testosterone (TT), calculated free T (cFT), Sex Hormone Binding Globulin (SHBG), and Luteinizing Hormone (LH) were determined.

Results Lower TT and cFT were found in the transferred to ICU/deceased in RICU group vs. groups of patients transferred to IM or maintained in the RICU in stable condition. Both TT and cFT showed a negative significant correlation with biochemical risk factors (i.e. the neutrophil count, LDH and PCT) but a positive association with the lymphocyte count. Likewise, TT was also negatively associated with CRP and ferritin levels. A steep increase of both ICU transfer or mortality risk was observed in men with TT < 5 nmol/L or cFT < 100 pmol/L.

Conclusion Our study demonstrates for the first time that lower baseline levels of TT and cFT levels predict poor prognosis and mortality in SARS-CoV-2 infected men admitted to RICU.

O 17

Does the sperm retrieval rate (SRR) result of a small test sample of each single mTESE (microsurgical testicular sperm extraction) sample reliably predict the SRR on the day of ICSI?

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Introduction The combination of mTESE and intracytoplasmic sperm injection (ICSI) has become the standard treatment of patients with azoospermia. For optimal management, we developed a stepwise approach for analysis of tissue samples. At day of surgery, a small piece of each of the eight TESE samples per testis (test sample, TS) is analysed for sperm occurrence. The outcome of the TS may help to assess the chances for ICSI and to decide how many samples have to be thawed at time of ICSI. Here we evaluate, if the retrieved sperm numbers of the TS correspond to the number of sperm in the cryopreserved specimen later on the day of TESE-ICSI.

Methods Of each TESE sample, about 1/10 is removed and digested immediately with collagenase for pre-analysis. The result-

ing single cell solution is screened for the occurrence of spermatozoa. Depending on the result, one or more TESE samples are thawed and processed for TESE-ICSI and the spermatozoa yield is counted again. The absolute number is recorded if less than 100 spermatozoa are found, otherwise the result is displayed as being 100 as a maximum value. These data were analysed for equality.

Results Sperm retrieval results of 872 mTESE samples thawed on the day of ICSI were compared to the respective TS. Comparing test and TESE-ICSI preparations 73.6% were identical or had a minor deviation of ± 5 spermatozoa, while in 12.9% of the samples less and 13.4% more spermatozoa were found at point of ICSI. Moreover, if no sperm were found initially this was confirmed in 93.1% (268/288) of the samples. TS with 1–4 spermatozoa had a 27.2% chance to result in a complete absence of spermatozoa.

Conclusion A small TS analysed on the day of TESE reliably reflects the probability of finding comparable numbers of sperm at time of ICSI. However, if only 1–4 sperm are in the TS, there is a relevant risk that no spermatozoa can be retrieved for TESE-ICSI and the couple should be carefully advised before start of treatment.

O 18

The role of Dead end 1 in early PGC development: combining human iPSC differentiation with genome editing

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Primordial germ cells (PGCs) are the earliest progenitors of the mammalian germline and thus the requisite cells for transmitting genetic and epigenetic information from one generation to the next. Several differentiation protocols have been established to derive early PGC-like cells (PGCLCs) from human induced pluripotent stem cells (hiPSCs). These cells resemble migrating PGCs in terms of epigenetic and transcriptomic profiles and represent the best available model to mimic early human developmental processes *in vitro*.

This study aimed to combine PGCLC differentiation with gene editing to elucidate the role of genes associated with male infertility during early germ cell development. Within our clinical research unit, both clinical and genomic data of infertile patients are available. Currently, more than 1000 exome datasets are available, mainly of infertile azoospermic men. Several interesting novel candidate genes have been prioritized through multiple levels of *in silico* evidence. Prospectively, these candidate genes will be

functionally analyzed in our PGCLC differentiation system.

For proof of concept, we used *DND1* (*DND1* microRNA-mediated repression inhibitor 1), a regulator of germ cell maintenance and differentiation in vertebrates. While *DND1* is extensively studied in zebrafish and mouse, little is known about its role in human PGC differentiation.

Mutations were introduced using CRISPR/Cas9 and three homozygous knockout (KO) lines were analyzed for differences in gene expression and differentiation capacity. *DND1* KO lines exhibited pluripotency gene expression, and kept their ability to differentiate towards all three germ layers *in vitro*. During PGCLC induction, we derived reduced numbers of PGCLCs with varying reduction depending on the hiPSCs line. KO PGCLCs are currently analyzed for differences in gene and protein expression using RNAseq and mass spectrometry to elucidate pathways affected by *DND1* KO during hiPSCs differentiation.

O 19

Increased sperm DNA damage leads to poor embryo quality and subfertility of male rats treated with methylphenidate hydrochloride in adolescence

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Methylphenidate hydrochloride (Ritalin®) is a psychostimulant that has been widely used in the treatment of Attention Deficit Hyperactivity Disorder (ADHD). Although the treatments are able either to alleviate or extinguish the symptoms, the disorder is chronic and has a 60% chance of accompanying the individual throughout life. Currently, little is known about the possible side effects of methylphenidate (MPH) on male fertility and the impact of post-treatment paternal inheritance. The present study aimed to investigate whether MPH poses a risk to the reproductive capacity and/or quality of embryos conceived after treatment during the adolescence of rats. Twenty four Wistar rats at 38 days of age were treated with distilled water, by gavage, in daily doses using an adequate volume for the animal weight (control group: n = 12); or 5 mg/kg body weight of MPH, in a single daily dose, via gavage (treated group: n = 12). Both groups were treated for 30 days. From the 100 days of age the animals were presented to cohabitation with untreated females. Pregnant females were divided into 2 groups, according to the stage of embryonic development: group E3 (2.5 dpc) and E20 (20 dpc). The effects of the paternal inheritance on the embryonic development in the pre- and post-implantation phases were analyzed. After the statistical analysis, the results showed that the group of males treated with MPH during adolescence presented alterations in most of the parameters of paternal inheritance analyzed,

namely: number of embryos containing 2-3 symmetric blastomeres at 2.5 dpc, level of embryo fragmentation, number of live pups at 20 dpc, number of implantations at 20 dpc and rate of implantation at 20 dpc. Thus, it is concluded that the subchronic use of MPH during male pre-pubertal phase may cause reduction of reproductive capacity and decrease in embryo quality, probably due to the production of irreparable genomic damage that took place during spermatogenesis.

O 20

Both comorbidity burden and low testosterone can explain symptoms and signs of testosterone deficiency in men consulting for sexual dysfunction

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Introduction Low testosterone (T) is frequent in men with chronic illnesses. The clinical features of T deficiency (TD) overlap with those of chronic diseases. The aim of this study is to evaluate the relative contribution of chronic disease score (CDS) and low T to the presence of TD symptoms.

Methods A consecutive series of 3862 men (aged 52.1 ± 13.1 years) consulting for sexual dysfunction was studied. Several clinical and biochemical parameters were collected, including the structured interview ANDROTEST, for the assessment of TD symptoms. Penile color Doppler ultrasound (PCDU) was also performed. Based on the medications taken, the CDS was calculated. For a subset of 1687 men, information on mortality was collected (follow-up of 4.3 ± 2.6 years).

Results Higher CDS was associated with lower free and total T (TT) as well as with higher ANDROTEST score. When introducing CDS and TT in multivariable models adjusted for age, severe erectile dysfunction and impaired morning erections were associated with both CDS (odds ratio [OR] = 1.25 [1.13; 1.37] and 1.38 [1.29; 1.48], respectively) and low TT (OR = 1.11 [1.00; 1.23] and 1.13 [1.06; 1.21], respectively). Similar results were obtained for PCDU parameters. Hypoactive sexual desire was associated with low TT (OR = 1.21 [1.13; 1.30]), whereas it was inversely related with CDS (OR = 0.91 [0.84; 0.97]). When considering mortality for major cardiovascular events, TT < 8 nmol/L, but not CDS, was a significant predictor (hazard ratio = 5.57 [1.51; 20.63]).

Conclusions Chronic illnesses are associated with an overt TD. Both chronic diseases and low T can be involved in determining symptoms present in subjects complaining for sexual dysfunction. This should be considered in the diagnostic workup for TD.

O 21

Hydroxyurea is not the major cause for the depletion of germ cells in immature testicular tissues of patients with sickle cell disease

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Introduction Cryopreservation of immature testicular tissues is explored worldwide for prepubertal boys as a fertility preservation strategy. Sickle cell disease (SCD) patients constitute a growing cohort in cryopreservation programs and are usually treated with hydroxyurea (HU). This drug is suspected to damage germ cells, including spermatogonia, which form the basis for spermatogenesis following puberty. The aim of this study was to evaluate whether the number of spermatogonia in SCD patients was impaired and whether this correlated with HU treatment.

Patients and Methods We included 23 SCD patients (age range: 2–15 years) which are part of the cryopreservation programs in Germany, Androprotect (n = 17), and in the Nordic countries, Nordfertil (n = 6). Two independent testicular tissue sections were stained with the germ cell-specific marker MAGE A4 to identify spermatogonia in round tubules. An age-independent Z-score was calculated by comparing the numbers of spermatogonia per round tubule (S/T) of SCD patients to published values of healthy boys (n = 309, age range: 0–18 years). Moreover, clinical parameters were collected, including HU dose, exposure time and age at treatment initiation. Considering all the clinical parameters and spermatogonial numbers, a Spearman correlation was performed.

Results The majority of SCD patients (n = 15) had S/T values below the reference values of healthy boys and therefore, a Z-score below -2. One patient scored above the reference values (Z-score above 2), and the remaining seven patients had spermatogonial counts within the normal range. No correlation was observed between HU dose (p = 0,936), exposure time (p = 0,816), and age at treatment initiation (p = 0,051).

Conclusion The results suggest that HU therapy is not the major cause of the reduction of spermatogonia in SCD patients. Therefore, other factors intrinsic to the disease, such as the disease severity or a prenatal influence of SCD on the testis need to be considered.

O 22

Safety of spermatogonial stem cell transplantation in mice: health at different life stages across two generations in a blinded longitudinal study

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Introduction Spermatogonial stem cell (SSC) in vitro propagation followed by autotransplantation (SSCT) is proposed as a restorative fertility treatment for childhood cancer survivors. Limited data is available on the impact of SSCT on the health of the offspring.

Materials and Methods Neonatal mice SSCs (DBA/2J) were cultured and transplanted into sterile males (W/W-v). Control (DBA/2J) and transplanted (W/W-v) males were placed in breeding with control females (DBA/2J) and bred for 2 generations (n=153 control, n=126 F1 SSCT, n=117 F2 SSCT) to assess health in a full-blinded study. At birth, the pups were checked for congenital abnormalities. During the first 28 days, weight, length, ear-opening, eye-opening, fur growth and incisor eruption were checked, along with behavioral reflex testing of negative geotaxis, grasp and righting reflexes. Fertility of the F1 animals was assessed by their ability to generate F2 and histological confirmation. The welfare and survival of the F1 animals were followed up to 18 months.

Results At birth and during childhood, no significant differences were found between control and SSCT offspring, for the majority of physical and behavioral tests. Congenital abnormalities were rare events (n = 8), with no statistically significant differences between groups in either generation (OR: 3.57 (95%CI: [0.13; 293.33]) for F1 and 4.19 (95%CI: [0.60–82.95]) for F2). During the first 28 days, SSCT F2 weight was lower than control (95%CI: [-0.42; -0.09]), while the eruption of their upper incisor occurred 1 day earlier (95% CI [-2.161; -0.167]). All

animals were fertile and life expectancy was similar between groups ($p = 0.22$).

Conclusion The general health and development of SSCT derived pups during birth, childhood and adulthood were similar to control pups, in both generations. Thus, our pre-clinical study takes an important step towards clinical translation of SSCT and improving quality of life in prepubertal male cancer survivors.

O 23

Uropathogenic *Escherichia coli* induced infiltration of immune cells disturbs testicular function

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The testis is an immune-compromised organ, however, infection and inflammation could lead to diminished fertility. Immunological and infectious infertility are major contributors (13–15%) to male infertility. Macrophages are important effector cells of the innate immune system and play a critical role in host defense. They also contribute to the maintenance of tissue homeostasis and promote inflammation resolution and tissue repair. In a mouse model of acute bacterial testicular inflammation, we demonstrated a massive infiltration of immune cells such as neutrophils (Ly6G+), monocytes (Ly6Chi MHC IIhi), and inflammatory macrophages (F4/80lo CD11bhi) into the interstitial space of the testes after 1, 3, 5, 7, and 10 days of infection with uropathogenic *Escherichia coli* (UPEC). The infiltration of immune cells resulted in the disturbance of spermatogenesis with a concomitant significant reduction in the weight of the infected testes in comparison to control from 5 days of infection. Importantly, in *Ccr2* deficient mice, which lack peripheral blood monocytes due to a defective egress of Ly6Chi monocytes from the bone marrow, the number of blood monocytes/inflammatory macrophages were reduced and disease progression was attenuated in the testis after 7 days of infection with UPEC. These findings suggest that blood monocytes/inflammatory macrophages impair testicular function under inflammatory conditions which may lead to reduced fertility.

O 24

Effect of bacteria, present in the male genital tract, on human sperm function: an in vitro study

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Introduction Microbial communities, present in male genital tract, could impact male fertility status. However, such issue is little investigated. The aim of the study was to

evaluate the in vitro effects of most common bacteria of the male genital tract (MGT) (*Proteus m.*, *Pseudomonas a.*, *E. coli* (25922 – 35218), *Klebsiella p.* (700608 – 13833), *E. cloacae*, *E. aerogens*) on sperm functions.

Methods Semen samples were divided in equal aliquots and incubated with bacterial strains (at a concentration ten times greater than spermatozoa) for 1 and 3 hours at 37° C, 5%CO₂. Sperm motility, viability, oxidative stress (by MitoSOX™), caspase 3 and 7 activity (by FLICA™) and DNA fragmentation (by Tunel) were evaluated.

Results All bacteria, except *Pseudomonas a.*, reduced significantly sperm progressive and total motility ($n = 16$). Sperm viability was affected only by *Proteus m.* and *E. coli* 35218 which determined also an increase of sperm oxidative stress, apoptosis and DNA fragmentation. To explain the possible mechanism responsible for decrease of motility, spermatozoa were incubated with *E. coli* knockout for genes of Sperm Immobilizing Factor (SIF) and with the corresponding wild type (*MG1655* strand). After incubation with *E. coli* wt, a reduction in sperm motility, but not in sperm viability, was observed, whereas incubation with KO did not affect both sperm parameters. The sequence analysis of alignment for SIF coding genes (respect to *E. coli* *MG1655*) showed the presence of homologous genes in all bacterial isolates (range of identity: 76–99%) except *Pseudomonas a.*

Conclusions Our results demonstrated that bacteria of MGT have a detrimental effect on sperm motility, likely due to the presence of SIF. Loss of viability, increase of oxidative stress, apoptosis and DNA damage are likely mediated by other bacterial factors requiring further investigation. Overall, our data suggest that bacterial semen infection may negatively influence fertility status.

O 25

Developing testes-on-chip model to study in vitro primate spermatogenesis and endocrine dynamics

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Testes evolved as a bi-functional and multi-compartmental organ, functionally regulated by the HPG axis-via gonadotropins. Conventional *in vitro* culture approaches like gas-liquid interphase systems, failed to recapitulate the complex in vivo structural physiology, endocrine regulation and stem-cell niche-driven microenvironment of the primate testes. To address this research gap, we aimed to control the tissue micro-environment to simulate in vivo-like physiological conditions and functional organization of the primate testes by using an organ-on-a-chip approach to develop testes-on-chip models.

Devices were fabricated from PDMS (polydimethylsiloxane) using soft-lithography and a 3D-printed mold, and bonded to thin glass slides to facilitate optical visualization of the samples during culture. To preserve/activate the cellular and clonal arrangements of the tubular sub-compartments, intact marmoset seminiferous tubules from prepubertal marmoset monkeys, and adult human seminiferous tubules (sourced from gender dysphoria patients) were loaded in a confined culture chamber to promote a higher concentration of autocrine secretions around the tubules. Culture was maintained for 9–12 days at 35 °C under perfusion of fresh medium from side channels, in a shear-free manner and in stimulatory (supplemented with FSH, LH) and non-stimulatory culture conditions. On-chip live imaging, off-chip live-dead cell assays and histological analysis demonstrated viability of tissues, preserved structural integrity in prepubertal marmosets and irregular maintenance of cultured human tubules, mostly due to the variation in initial maturation status of the patient testicular samples. Testosterone measurements by ELISA assay demonstrated higher testosterone readouts in stimulated culture samples, compared to non-supplemented culture samples. Our testes-on-chip model can be further used to understand spermatogenesis and endocrine regulation in primates under stimulatory conditions.

O 26

An invertebrate in vivo platform for the high-throughput analysis of evolutionarily-conserved spermatogenesis genes

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Introduction Recent advances in genome sequencing techniques have led to an increasingly higher number of genes being tentatively linked to spermatogenic function. Nevertheless, generating the functional data to confirm these associations remains costly and time-consuming. In this study we tested the feasibility of using a simple invertebrate organism (the fruit fly *Drosophila melanogaster*) as a fast, cost-effective screening tool to identify new spermatogenesis genes.

Methods We identified 908 evolutionarily-conserved genes whose expression is dynamically regulated during human male meiosis. We silenced each one of them in fruit flies (*in vivo* RNAi), specifically as male germ cells prepare to enter meiosis (using the bam-GAL4 driver). The reproductive fitness of these gene-silenced males was assessed by four independent fertility tests, with those having average fertility rates below 75% being selected for subsequent testicular pheno-

type characterization via differential interference contrast microscopy. Phenotypes were ascribed to four main classes (pre-meiotic, meiotic, post-meiotic or gametic defects) and results were integrated with all publicly-available information on the human and mouse orthologs of these genes.

Results Out of the 908 genes analysed, we identified 244 that are required for male fertility, 177 of which have never been previously associated with spermatogenic functions in any of the three species. Most of the recorded phenotypes were at the post-meiotic stage (113/244), suggesting a link between meiotic expression and later functions in spermiogenesis. Of note, we found 32 cases where the fruit fly phenotype recapitulates the previously reported infertility of the corresponding mammalian ortholog. Of these, 20 represent new invertebrate models of known causes of human and mouse infertility.

Conclusion Our data support *Drosophila melanogaster* as a simple, high-throughput ancillary tool to accelerate research in genetics of spermatogenesis.

O 27

Loss of CCR2 inhibits the development of testicular fibrosis – a possible role for activin A

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Experimental autoimmune orchitis (EAO) is a mouse model of chronic testicular inflammation that features fibrosis, an important characteristic of testicular disturbances in men. Pro-inflammatory and pro-fibrotic mediators, such as TNF, CCR2 (MCP-1) and activin A, are increased during EAO followed by the infiltration of leukocytes into the testicular interstitium. Sertoli cells produce activin A upon stimulation with inflammatory factors, including TNF. Recruited fibrocytes expressing hematopoietic markers and extracellular matrix proteins as well as dysregulated expression of matrix metalloproteases (MMPs) contribute to fibrogenesis in many organs. In this study, the interaction of the CCR2 axis and activin A on the development of testicular fibrosis was investigated. EAO was induced by active immunization with testicular antigens in WT and CCR2^{-/-} mice (n=5-8/group). Organ damage due to EAO and collagen deposition detected by Sirius red staining were reduced in CCR2^{-/-} testes. Flow cytometry indicated that the induction by EAO of CD45+ fibrocytes expressing collagen I was also reduced in the CCR2^{-/-} testes compared with WT testes. Bone marrow-derived macrophages (BMDMs) were used as a surrogate of testicular macrophages to test

the influence of activin A on the production of fibronectin and MMPs. BMDMs cultured in the presence of 50ng/ml activin A or with conditioned medium from cultured Sertoli cells (SCCM) stimulated with TNF were analyzed by qRT-PCR and zymography. Preliminary results show TNF-stimulated SCCM induced fibronectin mRNA in BMDMs (n = 3). Moreover, activin A increased MMP-2 and MMP-14 mRNA levels with a concomitant decrease of MMP-9 and TIMP1 in BMDMs (n = 8). Activin A also upregulated the enzymatic activity of MMP-2, and downregulated MMP-9 activity (n = 5). Follistatin, a potent activin A antagonist, inhibited these effects. Taken together, these data indicate that CCR2 and activin A may interact to regulate fibrosis during testicular inflammation.

O 28

Reconstitution of prospermatogonial specification in vitro from human induced pluripotent stem cells

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Establishment of spermatogonia throughout the fetal and postnatal period is essential for production of spermatozoa and male fertility. Here, we established a protocol for in vitro reconstitution of human prospermatogonial specification whereby human primordial germ cell (PGC)-like cells (hPGCLCs) differentiated from human induced pluripotent stem cells were further induced into M-prospermatogonia-like cells (MLCs) and T1 prospermatogonia-like cells (T1LCs) using long-term cultured xenogeneic reconstituted testes. Single cell RNA-sequencing was used to delineate the lineage trajectory leading to T1LCs, which closely resemble human T1-prospermatogonia in vivo and exhibited gene expression related to spermatogenesis and diminished proliferation, a hallmark of quiescent T1 prospermatogonia. Notably, this system enabled us to visualize the dynamic and stage-specific regulation of transposable elements during human prospermatogonial specification. Together, our findings pave the way for understanding and reconstructing human male germline development in vitro.

O 29

Development of an assay to measure PLC ζ activity in human sperm

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Introduction The sperm-specific phospholipase C ζ (PLC ζ) plays an important role during fertilization. Upon sperm-oocyte fusion, PLC ζ is released from the sperm head into the oocyte. PLC ζ -induced repetitive increases of the intracellular Ca²⁺ concentration orchestrate the activation and development of the oocyte. Patients with reduced or lacking PLC ζ activity suffer from sub- or infertility, also involving fertilization failure upon ICSI. However, PLC ζ activity is currently not routinely assessed in sperm from patients undergoing assisted reproduction.

Patients and Methods We developed a fluorescence-based assay to quantify the PLC activity in human sperm samples. Furthermore, we analysed the prevalence of alterations in the *PLCZ1* gene in 27 selected patients with a low fertilization rate in ICSI via PCR and Sanger Sequencing.

Results Using the PLC assay, we quantified PLC activity in sperm samples from healthy donors under different experimental conditions, and we compared PLC activity between sperm from healthy donors and patients lacking PLC ζ activity. This revealed that the assay detects the global PLC activity in sperm, which involves several PLC isoforms including PLC ζ . Moreover, the genetic screening identified two patients with novel *PLCZ1* mutations that are currently analysed in more detail.

Conclusion We describe a fluorescence-based assay to measure PLC activity in sperm as well as of recombinant PLC protein. This assay may help to better understand the functional impact of mutations in the *PLCZ1* gene.

O 30

Transcriptomic differences between fibrotic and non-fibrotic testicular tissue

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Introduction Klinefelter syndrome (KS; 47,XXY) affects 1–2 in 1000 newborn males. Most (95%) KS men suffer from azoospermia due to a loss of spermatogonial stem cells. Additionally, testicular fibrosis is detected from puberty onwards. **However, mechanisms responsible for fibrosis and germ cell loss remain unknown.** The aim of this study is to identify factors which may be involved in the fibrotic remodeling of KS testes by analyzing the transcriptome of fibrotic testicular tissue.

Patients and Methods RNA sequencing was performed to compare the genetic profile of testicular biopsies from patients with

(KS and testis atrophy patients) and without (Sertoli cell only patients and fertile controls) testicular fibrosis. Five testicular samples were included in each group. Differentially expressed genes (DEGs) were considered significant when $p < 0.05$. To gain insight in the potential functions of the DETs, gene-ontology and KEGG analyses were performed.

Results A total of 167 upregulated and 567 downregulated DEGs were identified between the two groups. Gene ontology analysis revealed that the top downregulated biological functions mostly included functions related to spermatogenesis and fertilization. In the top 10 upregulated biological functions, we could find DEGs involved in the extracellular structure organization, including decorin, lumican and vascular cell adhesion molecule 1 (VCAM1). In addition, the upregulation of delta like non-canonical notch ligand 1 (DLK1) was found.

Conclusion We compared DETs between fibrotic and non-fibrotic testis tissue to find genetic components related to the initiation of testicular fibrosis. Several genes were found upregulated, including DLK1, which was also found in previous KS-related gene expression studies. Furthermore, VCAM1, which plays a role in the inflammation pathway, as well as other genes involved in the extracellular matrix composition were found differentially expressed between the two groups.

O 31

Activin A modulates the pace of germ cell development at the onset of spermatogenesis

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Male infertility and testicular cancer are thought to result from disruptions to testis development in utero. Physiological perturbations during human pregnancy can feature high activin A levels, and this study investigates how elevated activin affects the initial stages of sperm formation. In fetal mouse testes, activin A affects Sertoli cell function and local steroid production. After birth, germ cells resume proliferation and transform into differentiating spermatogonia which initiate the first round of spermatogenesis, or into the spermatogonial stem cells (SSCs) vital to ongoing spermatogenesis in adults. We studied a mouse model with elevated activin A bioactivity (*Inha* KO; lacks the inhibin A subunit, a potent activin inhibitor), to discern whether this selectively affected establishment of SSC or spermatogonia initiating the first spermatogenic wave. Immunofluorescence was used to enumerate germ cells (SALL4+), nascent undifferentiated spermatogonia (GFRA1+), and proliferation (KI67+) in testis sections from *Inha* WT and KO mice at P0 and P3. Although *Inha* KO testes have significantly

fewer germ cells at birth, a higher proportion of cells were GFRA1+ and KI67+, indicating that their development was advanced in fetal stages by high activin A. By P3, these parameters did not differ, suggesting overexpression of activin A doesn't interfere with the capacity of surviving germ cells to develop further. Altered levels of transcripts associated with both SSCs (*Eomes*, *Etv5*, *Bcl6b*) and differentiated spermatogonia (*Stra8*, *Kit*) in P0 *Inha* KO testes were consistent with the hypothesis of accelerated germ cell development prior to birth, and also indicated altered retinoic acid signalling activity. Our observation that systemic activin A elevation modulates the pace of germ cell development in mice prior to birth suggests that pregnancy conditions with elevated activin A may affect male reproductive health by influencing the male germline.

O 32

Whole-genome methylation analysis of testicular germ cells from cryptozoospermic men points to recurrent and functionally relevant DNA methylation changes

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In the last 15 years several studies have described a prevalence of DNA methylation changes in sperm of infertile men. However, more recently using whole genome bisulfite sequencing we were able to refute this theory by demonstrating that somatic DNA contamination and genetic variation confound methylation studies in sperm of severely oligozoospermic men. As we cannot exclude the existence of testicular germ cells (TGCs) carrying aberrant DNA methylation in such patients, we compared the TGC methylomes of four men with cryptozoospermia (CZ) and of four men with obstructive azoospermia, who served as controls (CTR). By analyzing the methylation levels at the imprinted regions, we confirmed that the samples were free of somatic cell DNA. Although there was no difference in the global DNA methylation level, we detected 271 differentially methylated regions (DMRs) between the two groups, which are associated with 132 genes. To evaluate the relevance of these DMR associated genes during germ cell development, we analyzed single cell RNA sequencing datasets of human testicular germ cells from CZ and CTR samples ($n = 3$ each) as well as two published RNA-seq datasets [Guo et al., 2018; Hermann et al., 2018]. By this, we found that 65 of these genes are expressed at various stages of spermatogenesis. Furthermore, 12 of them

resulted to be differentially expressed between the patient groups. In conclusion, we found that impaired spermatogenesis is associated with DNA methylation changes in testicular germ cells at functionally relevant regions of the genome, which points to an important role of DNA methylation in normal spermatogenesis. We hypothesize that the described DNA methylation changes may reflect or contribute to premature abortion of spermatogenesis and therefore not appear in the mature, motile sperm.

O 33

Prediabetes induces transgenerational effects in testicular metabolome and sperm quality: a silent comorbidity?

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Adoption of fat-rich diets, often starting at an early age, is a main cause for “fat epidemic” and related comorbidities. Several studies have reported a decrease in sperm quality and correlate it with the onset of metabolic diseases. Recently, evidence has emerged about the intergenerational (sons) and transgenerational (grandsons) effects of ancestral paternal fat-rich diet and metabolic status in sperm parameters. Hereby, we postulate that a prediabetic state induced by a fat-rich diet is related to sperm defects and testicular metabolic remodeling, for several generations.

Our methodology is schematically described in **Figure 2**.

The consumption of fat-rich diets in F0 induces a prediabetic state that is reversed by dietary intervention. However, both mice fed with fat-rich diet (FR) and mice subjected to diet intervention (DI) presented more sperm defects and lower sperm viability and motility than mice fed with standard chow (CTRL). Even transient consumption of a fat-rich diet affected testicular metabolism, particularly aminoacids metabolism, the energy-obtaining pathways, and the lipid precursors. The sons (F1) of FR and DI mice have not displayed sperm defects, but testicular metabolic profile remained abnormal. Interestingly, grandsons (F2) of F0 mice fed a fat-rich diet (both transiently and permanently), presented

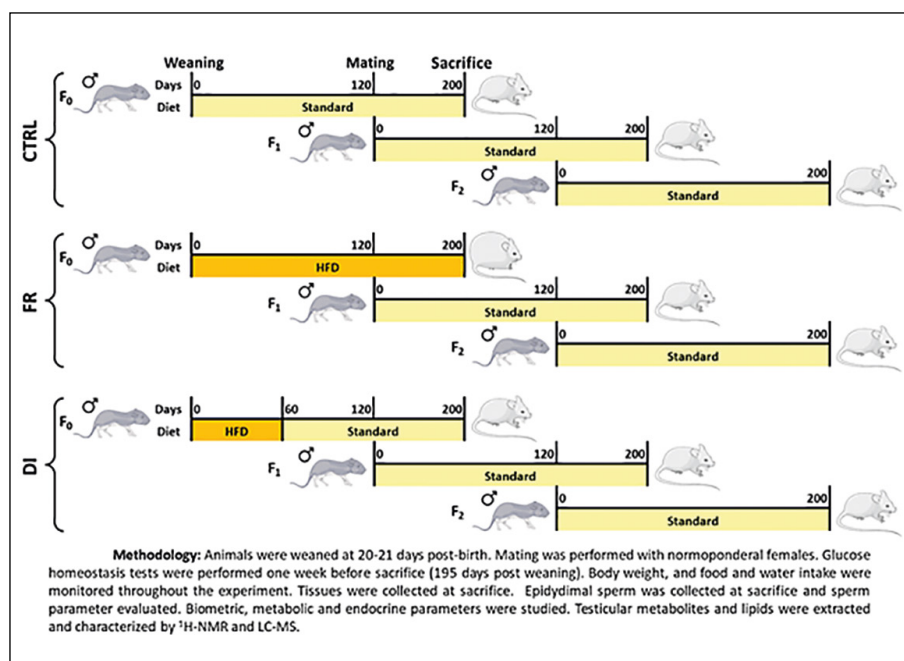


Figure 2. L. Crisóstomo, et al. © L. Crisóstomo

lower sperm counts and a higher proportion of sperm defects than the CTRL counterparts. Those results were found to be correlated with abnormal testicular metabolic signatures of ancestors.

Our findings show that a prediabetic state promoted by unhealthy food intake induces irreversible metabolic signatures in testis, which are related to sperm defects. Testicular metabolome remodeling is inheritable via paternal lineage for up to grandsons, perpetuating sperm defects. Prediabetes may cause a silent transgenerational imprinting that compromises male fertility potential.

O 34

Online searches for pornography, dating apps and sex work during and after lockdown measures for COVID-19 in Italy

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Introduction Lockdown and isolation measures have been necessary to contain spread of the SARS-CoV-2 coronavirus disease (COVID-19). In Italy, the most stringent lockdown measures were kept from March 9 to May 18, 2020. In the meantime, an increase in global traffic on several pornography websites was reported. We aimed to investigate whether this trend (search query: “Pornhub”) was also associated with a de-

cline in Google queries for sex work (search query: “Escort”) and dating apps (search query: “Tinder”), and whether reversal of such trends occurred at the end of lockdown.

Materials and Methods Analysis was conducted on data from Google Trends, a tool to explore the relative popularity of selected search queries in selected regions over a specific duration. Non-parametric regression via Mann-Kendall trend test and pairwise comparisons via Wilcoxon rank sum test with Benjamini-Hoch correction were used to measure differences in trends before, during and after lockdown.

Results Data between January 1 and September 4, 2020 were retrieved. All search items showed significant trends (*Pornhub*: $p < 0.0001$; *Escort*: $p < 0.0001$; *Tinder*: $p = 0.0187$). Queries for *Pornhub* were more prevalent during lockdown than either before or after ($p < 0.0001$ and $p = 0.0003$, respectively), and more prevalent after than before lockdown ($p < 0.0001$). Queries for *Escort* significantly declined during lockdown ($p < 0.0001$) and steadily increased after measures were withdrawn ($p < 0.0001$), despite being still lower than before ($p < 0.012$). Queries for *Tinder* decreased during lockdown ($p < 0.0001$) and increased soon after ($p < 0.0001$), with no significant change between before and after lockdown ($p = 0.15$).

Conclusions Interest in regards to pornography increased in Italy during lockdown, and, while declining, remained high once lockdown measures were withdrawn. Interest concerning dating apps and sex work declined abruptly during lockdown, and increased once again following the end of restrictions.

O 35

Tumour infiltrating T lymphocytes in human testis cancer – identification and functional analysis

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T cells are critical to tumour development and associated immune surveillance. Different types of T cells, specifically, CD4+ regulatory (Treg) and follicular helper T cells (Tfh) are strongly associated with poor prognosis of different cancers, but their involvement in human testicular germ cell tumours (TGCT) is unknown. This study aim is to identify and characterise Treg and Tfh involvement in TGCT development and progression. For this, human testis samples with seminoma (SE; $n = 10$), or germ cell neoplasia in situ (GCNIS) with/without lymphocytic infiltrates (LY) ($n = 10$, each) were compared to samples revealing normal spermatogenesis (NSP; $n = 10$), or hypospermatogenesis with focal infiltrates (HYP+LY; $n = 9$). Bouin-fixed paraffin-embedded specimens were processed for immunohistochemistry (IHC)/immunofluorescence (IF), and cryo-preserved samples were used for RNA extraction and RT-qPCR. Furthermore, flow cytometry was performed on fresh TGCT samples ($n = 4$, in a range of 3×10^3 – 4.9×10^4 cells). A panel of antibodies against CD3, CD4, CD8, CD20cy, CD68, CD25, FOXP3, CXCR5 and BCL6 was used to detect and sort specific immune cells. Both IHC and flow cytometry showed a high abundance of T cells relative to other immune cell types in SE. Flow cytometry data revealed that, besides the predominant CD4+ and CD8+ T cell subsets (36–60% and 21–44% of CD45+ cells, resp.), Treg and Tfh cells were detected in all tumour samples (up to 0.5% of CD45+ cells), suggesting that Treg and Tfh cells might play a role in TGCT biology. RT-qPCR confirmed that Treg and Tfh associated cytokines/chemokines are highly expressed in SE. This study has demonstrated the complexity and indicated the possible importance of rarer T cell subtypes in these tumours. Future experiments will use sorted T cell populations to extend our understanding of their functional roles in TGCT.

Grants Supported by DFG IRTG GRK 1871/2.

O 36

Whole-exome sequencing in testicular germ cell tumor: identification of novel genetic factors related to Lynch syndrome

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Testicular Germ Cell Tumor (TGCT) is a multifactorial and polygenic disease. Although there is strong evidence for a significant hereditary component of TGCT, the

genetic etiology is still undiscovered. Disruption of the DNA Repair genes results in the dysfunction of the overall repair system. Germline mutations in the DNA Mismatch Repair (MMR) gene family lead to Lynch syndrome (LS), which is a cancer (cc) prone disease. The most frequent malignancies associated with LS are colorectal and endometrial cc, while less commonly affected organs are hepatobiliary tract, urinary tract. Recently, TGCT has also been linked to LS.

Our objective was to test the diagnostic performance of Whole Exome Sequencing (WES) in identifying monogenic causes of TGCT in patients with family history of multiple ccs.

DNA was extracted from peripheral blood lymphocytes of two unrelated patients (18-1040, A2301) affected by TGCT and both of them with two or more family members suffering from different types of cc. WES was carried out on the variants in the coding

sequences. Autosomal dominant and recessive model have been applied to the obtained variants (n = 657 SNVs, 214 indels). Bioinformatic analyses allowed the prioritization of the most promising variants.

A heterozygous frameshift deletion (c.2906_2907delAT) in the *MSH6* gene was identified in patient 18-1040, whose mother had endometrial cc and his grandfather had pancreas cc. This variant has already been reported in association with LS. In patient A2301, a novel heterozygous non-frameshift deletion (c.3232_3237delTGACT) of *MLH3* was found. In his family endometrial, breast and pancreas cc has been detected.

Our findings provide novel evidence for a genetic link between TGCT and LS, indicating that TGCT may be part of the LS associated urological malignancies. We therefore, advise an onco-andrological screening of the male family members of LS families and genetic testing for MMR status in TGCT patients.

Poster

P 1.1

Loss-of-function mutations in TRIM71 as novel cause of early germ cell depletion in men and mice

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Introduction Infertility affects ~15% of couples worldwide. Sertoli cell-only (SCO) phenotype is a subtype of azoospermia without any germ cells and, if universally present, no chance to father children. Despite its severity and suspected genetic origin, the majority of causes remain unknown. Candidate gene approaches have been largely unsuccessful, substantiating the need for more systematic approaches, e.g. whole exome sequencing (WES).

Patients and Methods We applied bioinformatic tools to WES data of 226 well-phenotyped SCO patients from the Male Reproductive Genomics (MERGE) study. Gene filtering was conducted using the in-house software Sciobase and a newly developed data analysis tool termed Haystack, yielding 706 candidate genes carrying loss-of-function variants. Protein-protein interactions were assessed employing STRING.

Results In STRING, a cluster of E3 ubiquitin ligases was discovered, including TRIM71. In this gene specifically, we identified a heterozygous duplication in an SCO patient, leading to a frameshift and a premature stop codon. Several missense variants in *TRIM71* were found in other patients. *TRIM71* is highly expressed in human and mouse testes and, within the adult testis, expression is restricted to spermatogonia in both species. *Trim71* gonad-specific Nanos3-conditional knockout (KO) mice of both sexes are infertile. Homozygous KO males present with reduced testicular volumes and an SCO-like phenotype in histological stainings, while heterozygous males also exhibit a significant reduction in testis size. The SCO-like phenotype is already apparent in newborn mice, and since female KO mice also present with drastically reduced ovary size, the presence of *TRIM71* may be essential in early germ cell development.

Conclusion These results point to a potential role of TRIM71 in causing early germ cell depletion in mice and men.

Grants This work was supported by the DFG Clinical Research Unit 326.

P 1.2

Characterisation of the infertility-associated gene M1AP

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We recently identified bi-allelic variants in the gene *M1AP* encoding meiosis I arresting protein by whole exome sequencing in 14 individuals originating from four independ-

ent cohorts and one consanguineous family [Wyrwoll, et al. Am J Hum Genet 2020; 107: 342–51]. The common phenotype between all men was non-obstructive azoospermia mainly associated with meiotic arrest, but sporadically spermatids and rarely very few spermatozoa in the semen were observed. Combined with a testis-specific mRNA expression pattern and a knockout mouse model that had been previously described to be infertile, *M1AP* displays a highly promising candidate gene relevant for spermatogenesis in mice and men. However, hardly anything is known about its cellular role and no protein motifs or domains were identified yet, making it impossible to reliably predict molecular functions. Addressing this gap, we first aim to investigate *M1AP*'s subcellular localisation in relation to cellular compartments such as the plasma membrane or mitochondria. Heterologous transfection of DYK-tagged *M1AP* cDNA for protein over-expression enables detection of *M1AP* even in the absence of a reliable commercial antibody. COS-7 cells present a suitable model for this attempt due to their large cell body. Assumed impaired protein function related to aberrant localisation of mutated *M1AP* will be identified by generating patient-specific variants via site-directed mutagenesis. With this approach, we were already able to describe protein truncation for the frameshift variant c.676dup (p.Trp226LeufsTer4), lacking 57% of its full-length as shown by Western Blot. In perspective, this system will be transferred to other cells simulating the *in vivo* situation more closely, like TCam-2 or NTERA-2 cells. By in-depth characterisation of the M1AP protein, we will contribute to understand its role and general mechanisms of meiotic processes, indisputably needed for the assessment of identified variants associated with male infertility.

P 1.3

The genetic portrait of male infertility: an *in silico* ethnic evaluation of haplotype distribution among populations

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Background Male infertility is a complex and multifactorial disease with an increasing incidence worldwide. Among the possible etiopathogenetic factors, a genetic cause is recognized in about the 15% of cases and 34% of them remain idiopathic, representing a diagnostic challenge, in which the genetic contribution is likely underestimated. The aim of the study was to analyse the world-wide distribution of genotypes associated with male infertility, evaluating their evolutionary conservation and selection in different populations.

Methods A literature search (PubMed database) was performed to identify all single nucleotide polymorphisms (SNPs) associated with male infertility. SNPs with linkage disequilibrium (LD) value higher than 0.8 were used as markers of a specific haplotype, representative of the entire genomic region, and submitted to genetic analysis. Selected SNP data of individuals from 26 populations were downloaded from the 1000 Genomes online database. According to the genetic homology calculated using SNPs associated with male infertility, Bayesian clustering of individuals was performed. The genetic distance (FST) of each population from the Kenyan “LWK” was calculated. LWK is assumed to be representative of the ancestral genotype populating African regions from which ancient human migrations began. FST and geographic distance were then interconnected with linear regression analyses.

Results Two hundred thirty-three SNPs referring to 2504 sample individuals from 26 different populations were included in the final database. The genetic analysis revealed that the FST calculated for 124 SNPs significantly increased together with the geographic distance, suggesting to have undergone a natural selection during human evolution.

Discussion The study demonstrated that SNPs associated with male infertility have ethnic-based distribution worldwide and natural selection impacted more than 100 SNPs likely contributing to modulate male reproductive functioning.

P 1.4

49, XXXXY syndrome: An infant presenting with ambiguous genitaliaP. Patel¹, R. Nerl², S. Ghagane²¹Jawaharlal Nehru Medical College, Belagavi, Urology, Belagavi, India; ²KLES Dr. Prabhakar Kore Hospital and research centre, Urology, Belagavi, India

The presence of normal genes on the Y chromosome is essential for normal sex determi-

nation and sex differentiation of male genitalia. Several genes on the X chromosome and other autosomes have been shown to be anti-testes and have a detrimental effect on the development process of the normal male genital system. The addition of X chromosomes to the 46, XY karyotype results in seminiferous tubules dysgenesis, hypogonadism, and malformed genitalia. We report an infant male with 49, XXXXY syndrome presenting with ambiguous genitalia and multiple extra-gonadal anomalies.

P 1.5

Ten-eleven-translocation enzyme 1 (TET1) and polycomb repressive complex 2 (PRC2): Roles in spermatogenesis and male fertilityS. A. Shipilu^{1,2}, H. C. Schuppe¹, P. Western³, D. Fietz⁴, K. Steger^{1,5}, U. Schagdarsurengin^{1,2}¹Justus-Liebig-University Giessen, Clinic of Urology, Pediatric Urology and Andrology, Giessen, Germany; ²Justus-Liebig-University Giessen, Epigenetics of Urogenital System, Giessen, Germany; ³Monash University, Germ Cell Development and Epigenetics, Melbourne, Australia; ⁴Justus-Liebig-University Giessen, Institute for Veterinary Anatomy, Histology and Embryology, Giessen, Germany; ⁵Justus-Liebig-University Giessen, Molecular Andrology, Giessen, Germany

TET1 and PRC2 regulate DNA and histone methylation (H3K27me3), respectively. In mice, TET1 and PRC2 dysregulations are associated with male infertility. TET1 expression level was significantly downregulated in infertile patients. We hypothesize, TET1 interacts with PRC2 and promotes DNA demethylation at H3K27me3-enriched genes. We aim to clarify, where TET1 and PRC2 co-express during spermatogenesis, and co-localize in the mature sperm genome and how this interaction is changed in the sperm of subfertile men and Tet1-KO mice.

Immunohistochemical analyses (IHC) have been performed for TET1, H3K27me3, core PRC2 components (EED, EZH2, SUZ12) on testis sections from men with obstructive azoospermia and mice (wild-type wt and Tet1-KO). Western blot (WB) analyses of core PRC2 components and H3K27me3 have also been done on human sperm.

In humans, IHC results showed that TET1, H3K27me3, and EZH2 co-express in the spermatogonia B, leptotene spermatocytes and round spermatids. Compared to wt, in Tet1 homozygous KO mice, Tet1 showed no expression in germ cells. H3K27me3 expression had shifted from stage V–VI spermatogonia B to stage VII–VIII pre-leptotene spermatocytes, and from stage IX–XI elongating spermatid to stage X–XI elongating spermatids. EZH2 had gained expression in stage VII–VIII pre-leptotene spermatocytes, in stage VI–X pachytene spermatocytes whereas SUZ12 had shown new expression pattern in the late elongating spermatids and elongated spermatids. EED lost expression completely. However, WB analyses have shown that PRC2 components were not preserved in the sperm except H3K27me3.

So, knocking out Tet1 has potential impacts on Suz12 and Eed. Eed mutant mice are lethal at gastrulation and Tet1 might be a potential regulator which leads to infertility. Analysis of putative TET1-binding sites and their methylation status will be performed for selected genes (e.g. HAND1, ESX1L) to determine if there is a link between male infertility and the interaction of TET1 and PRC2.

P 1.6

Disorders in meiosis, spermatogenic cells and Sertoli cells in rats with experimentally induced metabolic syndromeO. Kolomiets², A. Kashintsova², Y. Korolev³, V. Spangenberg¹, E. Bragina²¹N.I. Vavilov Institute of General Genetics RAS, Moscow, Russian Federation; ²A. N. Belozersky Research Institute of Physico-Chemical Biology MSU, department of electron microscopy, Moscow, Russian Federation; ³National Medical Research Center for Rehabilitation and Balneology, Moscow, Russian Federation

Introduction Metabolic syndrome (MS) is characterized by the formation of a complex of disorders among which the most characteristic is a combination of abdominal obesity, hyperglycemia. MS can cause impaired spermatogenesis and lower sperm counts. However, the details of the effect of MS on spermatogenic cells remain unclear. The aim of this work was to study some spermatogenesis characteristics in rats with an experimental MS.

Materials and Methods Eight male rats were divided into two groups: group I rats received a standard diet, group II received a diet high in glucose and fat for 60 days. Methods of immunocytochemistry and electron microscopy were used.

Results The study of meiosis was carried out in spreading nuclei of I-order spermatocytes (Sp) with antibodies to SCP3 – the protein of the synaptonemal complex (Sc). Significant disturbances of the nuclear architecture were found in pachytene Sp of rat with MS as well as ring structure and Sc fragmentation. In 50% of Sp defects of the sexual body structure were observed, which is manifestation of pachytene arrest resulting in apoptosis [Turner et al., 2005].

The staining of the crushed cells of the seminiferous tubules with antibodies to LC3B protein, a marker of autophagosomes, as well as ultrastructure research revealed many autophagosomes in Sertoli cells, Sp and spermatids of group II rats. Electron microscopic examination of the Sertoli cells of group II rats, in contrast to group I, revealed lipid drops and numerous phagolysosomes.

Conclusion MS can be the cause of a violation of meiosis at the pachytene stage. It has been hypothesized that the activation of autophagy is an adaptive mechanism that removes of spermatogenic cells remnants selected as a result of MS. Meiosis disorders and the autophagy activation may lead to a

decrease in sperm count found by some researchers in experimental MS.

P 1.7

TREGEL – a shiny tool to identify type, composition and location of gene regulatory elements

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Hormones can act through signal transduction pathways or through modulation of gene expression, which can be accompanied by DNA methylation changes. Sertoli cells, which play a major role in supporting spermatogenesis, are the target of the follicle-stimulating hormone (FSH). We have analysed transcriptional changes associated with FSH stimulation of the SK11 Sertoli cell line. RNA-seq analysis revealed a total of 185 genes at least 2-fold up- or downregulated ($p \leq 0.01$) upon stimulation with FSH. We performed an analysis of the regulatory elements in the genome involved in these transcriptional changes. This type of analysis is tedious and often difficult. Therefore, we developed the novel tool TREGEL (Transcription Regulatory Elements) to search for regulatory elements in a set of genes. It does so by using public available databases (UCSC, Ensembl, JASPAR) and internal R routines which calculate overlaps between annotations and input genes. This tool allowed us to identify genes containing CpG sites and transcription factor binding sites close to their promoters. The gene list was further restricted by supplying TREGEL with a list of Sertoli cell-specific transcription factors. Using TREGEL, we obtained information about type, composition and location of regulatory elements for the resulting 88 candidate genes. TREGEL also offers the option of uploading a user-curated list of regulatory elements. This feature has the potential to improve current understanding of how each gene is regulated. Results are visualized through an UCSC genome browser interface and can be conveniently obtained as image and table files. With the help of this tool, we are now able to conduct a follow up analyses and validation to better understand Sertoli cell regulation. In the future, we intend to share this tool, which we believe is useful to other researchers studying hormone-dependent transcriptional changes.

P 1.8

Upregulation of MTHFR gene expression by 3 weeks of yoga based lifestyle intervention in infertile men

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Introduction A key enzyme of the folate metabolism is methylenetetrahydrofolate reductase (MTHFR). MTHFR is required for uninterrupted supply of S-adenosyl methionine for methylation reactions, replenishment of glutathione and nucleotide synthesis. Decreased activity of MTHFR enzyme can be due to diminished expression of MTHFR. Aberrant DNA methylation with oxidative DNA damage can decline semen quality. Yoga, a mind body intervention is gaining wide acceptance globally due to its benefits on chronic lifestyle disorders including male infertility. Yoga practice lessens free radical levels and up-regulate antioxidant defence capacity of body and also improves DNA integrity. Yoga upregulates anti-inflammatory & DNA repair genes and downregulates pro-inflammatory genes. Present study was aimed to examine the impact of 3 weeks of yoga based lifestyle intervention (YBLI) on MTHFR gene expression in infertile men.

Materials and Methods 30 infertile men were enrolled for the prospective clinical trial. Supervised daily sessions were conducted for 2 hours per day by a certified yoga instructor. Blood and semen samples were analyzed pre and post intervention for the expression of MTHFR gene and estimation of seminal reactive oxygen species (ROS). cDNA was synthesized by Agilent kit by reverse transcribing total RNA extracted from blood samples by phenol chloroform method. Expression of MTHFR in subject's blood samples were determined by quantitative real time polymerase chain reaction.

Results Expression MTHFR gene was up-regulated with 5.517974 axis fold change after 21 days of YBLI in comparison to day-0. Seminal ROS decreased significantly (p -value < 0.05) post intervention.

Conclusions This is the first report of up-regulation of MTHFR gene following practice of yoga. This simple and easy to adopt lifestyle intervention can be integrated as an adjunct to the standard therapy in a resource limited set up considering the burden of infertility and cost of treatment.

P 1.9

CFTR genotypes and spermatology in Cystic Fibrosis patients without CBAVD

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We evaluate the semen parameters, sperm ultrastructure and chromatin in 5 pancreatic-sufficient cystic fibrosis (PS-CF) patients without bilateral obstruction of the vas deferens (CBAVD), aged from 16 to 29 (22.6 ± 5.0) years. Semen analysis, fructose test, quantitative karyological analysis (QKA) of immature germ cells (IGCs) from the ejaculate sediment (the patent № 2328736, Russia, 2007) and transmission electronic microscopy (TEM) were done. The patients have follow-

ing CFTR genotypes: F508del/3849+10kbc > T ($n = 3$), CFTRdele2,3/3849+10kbc > T ($n = 1$), 3849+10kbc > T/Q493R ($n = 1$). Pathozoospermia was found in 4 patients (asthenoteratozoospermia, AT, $n = 2$; asthenozoospermia, A, $n = 1$; oligoasthenoteratozoospermia, OAT, $n = 1$), the patient with 3849+10kbc > T/Q493R genotype had normozoospermia. Semen volume was 2.5 ± 2.3 (0.3–6.0) ml, sperm concentration -149.2 ± 78.0 (64–243) mln/ml, total sperm count -432.5 ± 485.6 (19.2–1224) mln. Oligospermia was in 2 patients with F508del/3849+10kbc > T genotype (OAT, V = 0.3 ml; AT, V = 1 ml). The fructose was low in the sample with lowest volume. Increased viscosity was found in one sample; pH was in normal range (7.0–7.8). Leukospermia (2.0–2.2 mln/ml) was detected in 3 samples. QKA of IGCs revealed increased % (3–8.5%) of spermatocytes I at meiotic prophase, decreased % of spermatocytes II and increased % of degenerated IGCs. High percentage of abnormal heads, 97–100% ($n = 3$), activated acrosome ($n = 3$), “immature” chromatin ($n = 2$), also, intra-gamete viral infection ($n = 2$) and bacteria ($n = 1$) were found by TEM. Sperm DNA fragmentation was high (32%) in 1 of 2 AT samples. 3849+10kbc > T mutation is compatible with the absence of CBAVD in CF patients. The patients have various spermatology with no specific sperm abnormalities. Oligospermia and low fructose indicate impaired seminal vesicles in some CF patients with no CBAVD. Signs of impaired prophase I of meiosis and sperm ultrastructure defects were found.

P 1.10

HDACi drugs hamper experimental mouse teratoma development

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Testicular Germ Cell Tumours (TGCT) are the most frequent malignancies in young males, driven by epimutations. Teratoma are TGCT composed of all three germ layer tissues. Mouse teratoma model, due to its heterogeneity and pluripotent core, is a very reliable *in vitro* model for investigating the effect of cancer drugs, including histone deacetylase inhibitors, on the epigenetic regulation of tumorigenicity.

After isolation, mouse egg cylinders were treated for two hours with Trichostatin-A (TSA) and Valproate (VPA), respectively. Developing teratomas were cultured *in vitro* and measured for seven days. Proliferation (Ki-67) and apoptosis (Caspase-3) were analysed by immunohistochemistry. Gene

expression of stemness/differentiation genes was analysed by qPCR.

TSA and VPA induced strong growth retardation despite unchanged proliferative or apoptotic activity. Treatments exhibited opposite effects on gene expression compared to control. TSA upregulates while VPA downregulates stemness/differentiation genes.

VPA downregulation of investigated genes could be responsible for reducing teratoma growth, initiating senescence. TSA upregulation of these genes points to a loss of expression regulation, with consequent growth retardation possibly being due to general teratoma dysregulation. This research confirms the effect of VPA and TSA strongly hampering cancer development. These treatments producing the same phenotypical effect of growth inhibition despite different molecular effects and no change in proliferative and mitotic activity illustrate the complex relationship between histone acetylation and regulation of biological processes.

Grants This study was supported by the Scientific Center of Excellence for Reproductive and Regenerative Medicine, Republic of Croatia, and by the European Union through the European Regional Development Fund, under grant agreement No. KK.01.1.1.01.0008, project “Reproductive and Regenerative Medicine – Exploring New Platforms and Potentials”.

P 1.11

A case 46,XY DSD in female patient with pseudotranssexualism

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Genital abnormalities and disorders of sex development (DSD) represent rare congenital diseases in which anatomical, chromosomal or gonadal sex is atypical. We report on 46,XY DSD patient, presented herself as female-to-male transsexual, who was referred for cytogenetic examination. Chromosomal analysis was not performed early. The patient was 40-years old female with below average stature and height, normal intelligence. There were no psychiatric diseases mentioned in the anamnesis and family history. Neonatal medical sex assessment was failed, because of genitalia ambiguous. The patient had intersex phenotype with severe hypospadias, urogenital sinus and no palpable gonads. At the age of 3–12 years the patient underwent several surgical corrections of genitalia abnormalities. After that she was medically assigned as a girl and raised as female. From early childhood she felt herself in the “wrong body” and played with stereotypically male toys. Since teenage years, she expressed herself in an opposite gender manner, got short

haircuts and enjoyed active sport and boxing. At present the patient is undergoing processes to affirm a gender identity different from previous. Chromosome analysis of peripheral blood lymphocytes showed 46,XY karyotype. Despite the normal male karyotype the presence of undefined 46,XY DSD form is evidence against female-to-male transsexualism. Due to the high genetic heterogeneity and non-specific clinical features of many genital abnormalities, the overlapping of phenotypes, reaching a diagnosis is often complicated, especially in 46,XY DSD patients. Early differential diagnosis using complex cytogenetic and molecular-genetic evaluation is the best strategy for management of various DSD forms.

P 1.12

Whole exome sequencing of Sertoli cell only syndrome patient provides novel insights on genetic background on male infertility

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Infertility affects around 15% of couples and male factor is responsible in up to 50% of cases. Genetic factor explains around 15–30% of cases, where some patients have well-defined abnormalities such as Klinefelter syndrome (XXY), deletions in AZF region or rearrangements in other infertility related genes such as CFTR, SRY, AR, NR5A1. However, vast majority of cases remains unexplained. The aim of this study was to examine idiopathic Sertoli cell only syndrome (SCOS) in infertile patient and healthy father by whole exome sequencing (WES) to determine whether genetic mutations or other abnormalities could provide explanation of the SCOS diagnosis. WES was performed on DNA isolated from peripheral blood samples using NovaSeq6000 platform. Variants were annotated and filtered based on family structure, functional impact and phenotype matches using human and model organism data. Results showed that son was compound heterozygote with missense variants (p.Gln317Arg; rs375644492/p.Leu57Phe; rs142007602) in both alleles while in father one allelic variant (p.Leu57Phe; rs142007602) was found. Subsequently to infertility examination, our patient developed brain tumor – astrocytoma. Recent findings suggest FANCM to play a role in telomere length regulation showing that loss of FANCM leads to telomeric replication stress. Association of FANCM variants with astrocytoma has not been reported to date, however, mutations in FANCM had been recently associated with SCOS and

cancer. This finding revealed a first case of male infertility with inherited FANCM variant from healthy father coupled with latter cancer development.

Grants This study is a part of project “Integrated test for detection of genetic variants causing male infertility” funded by European Regional Development Fund and co-supported by the Center of Excellence for Reproductive and Regenerative Medicine, Croatia (grant KK.01.1.1.01.0008, “Reproductive and Regenerative Medicine – Exploring New Platforms and Potentials”).

P 1.13

Reproductive decline across generations due to paternal MTHFR deficiency and linked to demethylation of young retrotransposons

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Introduction Methylenetetrahydrofolate reductase (MTHFR) is a crucial enzyme in one-carbon metabolism with an important role in the production of S-adenosyl methionine and methyl groups for cellular processes including DNA methylation. As mouse and human studies have shown that MTHFR deficiency can impact male fertility and sperm DNA methylation, there is the potential for the intergenerational passage of epimutations. Here, our aim was to determine whether the effect of MTHFR deficiency on the testis or sperm DNA methylation was similar or exacerbated from one generation to the next.

Materials and Methods First generation (F1) C57BL/6 *Mthfr*^{-/-} male mice were bred with *Mthfr*^{+/-} females to produce F2 generation *Mthfr*^{-/-} sons. Reproductive parameters were evaluated and sperm were collected and used for genome-wide DNA methylation analysis.

Results While F1 *Mthfr*^{-/-} fathers had minor effects on testis weights and sperm counts with a small increase in abnormal tubules (20%) in the testis, F2 *Mthfr*^{-/-} sons showed a further deterioration in reproductive parameters with more of a decrease in testis weights and sperm counts and an increase in abnormal tubules. F1 sperm DNA methylation was dramatically affected, with nearly 30,000 CpGs affected, most (99.2%) showing a loss of methylation. Compared to their fathers, > 80% of F2 sperm DNA methylation defects overlapped with regions affected in F1 sperm suggesting that there are regions consistently susceptible to MTHFR deficiency. These regions were in genomic loci that are normally methylated late during prenatal germ cell development and highly enriched in young retrotransposons.

Conclusions The worsening of reproductive parameters in MTHFR-deficient sons versus their fathers suggests that epigenetic defects can accumulate across generations. Loss of methylation at retrotransposons could contribute to this effect, findings reminiscent of epigenetic inheritance (funded by CIHR).

P 1.14

Small RNA library preparation for human sperm of young adults

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Introduction The isolation of small non-coding sperm RNA (sncRNA) and library preparation for NGS challenging from semen with a small quantity of RNA present in sperm and the need to use DNase. This was addressed by comparing two sncRNA library preparation kits.

Patients and Methods 24 sperm samples from the Russian Children's Study biobank were prospectively collected at 18–20 years of age. After density gradient centrifugation sperm RNA was extracted and treated by Qiagen DNase and by TurboDNase. 24 pairs of NEBNext and NEXTFlex sncRNA libraries were constructed and sequenced on NextSeq 500. Unique sncRNA reads were profiled, included miRNA, piRNA and tRNA, and the 8 pairs that had > 240 different sncRNAs with ≥ 10 counts were analyzed. Variance stabilizing transformation (VST) was applied to the sncRNA counts for Bland-Altman (BA) analysis.

Results Additional treatment by Turbo DNase decreased the yield of RNA by 42%. The median input sperm count and miRNA yielded was 28.4 (15.9–44.7) mill and 0.65 (0.31–13.1) ng/ul, respectively. Although level of input miRNA measured by Qubit in both the NEBNext and NEXTFlex libraries was the same, 7.2 (2.5–105) ng, the concentration of DNA in library was significantly higher using NEBNext, 11.8 (4.9–21.3) nM, in comparison with NEXTFlex, 0.2 (0–2.1) nM. Low library quantity was critical for successful sequencing. The minimum number

of unique reads for a sample to be included in that study was set at > 250,000. This was observed in 11 (46%) samples prepared by NEXTFlex and 20 (83%) by NEBNext. According to BA analysis of 8 pairs that met sequencing quality, the average concordance correlation coefficient was 0.52 for miRNA, 0.58 for piRNA and 0.65 for tRNA, $p < 0.05$.

Conclusion Using NEBNext kit we able to generate libraries with higher number of reads uniquely mapped to sncRNAs. The highest reproducibility between kits was found for tRNA and lowest for miRNA.

Funding: RSF #18-15-00202; for parent RCS – NIEHS #R01 ES014370.

P 2.1

Ultrasonic sonoelastography of scrotum in the diagnosis of male fertility

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Sonoelastographic method is widely used in urology for the past 9 years. With it assessed the extent of fibrotic processes and differentiate neoplastic diseases of the urinary organs (bladder, kidney), prostate, penile and scrotum.

The aim of our study was to determine the possibility of using this method for detecting the scrotum in assessing reproductive function of men.

This research was conducted in men aged from 20 to 44 years old. They were divided into three groups. The first group of men consisted of 25 patients with secretory infertility. The second group included 25 male with infertility caused by varicocele. The control group was presented by 12 men in the age range from 22 to 31 years old with the implementation of the reproductive function of 1 or more children. The study was performed by ultrasonic scanner expert class Acuson S2000 manufacture of the company Siemens, was used microwave sensor 18L6 HD. Sex hormones were studied on immunochemiluminescence analyzer Access 2 production Beckman Coulter (USA). Obtaining and study of ejaculate was carried out according to Recommendations of the WHO (5th edition).

For every group were identified the following indicators: speed of spread of shear wave, mapping the area of “soft” tissue, the concentration of spermatozooids, mobility (rapid forward movement, slow forward movement), volume of ejaculate and testosterone.

In the end we obtained the following results. The application of research shear transverse wave Vs men with secretory infertility and varicocele is accompanied by the increase of its value to 1.5–1.6 m/s and is characterized by 35–45% from similar parameters of reproductive healthy men. In half of the cases studies such an increase in performance and decrease in the area of mapping the so-called “soft” areas of the epididymis is accompa-

nied by a decrease in the concentration and actively motile sperm.

So, that's why we definitely can make the following conclusions:

1. Sonoelastography become widely used in andrology, reproductology.
2. Application of such a method of instrumental diagnostic allows to reveal reproductive potential damage in men on the stage of preliminary ultrasound screening.

P 2.2

X-ray endovascular treatments of vascular erectile dysfunction

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Objective Penile prosthesis is the method of choice in the surgical treatment of vascular erectile dysfunction. However, in early stages of disease it seems appropriate to apply a new endovascular treatment.

Method We have operated 14 patients with vascular ED at the age of 25–48 (mean age 36.4 ± 7.4). 9 of them had veno-occlusive erectile disruption, 3 of them had arterial insufficiency of cavernous body and 2 of them were with arteriovenous ED.

The diagnosis is based on Doppler ultrasound study of the corpora cavernous of the penis with penile farmtests, Multispiral computed pharmacocavernosography and multispiral computed tomography of the arteries of the pelvis. The study is performed on the unit “Toshiba Aquilion 64”.

Results We have made vesels stanting in isolated proximal atherosclerotic lesion iliac vessels. Unilateral puncture access was used. Balloon dilatation was used when defeat of pudendal arteries was length. We used balloon catheter Sim2 № Fr 5 with the pressure 10 atm in balloon and time for exposition was 4–5 min. The operation was performed on the periphery of the proximal area of the vessel. In vienocclusive abnormality of erection in the form of abnormal venous drainage of the corpora cavernosa of the penis proximal direction we were taking venous embolization.

Conclusions These techniques of operations are effective ways of sexual rehabilitation of patients with erectile dysfunction.

P 2.3

The effect of salubrinal on ER stress pathway in heat-stressed spermatogenic cells in vitro

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Aim It was aimed to investigate the effect of SAL on endoplasmic reticulum (ER) stress on experimental in vitro heat stress model (HSM) of spermatogenic cells.

Materials and Methods In the study Mouse spermatogonium (GC1) and spermatocyte (GC2) cell lines were used. IC50 dose of SAL were calculated via MTT assay. Each cell lines were separated into 4 different groups which are; control (GC1-C, GC2-C), SAL treated (GC1-SAL, GC2-SAL), HSM experimental model (GC1-HSM, GC2-HSM) and SAL treated HSM model (GC1-HSM-SAL, GC2-HSM-SAL). Control cells were incubated under standard culture conditions. HSM model group cells were incubated under 43°C for 60 minutes. In the SAL group, cells were incubated with 20 μ M SAL containing culture medium for 24 hours. After the experiments, all groups were stained with immunofluorescence probes of p-PERK, GRP78, p-eIF2 α , HSP70 antibodies. Moreover, mRNA levels of GRP78, PERK, eIF2 α were evaluated with qRT-PCR.

Results It was observed that HSM model has cytotoxicity. In the experimental model of HSM cells, all the markers showed elevated levels of immunoreactivity, that they were agreed upon to have ER stress. With the treatment of SAL, decreased levels of ER stress detected. GRP78, PERK and eIF2 α mRNA levels were upregulated in the HSM model group. Even though there was a downregulation following SAL treatment, the difference was not statistically significant.

Conclusions In the view of such findings, it was thought that heat stress triggered ER stress on spermatogenic cells, and SAL has effect on these markers. Studies of ER-related stress mechanisms on spermatogenic cells will be important for the therapeutical strategies with the forthcoming advanced molecular analyses.

P 2.4

Electrophysiological modeling of Vas deferens smooth muscle cells: Role of ion channels in generating electrical activities

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The coordinated activation of several ion channels generates a wide range of electrical activities in the Vas deferens smooth muscle (VSDM) cell. Any mutation or dysfunction to VSDM ion channels causes premature ejaculation, which is a male sexual disorder. To elucidate the quantitative contribution of individual ionic current to the action potential (AP) generation, a biophysically constrained single guinea-pig VSDM cell model is proposed. The model includes voltage-gated Na⁺ channel, Ca²⁺ channel, K⁺ channel, Ca²⁺ activated K⁺ channel and nonselective cation current (NSC). The contributions of each membrane current were investigated by sensitivity analysis and modification of the current parameters. The ion channel conductances are set to maintain the resting membrane potential (RMP) at -50 mV as it is documented that the resting membrane potential (RMP) in VSDM cell varies between -45 to -70 mV.

AP was simulated in the whole-cell model by applying an external stimulus current (10–30 pA), as a brief square pulse of 10 ms duration. The AP exhibits depolarization, repolarization, and hyperpolarization phases as found in experiments. The simulated ionic currents and AP show good agreement with the experimental recordings. Therefore, this electrophysiological model can be a powerful platform to investigate the various electrical properties of VSDM cells in both normal and pathological conditions.

P 2.5

Protective effect of astaxanthin on testicular ischemia-reperfusion injury in rats

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Introduction Testicular torsion is one of the conditions of the acute scrotum that requires immediate surgical intervention. If not recognized at time, it can result of ischemic injuries and testicular loss. Astaxanthin (C40H52O4) is a pigment from the xanthophyll family, oxygenated derivatives of carotenoids whose synthesis in plants originates from lycopene. The antioxidant activity of astaxanthin is 10 times higher than zeaxanthin, lutein, cantaxanthin, β -carotene and 100 times higher than α -tocopherol. Since to date there is no drug given to patients with torsion-detorsion testicular injury, we have investigated the effect of this powerful antioxidant.

Materials and Methods Thirty-two male Fischer prepubertal rats were divided into 4 groups of 8 individuals. Group 1 underwent sham surgery to determine basal values for histological evaluation. In group 2 (torsion-detorsion group), right testis was twisted at 720° for 90 min. After 90min of reperfusion, the testis was removed. Astaxanthin was administered intraperitoneally at the time of detorsion (group 3) and 45 minutes after detorsion (group 4) in the treatment groups. Using software ImageJ[®], histological morphometric values were measured.

Results There is a statistically significant difference in all observed parameters; MSTD (193,489 $\pm$ 21,127) (χ^2 = 55,733, DF = 3, p < 0.0001), MSLD (89,057 $\pm$ 15,187) (χ^2 = 65,687, DF = 3, p < 0.0001), epithelial height (47,877 $\pm$ 12,800) (χ^2 = 66,321, DF = 3, p < 0.0001), tubular area (30920,292 $\pm$ 4901,310) (χ^2 = 59,290, DF = 3, p < 0.0001), luminal area (6361,205 $\pm$ 2040,415) (χ^2 = 67,882, DF = 3, p < 0.0001), Johnsen score (6,563 $\pm$ 1,101) (χ^2 = 71,018, DF = 3, p < 0.0001). *Post-hoc* analysis found that a statistically significant difference existed between all groups (p < 0.0001).

Conclusion By measuring all histological morphometric parameters it can be concluded

that astaxanthin has a protective effect on testicular torsion-detorsion (ischemia-reperfusion) injury in rats.

P 2.6

The Novoglan-01 clinical trial of a conservative treatment for adult phimosis

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Adult phimosis can affect up to 10% of the male population over 18. Common conservative treatments with steroid creams are frequently unsuccessful, requiring repeat interventions. Circumcision in adults is much more delicate than in children, with potential scarring and other undesirable side effects. Foreskin tissue expanders have been advocated as a conservative treatment for adult phimosis and an alternative to steroid creams or circumcision for several years but their relative safety and efficacy has never been proven. Conservative treatments such as foreskin tissue expanders are particularly attractive in the current pandemic when elective surgeries such as circumcision are often unavailable or avoided. The Novoglan product is a foreskin tissue expander commercialised for over 15 years with a very high safety record. We describe the Novoglan-01 clinical trial, started in 2019 at Macquarie University Hospital, Sydney, Australia. In line with Good Clinical Guidelines, the Novoglan-01 clinical trial aims to assess the safety, tolerability and efficacy of the Novoglan product in treating adult phimosis. A second study site at Princess Alexandra Hospital, Brisbane, Australia has recently been set up. The study is expected to conclude and report its findings in 2021.

P 2.7

Machine learning applications in the domain of male infertility: development of a prediction model for Klinefelter Syndrome in azoospermic men

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Introduction With successful digitalization in hospitals over the past years, large sets of patient records labelled with diagnoses are now becoming available, facilitating the application of machine learning (ML) based models to predict conditions of patients. ML based models could present an ideal tool to improve diagnostic precision and therefore

patient treatment. With the in-house developed database Androbase, the Centre of Reproductive Medicine and Andrology (University Hospital Münster) has thousands of records on infertile men available that contain information on e.g. hormone levels, semen analysis, and ultrasound. In order to evaluate the potential of ML for identifying the Klinefelter syndrome (KS) in azoospermic men, multiple models from different algorithms have been trained and benchmarked.

Patients (or Materials) and Methods Computations were executed in R based on the package MLR3. For this task, a cohort of 1476 azoospermic patients (1099 controls with karyotype 46,XY and 377 KS with karyotype 47,XXY) was extracted from Androbase. A subset of patients (105 controls and 32 KS) was used for testing the model performances and additionally evaluated by 16 andrologists/urologists.

Results Comparison of the models regarding sensitivity, specificity, classification error and area under the curve (AUC) indicated that support vector machines, random forests, linear classifiers and logistic regression all perform particularly well on this task. For the independent test data set, a sensitivity of 1 and a specificity of > 88% was achieved. The andrologists/urologists achieved similar results regarding specificity (~88%) but detected fewer KS patients than ML-based models (on average: 81%).

Conclusion These results indicate that ML models might be able to increase the diagnostic rate of KS among azoospermic patients if respective supporting algorithms are routinely included into database systems.

P 2.8

Evaluation of functional and genetic integrity of spermatozoa extracted by migration-sedimentation (MS) method

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Introduction Centrifugation-based sperm selection techniques used in assisted fertilization primarily involve selection of motile and viable sperm from centrifugal pellets of spermatozoa. The swim up (SU) method is routinely used either singly or in combination with density gradient (DG) method to select the most active and motile spermatozoa but these methods don't replicate all complex natural barriers which sperm encounter *in vivo*. Migration-Sedimentation (MS) method isolates spermatozoa based on motility with sedimentation of gravity, thus, the deleterious effects of centrifugation are believed to be avoided. Hence, the main aim of this study is to understand the functional and genetic integrity of spermatozoa extracted by migration-sedimentation (MS) method when compared

with density gradient (DG) method and Swim Up (SU) method using split human samples.

Methods This study included 35 men having normal semen parameters with a minimum of 80 million total sperm number. Spermatozoa isolated by SU, DG and MS methods were compared for sperm yield, vitality, motility and longevity as well as functional characteristics like mitochondrial integrity, protein tyrosine phosphorylation and acrosome reaction. The ability of selection techniques in reducing spontaneous and radiation-induced sperm DNA lesions was assessed by TUNEL assay.

Results MS-selected spermatozoa had higher viability ($p < 0.001$), longevity in terms of total motility at the end of 6 and 18h post-extraction ($p < 0.001$), and mitochondrial integrity ($p < 0.001$) compared to those selected by DG. Furthermore, spontaneous DNA lesions were significantly reduced in MS and SU fractions compared to NE ($p < 0.001$). Similarly, radiation-induced sperm DNA lesions were significantly lower in MS and SU-fractions ($p < 0.001$) compared to DG.

Conclusion MS-based device offers a centrifugation-free, efficient, sperm selection method, making it suitable for partially equipped intra-uterine insemination (IUI) laboratories.

P 2.9

Multispiral computed pharmacocavernosography in the diagnosis of venoocclusive erectile dysfunction

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Introduction Causes of surgical treatment failure for venoocclusive erectile dysfunction (ED) are not only the formation of new collaterals, but also the functioning of residual veins. The purpose of this study was visualization of the veins, which are mainly involved in the blood leakage from the corpora cavernosa.

Methods and Materials Eighty-eight men with ultrasound signs of venous insufficiency of the penis were examined. The average

age was 36.2 ± 9.6 years (18–56 years). The study performed on a multislice computed tomography “Toshiba Aquilion”. We used a protocol Pelvis HCT Native; 120 KV; 60 mA; Rot. Time 0.5. After intracavernosal injection 10–20 micrograms of “Caverject” penile Doppler was performed. When the maximum pharmacological response obtained, intracavernosally injected 5 ml of contrast “Vezi-pak 320”, diluted with 15 ml of saline.

Results Abnormal venous leakage was confirmed in 72 (81.8%) patients, pathological shunts between the spongy and cavernous bodies in 26 (29.5%), abnormal structure of the corpora cavernosa in 13 (14.8%) patients. We identified several types of pathological venous leakage from corpora cavernosa during study: distal – via deep dorsal vein in 28 (31%), proximal – via deep penile veins in 49 (55.7%), and mixed type in 11 (12.6%). Additionally, revealed signs of sclerosis of the corpora cavernosa in 19 patients (21.5%), clarified the status of the cavernous channels, urethra, and the integrity of the rigid and 3-component implants in 2 (2.28%). The sensitivity of this method was 98%, specificity –96%.

Conclusion This study demonstrated a high diagnostic value of dynamic pharmacocavernosography performed with computed tomography in detecting pathological venous leakage from the cavernous pool and its advantage comparing ultrasound techniques.

P 2.10

Satisfaction with the semirigid penile prosthesis among couples from a Semiurban Indian population

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Introduction Insertion of penile prosthesis for treatment of irreversible erectile dysfunction (ED) is a common and well-established treatment in the western countries. Even in the times of the newly available oral medications, penile prostheses continue to have such a nonoptional place in the management

Characteristics	n
Patients	78
Age (years), mean±SD (range)	44±7.30
Preoperative IIEF-5 score, mean±SD	5.33±1.69
Previous oral treatment (%)	100
Previous ICI treatment (%)	17.94
Duration of ED before surgery (months), mean±SD	38.69±12.44
Follow-up (months) after PPI (mean±SD)	32.9±10.50
SD=Standard deviation, IIEF=International index of erectile function, ICI=Intracavernosal injection, ED=Erectile dysfunction, PPI=Penile prosthesis implantation	

Figure 3. P. Gupta

of severe ED. In this study, we have assessed the satisfaction among semiurban Indian couples following insertion of the semirigid penile prosthesis.

Materials and Methods Between January 2000 and December 2015, 78 men with ED underwent semirigid penile prosthesis implantation (PPI) at our hospital. The satisfaction of patients and partners was evaluated using the ED Inventory of Treatment Satisfaction (EDITS) questionnaire and EDITS partner survey.

Results The mean age of the patients was 44.84 ± 7.30 years. The mean duration of time with ED in the preimplantation period was 38.69 ± 12.44 months. The satisfaction of patients and partners as assessed by EDITS questionnaire and EDITS partner survey was 80.66 ± 4.49 and 75.66 ± 6.57 , respectively, at 12 months after surgery and 71.73 ± 8.10 and 65.6 ± 6.49 , respectively, at 24 months after surgery.

Conclusions This study showed a high degree of satisfaction among patients as well as their partners with semirigid PPI. More than 80% of the men reported being very satisfied with their penile implantation surgery (Fig. 3).

P 2.11

Two piece inflatable penile prosthesis surgery: operative steps

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Introduction The placement of an inflatable penile prosthesis has been considered the most reliable and effective treatment for erectile dysfunction in men whose medical treatment has failed.

Materials and Methods We report the use of this two-piece prosthesis in a 50 year old patient with erectile dysfunction and describe the surgical steps of the same.

Results The 2-piece system eliminates a separate reservoir making the surgery easy for the treating surgeon/urologist.

Conclusion In an appropriately selected patient, the 2-piece penile prosthesis is a reliable, user-friendly prosthesis with high patient and partner satisfaction rates.

P 2.12

Fluorescence microscopy and FE-SEM as complementary tools to detect terminal fucose residues on human sperm glycocalyx

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Introduction The redistribution of glycoconjugates stands out as a major feature of sperm capacitation. However, terminal fucose changes during human in vitro capacitation remain poorly described. This study aimed to locate and quantify terminal fucose residues on ejaculated and in vitro capacitated human sperm using fluorescence microscopy and field emission scanning electron microscopy (FE-SEM).

Materials and Methods Sperm samples were obtained from three normozoospermic donors and spermatozoa were capacitated by swim-up during one hour. Two physiological sperm conditions were used, uncapacitated (UC) and one-hour capacitated (C1). For fluorescence microscopy, surface fucose residues were detected by *Aleuria aurantia agglutinin* (AAA) lectin conjugated to fluorescein-5-isothiocyanate and a minimum of 300 cells were analyzed. For FE-SEM, AAA-binding sites were quantified by indirect immunogold-labeling and a total of 100 cells were included. Data were analyzed statistically.

Results Fluorescence microscopy identified a high subpopulation of cells (~45.0%) with terminal fucose residues distributed in a dotted way around the head in UC cells. However, after capacitation we observed a significant increase in the percentage of spermatozoa with fluorescence in both acrosomal and postacrosomal region (~70.0%; $p < 0.05$). Otherwise, we quantified that the number of colloidal gold particles decreases significantly ($p < 0.05$) from ~65.0 in UC to ~40.0 in C1 by FE-SEM.

Conclusion Surface fucose residues undergo redistribution and decrease after in vitro sperm capacitation. Overall, fluorescence microscopy combined with FE-SEM allows accurately identify the location pattern and the quantification of surface fucose residues on human spermatozoa.

P 2.13

Evaluation of the patients' genomic and metabolomic profile to improve the adherence to drug therapy: a study on PDE5 inhibitors.

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Type V-phosphodiesterase-inhibitors (PDE5i) are the first choice drugs used for the treatment of erectile dysfunction (ED), being effective in 60–70% of patients. However, proximately 50% patients per year discontinue the treatment PDEi reporting poor drug efficacy or major adverse drug reactions (ADR). In or-

der to identify early markers of efficacy/safety for the therapy of ED with PDE5i, the basal clinical characterization of patients, integrated with metabolomics analysis of serum and urine and genomic data, were here correlated with the PDE5i efficacy and the occurrence ADR upon administration.

Thirty-six males with new diagnosis of ED were consecutively recruited and characterized at basal for anthropometrics, blood pressure, blood glucose, lipid profile, serum levels of thyroid/sex hormones and erectile function evaluated by IIEF-15 questionnaire. Targeted Next Generation Sequencing (NGS) was applied to genes involved PDE5i pharmacodynamics and pharmacokinetics. Fasting metabolic profile of serum and urine were assessed by NMR-based metabolomics analysis. Patients were prescribed on-demand therapy with Sildenafil oro-dispersible film and followed-up after 3 months from recruitment. Basal data were correlated with IIEF-15 score at follow-up and with the occurrence of ADR recorded by a dedicated questionnaire.

Twenty-eight patients were finally included in the analysis. Serum LDL-cholesterol levels were increased in those reporting ADR (143.3 ± 13.2 mg/dL ADR vs 133.1 ± 12.4 mg/dL No ADR; $P = 0.046$). NGS data showed that specific variants of *PDE11A* and *CYP2D7* genes were more represented in drug responders (both relative risk=2.7 [0.9-5.1]; $p = 0.04$). NMR-based metabolomics showed the highest association between serum LDL-cholesterol metabolites and the occurrence of ADR (Hazard ratio = 17.5; $p = 0.019$).

The association between lipid profile and the ADR pattern suggests major cues in the tailoring of ED therapy with PDE5i.

P 3.1

Handling temperature and time interval prior to testicular-tissue organotypic culture in prepubertal mice have a differential impact on cellular homeostasis

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Prepubertal boys undergoing chemotherapy are at high risk of gonadotoxicity. Cryopreservation of immature testicular tissue (ITT), yet experimental, is the only recommended option for fertility preservation, prior to cancer treatment. In optimizing the fertility restoration procedures, care should be taken in handling and manipulation of ITT in *ex vivo* conditions, as a suitable holding condition is of primary importance in the maintenance of

cellular homeostasis, prior to cryopreservation. To explore this, ITT from 6 day old mice were held and manipulated at ultra-profound-hypothermic, profound-hypothermic, and mild-warm-ischemic temperatures for varying time periods, prior to 14-days organotypic culture. Cell viability and functionality along with the expression of synaptonemal complex and chromatin condensing proteins were assessed. Holding of ITT at ultra-profound-hypothermic temperature up to 24 h did not change the cell viability, levels of testosterone, and *in vitro* proliferation ability, whereas ITT held at profound-hypothermic temperature for 24 h significantly reduced the number of viable cells ($p < 0.01$). However, the mild-warm-ischemic temperature was found detrimental for holding of ITT even up to 6 h. Furthermore, the holding of ITT at ultra-profound-hypothermic had no significant negative effect on 14-day organotypic culture, whereas ITTs from a profound hypothermic group showed reduced post-meiotic transcripts ($p < 0.01$). Therefore, holding of ITT at ultra-profound-hypothermic-temperature was found to be most suitable; whereas profound-hypothermic-temperature may compromise post-meiotic germ cell yield, post *in vitro* culture. This data, albeit in the mouse model, will have immense value in human prepubertal fertility restoration research.

P 3.2

The influence of different ways of education on the home injection of assisted reproduction patients

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Objective To compare the effects of different education methods on home injections of assisted reproduction patients to ensure the effectiveness and safety of home injections.

Methods 350 patients were randomly selected using the following methods: plain text, graphics and text, video, and nurse operation demonstration. The comparison of the accuracy of the first injection after the education was conducted between different education methods. And on the first day after the home injection, the patients' satisfaction with different ways of education was followed up.

Results After using different education methods, the nurses assessed the patient's injection preparation process, drug exhaust method, dose adjustment, skin disinfection, injection site, injection method, drug storage, needle handling accuracy, and patient satisfaction. The injection accuracy rate and patient satisfaction rate of patients with text education were the lowest, while the injection accuracy rate and patient satisfaction of patients with video education combined with nurse operation demonstration were the highest.

Conclusion The use of video combined with nurses' demonstrations during the first home injection of assisted reproductive patients is more beneficial for patients to master the correct injection method. At the

Efficiency of different ways of different education methods on assisted reproduction patients (n=350)									
group	cases	injection preparation ratio (%)	drug exhaust method (%)	dose adjustment (%)	skin disinfection (%)	injection site (%)	injection method (%)	drug storage (%)	needle handling accuracy (%)
Plain text	50	24/50 (48%)	17/50 (34%)	38/50 (76%)	21/50 (42%)	27/50 (54%)	32/50 (64%)	39/50 (78%)	22/50 (44%)
graphics and text	50	32/50 (64%)	26/50 (52%)	40/50 (80%)	27/50 (54%)	30/50 (60%)	34/50 (68%)	40/50 (80%)	26/50 (52%)
Video	50	35/50 (70%)	33/50 (66%)	43/50 (86%)	33/50 (66%)	34/50 (68%)	39/50 (78%)	42/50 (84%)	31/50 (62%)
Nurse operation demonstration	50	41/50 (82%)	39/50 (78%)	41/50 (82%)	35/50 (70%)	40/50 (80%)	41/50 (82%)	45/50 (90%)	35/50 (70%)
Text and nurse operation demonstration	50	45/50 (90%)	41/50 (82%)	44/50 (88%)	40/50 (80%)	43/50 (86%)	44/50 (88%)	48/50 (96%)	41/50 (82%)
Graphics, text and operation demonstration	50	42/50 (84%)	44/50 (88%)	46/50 (92%)	45/50 (90%)	46/50 (92%)	47/50 (94%)	48/50 (96%)	46/50 (92%)
Video and operation demonstration	50	48/50 (96%)	49/50 (98%)	50/50 (100%)	47/50 (94%)	50/50 (100%)	50/50 (100%)	50/50 (100%)	50/50 (100%)
χ^2		45.864	71.673	16.37	51.498	49.421	35.230	23.085	63.383
P		< 0.001	< 0.001	0.012	< 0.001	< 0.001	< 0.001	0.001	< 0.001

Chi-square test

Satisfaction of different education methods on assisted reproduction

Figure 4. H. Pan, et al.

same time, nurses were able to observe the patient's experience psychology during the health promotion process, and effectively conduct psychological counseling to ensure the medicine Effectiveness and safety during injection (Fig. 4).

P 3.3

Follow-up management of pregnant women undergoing pre-implantation genetic testing under the new coronavirus epidemic

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Objective To explore the appropriate follow-up management of pregnant women with preimplantation genetic testing (PGT) during COVID-19 epidemic.

Methods The follow-up managements of pregnant women with PGT was carried out during the epidemic period, including con-

sultation guidance, psychological guidance, online consultation guidance and nursing services, and these managements were compared with the follow-up managements before the epidemic.

Results During COVID-19 epidemic, PGT pregnancy patients who failed to arrive at the hospital on time increased. However, the number of people who visited online through inquiries and active contacts increased significantly, with a follow-up rate of 100%. In addition, through the way of online contact, we can provide consultation, protection and psychological guidance for PGT pregnant women to ensure their safe pregnancy.

Conclusion Medical staff can follow-up the pregnant women with PGT through online consultation, and provide them with scientific consultation guidance and psychological guidance during pregnancy, which not only reduces the psychological burden of pregnant women with PGT during the epidemic, but also improves their compliance, so as to ensure the maternal and infant health during pregnancy.

P 3.4

Understanding the role of selective inhibition of Arachidonate 15-lipoxygenase (ALOX15) during human semen cryopreservation

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Introduction One of the deleterious effect of semen cryopreservation is oxidative stress which can compromise the sperm functional and fertilizing ability. The oxidative stress in sperm can trigger lipid peroxidation thereby producing cytotoxic aldehydes such as malondialdehyde (MDA) and 4-hydroxynonenal (4HNE). The metabolism of 4HNE is through a class of lipoxygenase enzymes mainly the human arachidonate 15-lipoxygenase (ALOX15) which are potentially capable of disrupting sperm function. Therefore, the aim of this study was to understand the effect of selective inhibition of ALOX15 enzyme by its inhibitor 6,11-dihydro[1]benzothiopyrano[4,3-b]indole (PD146176) during human semen cryopreservation.

Methods This study included 20 men having normal semen characteristics. The semen samples were pretreated with 0.25 µM PD146176 for 20 min before rapid freezing. Post thawing, spermatozoa were assessed for motility, morphology, mitochondrial potential, DNA damage and acrosome reaction.

Results Ejaculates cryopreserved with PD14616 showed a moderate but non-significant improvement in sperm motility and mitochondrial potential whereas slight reduction in morphological abnormalities and DNA damage was observed in comparison to the control.

Conclusion The selective inhibition of ALOX15 during human sperm cryopreservation did not demonstrate any benefits in improving the sperm functional and genetic integrity indicating it is not useful in protecting sperm from freeze-thaw induced damage.

P 3.5

Loss of CatSper-channel function leads to male infertility and IVF failure: a case report

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Introduction The sperm-specific Ca²⁺ channel CatSper controls the intracellular Ca²⁺ concentration and, thereby the swimming behaviour of sperm. Genetic aberrations of genes encoding CatSper subunits are associated with male infertility. We identified a CATSPER2-deficient infertile patient that underwent with his female partner assisted reproduction at our centre. Although semen

quality when assessed by classical semen analysis indicated an IVF we decided to do a combined IVF/ICSI to avoid an expected fertilisation failure.

Patients and Methods We used the standard long protocol with 14 days GnRH agonist prior to a daily dose of 125 IU recombinant FSH for controlled ovarian hyperstimulation. Follicular development was monitored by vaginal ultrasonography and ovulation was induced by 6500 IU recombinant hCG when at least one follicle reached a diameter ≥20 mm. Ovum pick-up was performed 36 h after hCG.

Results We retrieved 20 oocyte-cumulus-complexes (COC) of which five were allocated to classical in-vitro fertilisation (IVF) and inseminated with >32,000 motile spermatozoa. The remaining 15 oocytes were injected each with a single sperm (ICSI). Fertilisation was checked 17 h after IVF or ICSI. All 5 IVF oocytes showed no sign of fertilisation, although the sperm were able to dissolve the COCs and bound to the Zona pellucida. The ICSI oocytes gave rise to 9 fertilised oocytes (PN stages). Due to a mild ovarian hyperstimulation syndrome and because of the Corona pandemic all PN stages were frozen. In the meantime, 4 PN stages were thawed and the transfer of two 6-cell stages yielded in a biochemical pregnancy, which unfortunately, resulted in an early loss.

Conclusion Loss of CatSper channel function leads to infertility. CatSper-deficient sperm fail to penetrate the Zona pellucida and, thus to fertilise the egg both *in vivo* and *in vitro*. This indicates that in these normozoospermic patients ICSI is required to overcome CatSper-related infertility.

P 3.6

Developing an in vitro model for toxicological study of chemotherapy on isolated mouse germline stem cells

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Background The overall incidence of mortality for childhood cancers is steadily decreasing thanks to improved diagnosis and treatments. Cisplatin is a chemotherapeutic drug used in paediatric oncology. Accumulating evidence shows that cisplatin impairs fertility in cancer survivors, by targeting the germ stem cells (GSCs) in the testis of young patients. The absence of clinical options to preserve children fertility means that strategies to protect the immature testis from chemotherapy-induced damage are urgently required. The work here aims to develop an *in vitro* model of GSCs to understand cisplatin cytotoxicity and identify key molecular pathways to be exploited for fertility preservation therapy.

Methods GSCs derived from pnd7 F1 (DBA/2 crossed C57BL/6) mouse testes were

cultured on mouse embryonic fibroblasts, using a protocol adapted from Kanatsu-Shinohara et al. BoR 2003. Roughly 2×10⁵ GSCs were plated in a 6-well plate, with medium replaced every 3 days. At day 6, half plate was exposed to cisplatin (1 µg/ml) or drug-free medium. At day 8, GSCs were harvested and sorted by flow cytometer, with DRAQ5™ dye used to identify dead cells. Four replicates were analysed using a paired t-test.

Results GSC colonies displayed the characteristic shape of proliferating cell chains and a slow proliferating rate. Cell viability analysis found more than 95% cells were alive in both control and cisplatin group. Cisplatin treatment significantly reduced the number of harvested cells, with an average drop of 56% (control 13×10⁴ cells vs cisplatin 7×10⁴ cells; p < 0.05).

Conclusion Dead cell count is unreliable as cell viability assay due to the early detachment of non-vital cells. Exposure to cisplatin, at a concentration within the range found in plasma of cancer patients, halved the number of mouse GSCs harvested within 48 h. This cisplatin exposure condition (dose/time) will be used as median lethal dose in an ongoing toxicological study using mouse germline stem cells.

P 3.7

Ex vivo testis explant culture of human intrapubertal testicular tissue

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Introduction Fertility preservation for boys subjected to gonadotoxic therapy, e.g. for cancer treatment, is increasingly in demand by patients and relatives, as long-term overall survival probability for pediatric cancer patients has risen to > 80% (German Childhood Cancer Registry, 2018). Consequently, most of the patients reach the reproductive age (nearly 50,000 in the GCCR long-term survivor cohort as of 2016). However, cryopreservation of sperm, applicable for later assisted reproduction techniques (ART) is no option for prepubertal boys since no spermatozoa are produced before puberty. Hence, the only possibility to restore fertility of young cancer survivors is cryopreservation of testicular tissue containing spermatogonial stem cells (SSCs).

Material and Methods In a first attempt to test the potential of juvenile human testicular tissue, we cultivated a rice grain sized testicular biopsy of a 13-year-old boy for 78 days. The tissue was fixed in modified Davidson's fluid and tissue cells were visualized using

immunostaining with cell-specific markers. The initial cellular status of the testis tissue prior to culture was determined on toluidine blue-stained semi-thin sections of glutaraldehyde-osmium-fixed and Epon-embedded tissue.

Results Both, germ cells, including SSCs, and somatic cells could be detected after the culture period. In one case, single PRM1+ structures resembling spermatids were focally detected. These cells are most likely remnants of individual spermatozoa already present in the boy's testicular tissue, as these were also identified in the testicular tissue before organ culture.

Conclusion These findings demonstrate the potential to develop methods for *in vitro* spermatogenesis and the propagation of human SSCs within cultured human juvenile testis biopsies. If these techniques can be successfully established, this would pave the way for young male cancer survivors to fulfill their wish for biological paternity in later life.

P 3.8

Novel organoid approaches to induce male differentiation of human PGCLCs

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The fetal gonad is characterised by its bi-potential nature, capable of developing into both, ovary or testis. The developing microenvironment precisely coordinates the differentiation of primordial germ cells (PGC) towards male gonocytes. However, fate decision towards the male lineage is not fully understood, since examination of these processes is limited for ethical and technical reasons.

We established a novel and standardized xeno-organoid approach combining somatic testicular cells from pre-pubertal rats and iPSC-derived human PGC-like cells (hPG-CLC). These cells resemble early, migratory PGCs and can recapitulate initial steps of sex differentiation. Our system enabled observation and manipulation of testicular cord-like re-assembly and by adapting medium and culture conditions, we facilitated hPGCLC integration into the somatic surrounding for up to two weeks. Human PGCLCs maintained their PGC-like morphology and expressed the PGC markers OCT4, SOX17, and AP2y. To assess their differentiation, we are analysing male specific gene expression and changes in methylation.

To transfer the organoid towards a homologous setting, we applied previously published protocols for the derivation of adult human testicular cells from testes obtained by gender-confirming surgery of male-to-female transgender patients. They maintain their

ability to re-organise into cord-like aggregates containing SOX9⁺ Sertoli cells. As we currently observe patient-to-patient variation in these cultures, we will first concentrate on increasing reproducibility. Then, hPGCLCs will be included to analyse male differentiation in this homologous organoid.

With this system, we have a model for early fate decisions of human germ cells and a platform to analyse genes associated with infertility. We will use hiPSCs with functional knockout of respective candidate genes and analyse their interaction with the somatic surrounding and male differentiation in comparison to isogenic control hiPSCs.

P 3.9

The optimization of freezing media with saccharides and/or antioxidants to improve the quality of cryopreserved boar spermatozoa

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Introduction Boar semen cryopreservation remains a suboptimal reproductive technology, optimization of freezing media with saccharides and/or antioxidants (AOX) are part of the strategies used to improve the quality of cryopreserved sample. This study evaluated the combined effect of replacement of lactose in the traditional medium by 0.25 M of sucrose (S) or trehalose (T), and the media supplementation with 2.0 mM of butylhydroxytoluene (B) or 1 µM of melatonin (M).

Material and Method The media used were composed of medium A (saccharide/AOX/egg yolk) and B (saccharide/AOX/egg yolk/glycerol/Equex). One ejaculate from eight different boars were split and cryopreserved in the traditional freezing medium (Control) and four experimental freezing mediums: SB; SM; TB and TM. The sperm function test were evaluated by flow cytometry. Total and progressive sperm motilities at 0, 30 and 60 min post-thaw were analyzed.

Results The groups TB and TM maintain the best viability, and only TM significantly decrease the membrane lipid disorder. The SM, TB and TM groups presented a better mitochondrial membrane potential and significantly lower production of O₂- and peroxynitrite than the control, only TM decreased the total ROS production, while SM reduced the lipid peroxidation. To total and progressive motility, the groups SM and TM were significantly higher than the control at T0, however, in all groups there was a negative effect of the incubation time (T0 vs T60).

Conclusion The replacement of lactose by sucrose or trehalose and the supplementation of the medium with melatonin, diminish the presence of nitrosative/oxidative stress markers, since they counteract the harmful effects of free radicals, in particular peroxynitrite,

they allow to preserve the fluidity and integrity of the membranes, which has repercussions in a better mitochondrial status and with it, the sperm motility.

Acknowledgements Supported by FONDECYT REGULAR N° 1180912, ANID, Chilean Government.

P 3.10

Gonocyte transformation into spermatogonial stem cells (SSC): The key to understand infertility and malignancy of cryptorchidism

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Undescended testis (UDT) is a major health problem, affecting over 2% of new-born boys with increased infertility (30–60%) and testicular cancer (5–10 fold > normal males) later in life. We have studied animal models in conjunction with human biopsies of UDT in order to understand the process of gonocyte transformation into SSC to elucidate how to prevent infertility and testicular cancer in cryptorchidism. We used testes from Oct4-promoter-driving GFP transgenic mice, androgen receptor knockout (ARKO) mice, hypogonadal (hpg) mice, Bax knockout (BaxKO) mice and human biopsies for gene expression, immunohistochemistry and confocal imaging analysis. Serum and testes were collected for hormone analysis. We have found that mouse gonocytes transformed into SSC between postnatal days 2–6 during minipuberty when testosterone, FSH receptor and Oct4 peaked. There was no difference for number of gonocytes transformed into SSC/tubule between ARKO mice and wild type (WT) littermates. Germ cells/tubule were significantly less in hpg mice comparing to WT. There were persisting gonocytes in BaxKO testicular tubules which were not present in WT. UDT biopsies showed empty tubules without germ cell significantly increased and number of germ cells decreased with increasing age of orchidopexy. There were persisting gonocytes in testicular tubules of congenital UDT after gonocyte transformation. In conclusion, we found that gonocytes transform into SSC at 2–6 days of age in mouse. Like human minipuberty does exist in mouse and coincides with gonocyte transformation into SSC. Gonocyte transformation in mouse is independent from androgen but gonodotrophin deficiency caused germ cell death. Disruption of apoptosis regulator, Bax, caused persisting gonocytes. Orchidopexy at older age showed significant germ cell depletion. These results suggest that gonocyte transformation into SSC is the key to understand infertility and malignancy of cryptorchidism.

P 4.1

Novel model of sex steroid deficiency in mice to study the physiological effects of delayed and suppressed puberty

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Introduction Sex steroids are critical for skeletal development and maturation during puberty as well as skeletal maintenance during adult life. However, the exact time during puberty when sex steroids have the highest impact as well as the ability of bone to recover from transient sex steroid deficiency is unclear. The latter is highly relevant in the clinical context of delayed puberty, since the impact of a delayed pubertal onset on adult bone health remains elusive. Surgical castration is a common technique to study sex steroid effects in rodents, but it is irreversible, invasive, and associated with metabolic and behavioral alterations. Hence, alternative approaches are needed to study timing and reversibility of sex steroid actions.

Materials and Methods We used a low dose (LD) or a high dose (HD) of gonadotropin-releasing hormone antagonist to either temporarily or persistently suppress sex steroid action in male mice, respectively. Growth, body composition and bone parameters were determined.

Results The LD group, a model for delayed puberty, did not show changes in linear growth or body composition, but displayed reduced trabecular bone volume during puberty, which fully caught up at adult age. In contrast, the HD group, representing complete pubertal suppression, showed a phenotype reminiscent of that observed in surgically castrated rodents. Indeed, HD animals exhibited severely impaired cortical and trabecular bone acquisition, decreased body weight and lean mass, and increased fat mass. In addition, the HD group was characterized by an increased linear growth, which is reminiscent of the clinical observation in patients with hypogonadotropic hypogonadism.

Conclusions We validated a new rodent model of chemical castration, which can be used as an alternative to surgical castration. Moreover, the transient nature of the intervention enables to study the effects of delayed puberty and reversibility of sex steroid deficiency. Our work suggests that, at least in mice, a delayed pubertal timing is associated with bone loss during puberty, but this deleterious effect does not persist at adult age.

P 4.2

Alphoma – a pituitary tumor causing infertility

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Introduction Pituitary tumors can cause infertility. Prolactin levels are not always very high. Macroadenomas (tumors ≥ 10 mm) additionally give neurological symptoms like visual disturbances and headaches.

Hyperprolactinemia in men most often leads to erectile dysfunction and decreased libido. Over time, gynecomastia may also develop. This is caused by hypogonadism and a decrease in the testosterone to estrogen ratio, which causes mammary gland hyperplasia. Testosterone substitution or treatment with dopamine agonists protects against the effects of hypogonadism. Erectile function and libido return but there is no improvement in spermatogenesis. An operation and exogenous gonadotropins can be used as an effective therapy in such patients.

Patient A 32-year-old man was referred for diagnostic workup due to infertility. Due to intensifying visual disturbances and headaches, the patient went to an ophthalmologist. A brain tumor was suspected based on a fundus examination. Pituitary tumor was confirmed in an MRI scan. The patient was referred for further diagnostics to our Department of Endocrinology.

A contrast-enhanced MRI confirmed a 3 cm pituitary tumor with pressure on the intersection of the optic nerves. The tumor completely destroyed the pituitary gland.

Result A successful neurosurgical procedure was performed involving the excision of a major portion of the tumor, thus saving the patient's sight and life. A histopathological evaluation and clinical examinations have allowed a new type of pituitary tumour – an alphoma, to be recognized.

Conclusion Alpha-subunit tests should also receive consideration in treating patients with a pituitary tumour. Perhaps the alpha-subunit concentration in patients with infertility and hyperprolactinaemia should be measured (Fig. 5).

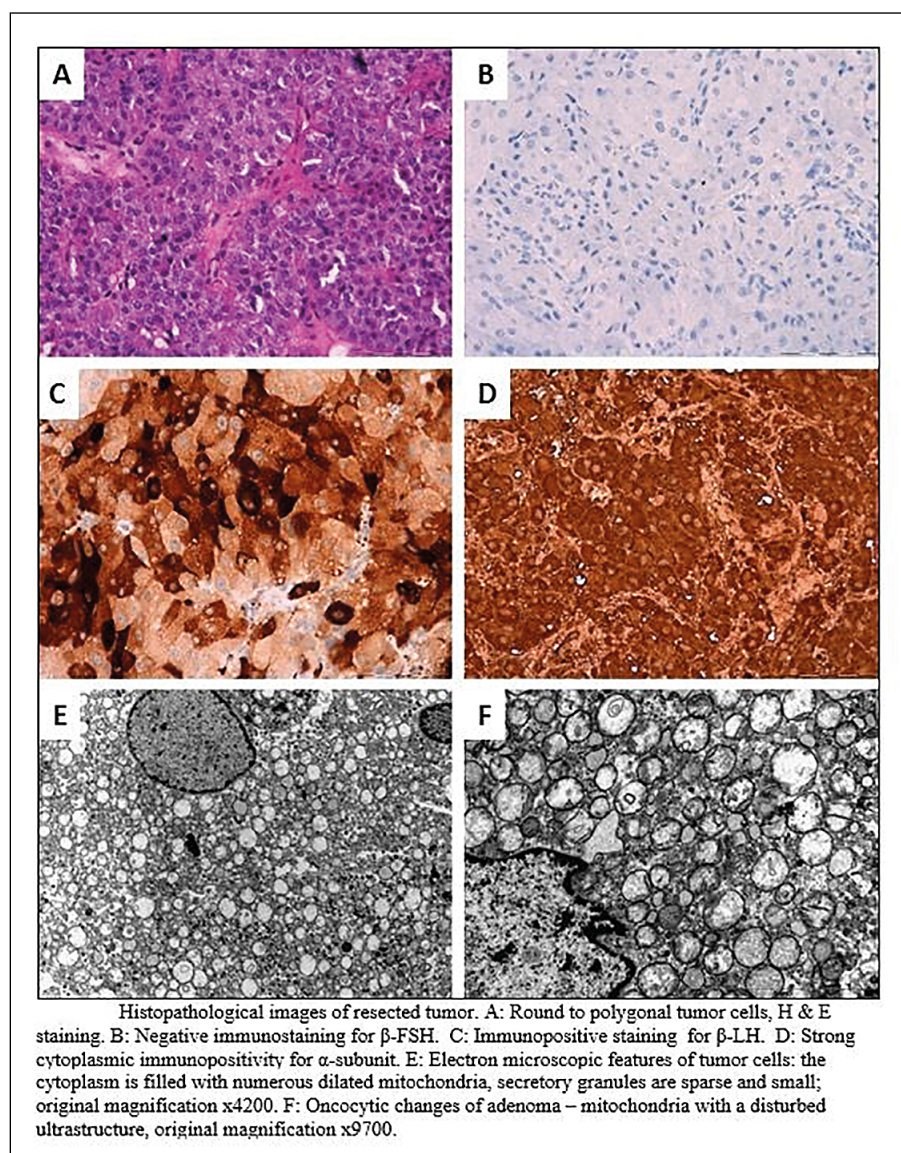


Figure 5. K. Jankowska, et al.

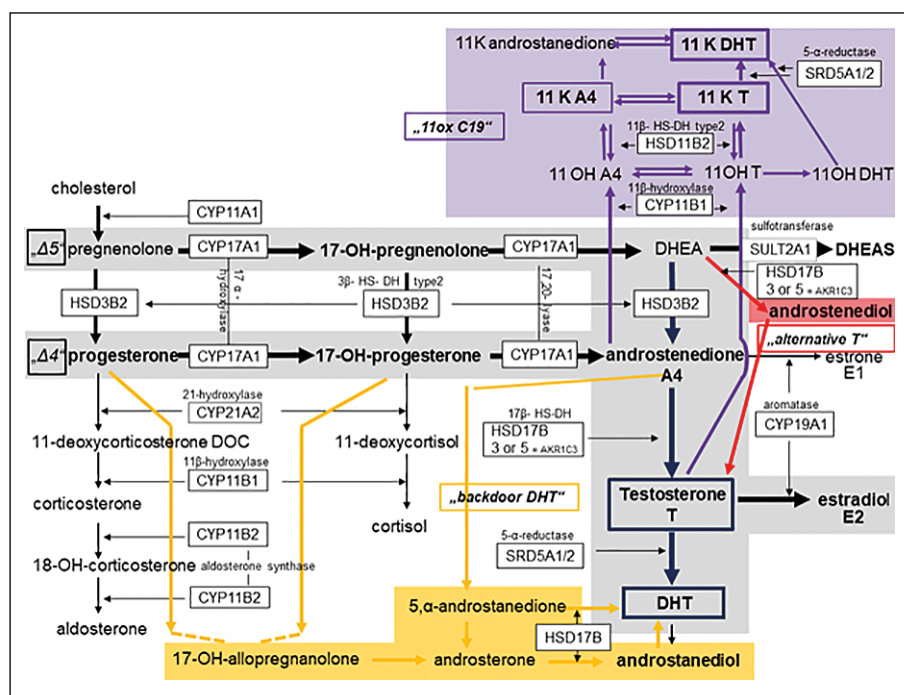


Figure 6. J. Rohayem, et al. © J. Rohayem

P 4.3

The role of gonadotropins in testicular and adrenal androgen biosynthesis pathway – insights from males with congenital hypogonadotropic hypogonadism on hCG/rFSH and on testosterone replacement

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Introduction By this case-control study we aimed to delineate the role of gonadotropins in male androgen biosynthesis pathways

Patients and Methods 25 males with congenital hypogonadotropic hypogonadism (CHH) underwent hCG/rFSH and testosterone treatment sequentially. Serum steroid hormone profiles (testosterone precursors and metabolites) on both replacement regimens were analyzed, using liquid chromatography-tandem mass spectrometry (LC-MS/MS) and compared to those of healthy controls, matched by age, BMI and serum testosterone.

Results On testosterone replacement, serum concentrations of the classic Δ4 pathway hormones progesterone and 17-hydroxyprogesterone (17-OHP), and the marker steroid of an alternative pathway of testosterone synthesis (androstenedione) were decreased, compared to controls. Androstenedione, a marker of the backdoor pathway of dihydrotestosterone (DHT) synthesis, was increased. 17-OH-pregnenolone, androstenedione and DHEA (Δ5 pathway), three 11-oxygenated C19 androgens (11-keto-A4, 11-keto-T and 11-keto-DHT)

and the testosterone (T) metabolites DHT and 17β-estradiol (E2) were similar to controls.

On gonadotropin replacement, 17-OHP, 17-OH-pregnenolone, DHEA and androstenedione, as well as DHT, androstenedione, and all 11-oxygenated C19 androgens were normal. Progesterone (Δ4 pathway) was slightly decreased, androstenedione (backdoor DHT pathway) and E2 (T metabolite) were increased.

Conclusions In males with CHH, serum steroid hormone profiles resemble those of healthy men, if hCG/rFSH is used for substitution. Gonadotropins contribute to steroid hormone production along the classic Δ4 pathway and co-activate an alternative pathway of testosterone biosynthesis via androstenedione. Backdoor DHT biosynthesis, Δ5 17-OH-pregnenolone, DHEA(S) and androstenedione synthesis and 11-oxygenated C19 androgen production are activated independently of gonadotropins (Fig. 6).

P 4.4

Relationship of vitamin D status with testosterone levels: a systematic review and meta-analysis

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Introduction In spite of the biological plausibility of a direct link between low vitamin D and androgen deficiency, the association remains inconclusive in epidemiological studies. Therefore, this systematic review and meta-analysis of case-control studies aims to assess whether and in what populations such an association can be demonstrated.

Materials and Methods A systematic search was performed in PubMed, EMBASE, Cochrane Library, Web of science, Science Direct and CINAHL. Standardized mean differences (SMDs) and 95% confidence intervals (CIs) in total testosterone (TT) levels between men with 25-hydroxyvitamin D (25[OH] D) < 20 and ≥ 20 ng/mL were combined using random effects models. Funnel plot and trim-and-fill analysis were used to assess publication bias. Heterogeneity source was explored by a sub-group analysis according to health-related characteristics of the study populations.

Results Eighteen included studies collectively gave information on 9892 men with vitamin D deficiency and 10675 controls. The pooled SMD revealed a slight, albeit just significant, positive association between 25(OH)D and TT (pooled SMD: -0.23, 95% CI: -0.45 to -0.01; p = 0.04) with a large between-study heterogeneity (I-squared = 98%, p-value for heterogeneity < 0.00001). At the sub-group analysis, a significant positive association, along with noticeable decrease in heterogeneity, could only be demonstrated in studies of patients with frailty states (pooled SMD: -0.19; 95% CI: -0.27, -0.10, p < 0.0001; I-squared = 51%, p-value for heterogeneity = 0.06). A sensitivity analysis revealed a high stability of the result and the trim-and-fill adjustment for publication bias did not affect the pooled estimate.

Conclusions The results of the present meta-analysis support the notion that hypovitaminosis D and androgen deficiency should be regarded as markers of a poor health status, sharing common underlying aetiologies and risk factors.

P 4.5

Gender dysphoria and hormonal treatment: Ten years' experience of a specialized center in Greece

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In response to the growing trend in the literature on developing a management framework for hormonal therapy (HT) in Gender dysphoria (GD), we present our department's latest relevant experience.

We conducted a retrospective case file study on patients with GD, who attended our outpatient clinic from 2009–2019. In total 56 patients were included. 26 were trans-females/TF (mean age ± SD 27.4 ± 10.0 years, HT duration 3.2 ± 2.1 years) and 30 were trans-males/TM (age 26.1 ± 5.6 years, HT duration 3.8 ± 2.6 years).

HT in TFs was an antiandrogen/estrogen combination. GnRH agonist was also given in 10 patients. No significant disturbance in the lipid, haematological or hepatic profile was noted. Total testosterone levels during HT were 0.28 ± 0.2 ng/ml (pre-HT 6.0

± 5.2 ng/ml). TSH levels during treatment were almost stable (from 2.3 ± 1.3 μ IU/ml to 2.8 ± 1.3 μ IU/ml), while 25(OH)VitD levels were 17.37 ± 9.2 ng/ml. PRL levels tended to increase at 32.4 ± 24.5 ng/ml, 6 months after HT ($p = \text{NS}$). In one case treatment with cabergoline was initiated. Osteoporosis treatment was initiated in one patient. Body weight change was -0.27 ± 5.6 kg.

HT in TMs was initially transdermal and then injectable forms of testosterone. In 4 patients, GnRH agonist was given for induction and maintenance of amenorrhea. Total testosterone levels, during HT, showed a tendency to stabilize at 4.9 ± 2.3 ng/ml (pre-HT 0.9 ± 1.4 ng/ml). Mean Hemoglobin levels increased at 15.2 ± 1.0 g/dl (pre-HT 12.9 ± 1.1 g/dl). In one case erythrocytosis occurred and treatment was stopped. Lipid and hepatic profile were constant, while a slight decrease ($p = \text{NS}$) in TSH levels was observed during HT (from 3.22 ± 1.7 μ IU/ml to 2.29 ± 1.1 μ IU/ml). 25(OH)VitD mean levels were 16.85 ± 8.8 ng/ml. Body weight change was $+3.6 \pm 5.5$ kg.

Five TFs underwent gender reassignment surgery (orchiectomy+colpoplasty) and 14 TMs respectively (7 mastectomy+hysterectomy, 5 mastectomy, 1 hysterectomy, and 1 mastectomy+hysterectomy+phalloplasty).

P 4.6

A combined form of hypothyroidism is present in Klinefelter syndrome from pre-pubertal children to adult subjects: a longitudinal retrospective study

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Introduction Hypothyroidism has been proposed to contribute to the clinical picture of Klinefelter syndrome (KS). However, the available studies are limited and fragmentary. The present study aimed at describing thyroid function and structure in KS, from pre-puberty throughout adulthood.

Patients and Methods 254 KS individuals were included, divided according to pubertal status. Adults were further classified into eugonadal, hypogonadal and those receiving testosterone replacement therapy. 300 age-matched healthy and 61 adult chronic lymphocytic thyroiditis (CLT) individuals served as control groups. Serum TSH, fT3, fT4, Ab-Tg/TPO, SHBG and testosterone concentrations were measured. The fT3/fT4 ratio was derived and free testosterone (cT_e) was calculated. Thyroid ultrasound was performed, and thyroid volume (TV) was measured, echogenicity was quantitatively assessed while echotexture parameters were estimated through grey level co-occurrence matrix (GLCM) analyses.

Results Compared to healthy controls, fT4 levels are reduced in pre-pubertal, pubertal

and adult KS ($p < 0.001$, $= 0.01$ and < 0.001 , respectively), whereas fT3 levels are reduced in pubertal children and adults ($p < 0.001$ and $= 0.01$) and TSH values are lower in adult KS ($p = 0.04$). An increased prevalence of Ab-Tg/TPO positivity is present in all age groups. A lower TV, alongside reduced echogenicity and increased parenchymal inhomogeneity is present in KS, similar to CLT subjects. Testosterone regulates the balance between thyroid hormones, as evidenced by group differences according to gonadal status, confirmed by linear regression and a positive correlation between cT_e levels and the fT3/fT4 ratio.

Conclusions KS is characterised by a combined form of hypothyroidism, already present in pre-pubertal children and potentially contributing to the overall clinical picture.

P 4.7

Pituitary response to LHRH stimulation tests in different FSHB -211G>T genotypes

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Introduction FSHB -211G>T variant (rs10835638) is known to affect pituitary FSH output by impairing the transcriptional activity of FSHB.

Materials and Methods This was a cross sectional, retrospective study on 67 consecutive males undergoing LHRH stimulation test for diagnostic purposes in cases of secondary hypogonadism, performed by administering an intravenous bolus of 100 μ g of the LHRH-analogue gonadorelin acetate, with blood samples drawn immediately prior to injection (T0), and after 30 (T1) and 45 minutes (T2). Clinical and genetic data were retrieved from an electronic database. Linear longitudinal mixed-effect models were used to assess the FSHB genotype's effects on FSH and LH levels over time via additive and recessive models.

Results An overall marked increase in serum FSH and LH following administration of 100 μ g of the LHRH-analogue was found ($p < 0.0001$ for linear trend, both models). Peak levels of LH were significantly higher in TT than in GT and GG carriers ($p = 0.012$); no significant between-groups difference was found concerning stimulated FSH levels. In both the additive and recessive models, the main effect of T allele(s) did not reach statistical significance concerning FSH levels ($p = 0.8564$ and $p = 0.7520$, respectively), yet interaction effects over time demonstrated an attenuated response in T-allele carriers compared to the GG carriers ($p = 0.0219$ and $p = 0.0276$). Main and interaction effects for LH were significant in both the additive ($p = 0.017$ and $p = 0.0013$) and recessive model ($p = 0.0019$ and $p = 0.0016$).

Conclusions Following LHRH stimulation, T-allele carriers for the FSHB -211 G>T variant reached higher concentrations of LH and lower concentrations of FSH than GG carriers, suggesting pituitary dysregulation. Especially for the latter, this is in accordance with reduced FSHB promoter activity leading to reduced FSH secretion and with different dynamics of LH release from the pituitary.

P 4.8

Serum and seminal leptin levels, serum testosterone levels and sperm parameters in obese men

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Introduction Leptin is a peptide hormone, secreted by white adipose tissue. The presence of leptin receptor on human spermatozoa and soluble leptin receptor in seminal plasma are well known. This receptor was significantly associated with the intactness of sperm plasma membranes. The aim of this study is to investigate the relationship between serum and seminal plasma leptin levels, serum testosterone levels and sperm parameters in obese and normal weight

Patients and Methods The study includes 60 obese men and 30 normal weight men, between 20 and 50 years. Semen samples were analysed for sperm volume, concentration, motility and morphology as well as for sperm DNA fragmentation index. Serum levels of sex hormones were determined by immunochemiluminiscence technique. Serum and seminal plasma leptin levels as well as estradiol and testosterone in seminal plasma levels were tested by ELISA.

Results The serum leptin level showed a statistically significant difference between the obese men group versus the normal weight men group ($p < 0.05$). In seminal plasma, in regard of leptin, such a difference was not found. Serum leptin levels in obese men correlate negatively with sperm motility. This study does not find a significant difference in serum testosterone levels between obese and normal weight men.

The significant differences in the average sperm concentration and teratozoospermia index were observed by comparison between obese and non obese men.

Conclusion This pilot study showed that obesity adversely affects sperm parameters such as decreased sperm motility and increased teratozoospermia index. Plasma leptin levels are significantly lower in patients with normal sperm parameters and showed an inversely proportional correlation with sperm motility.

The pandemic spread of obesity necessitates further studies to clarify the additional links and mechanisms that are manifested at an early stage and are related to violations of reproductive function in obese men.

P 4.9

Action of progesterone on 2-arachidonoylglycerol (2-AG) levels and enzymes metabolizing 2-AG in human spermT. Depmeier¹, T. Strünker², M. Lehr¹, J. Fabian¹, T. Philipps¹¹Institute of Pharmaceutical and Medicinal Chemistry, University of Münster, Münster, Germany;²Centre of Reproductive Medicine and Andrology, University of Münster, Münster, Germany

Introduction The sperm-specific CatSper controls the intracellular Ca²⁺ concentration and, thereby, the function of sperm. Human CatSper is activated in a non-genomic fashion by oviductal steroids, e.g., progesterone. It has been proposed that progesterone stimulates the activity of membranous serine hydrolase α/β hydrolase domain-containing protein 2 (ABHD2), which in turn degrades 2-arachidonoylglycerol (2-AG) in the sperm membrane, relieving CatSper from inhibition by 2-AG.

Material and Methods We investigated the progesterone-control of 2-AG turnover in human sperm using the fluorescent 2-AG mimetic 1,3-dihydroxypropan-2-yl 4-pyren-1-ylbutanoate (DPPB) and the serine-hydrolase inhibitor methyl arachidonyl fluorophosphate (MAFP). Moreover, we analyzed the action of progesterone on 2-AG levels in human sperm using liquid-chromatography combined with mass spectrometry (LC/MS).

Results We show that human sperm metabolize DPPB. The turnover of DPPB is suppressed by MAFP as well as by 2-AG, confirming that DPPB is metabolized by serine hydrolases and that DPPB competes with 2-AG for the same active sites, respectively. Progesterone does, however, not enhance DPPB turnover. Moreover, we show that 2-AG levels in human sperm are similar before and after stimulation with progesterone.

Conclusion We conclude that the enzymes metabolizing 2-AG in human sperm are, in fact, insensitive to progesterone. In line with this finding, progesterone does not decrease 2-AG levels in human sperm. These result contest the proposed mechanism of steroid-activation of CatSper Ca²⁺ channels.

P 5.1

Improving the HPO terms for non-syndromic male infertilityM. J. Wyrwoll^{1,2}, G. W. van der Heijden³, L. Ramos³, S. Kliesch², F. Tüttelmann¹¹University of Münster, Institute of Human Genetics, Münster, Germany; ²University Hospital Münster, Centre of Reproductive Medicine and Andrology, Münster, Germany; ³Radboud University Medical Center, Division of Reproductive Medicine, Department of Obstetrics and Gynecology, Nijmegen, Netherlands

The Human Phenotype Ontology (HPO) is a formal dictionary of human disease phenotypes and widely applied in genetics. It allows phenotype driven differential diagnosis

tics in clinical routine as well as in research. The current hierarchy of terms concerning infertile patients warrants improvements as several phenotypes are not annotated and others are not linked in the correct hierarchy. Thus, we designed a revised HPO framework for male infertility that is easy to understand and straightforward in its application but does contain most relevant information to study the impact of an identified mutation on spermatogenesis. To this end, an overview of diagnoses for male infertility was made. From these, a branching structure was conceived: the proposed “HPO tree”.

Currently, we focus on a framework for testis histopathology that is based on the presence of germ cells and Sertoli cells, comprising five main categories. In addition to Sertoli cell-only phenotype, we add the classification of tubular shadows, when also Sertoli cells are absent. When germ cells are present, their stage of arrest, number and distribution is informative about the effect of the causal gene with the causal mutation. Hence, we propose to distinguish Germ Cell Arrest (GCA), in which no mature spermatozoa are observed, from hypospermatogenesis (HS). To establish a lower threshold for complete spermatogenesis, we determined the number of tubules that contain elongating spermatids (ES) in a collection of men with obstructive azoospermia (N = 117). By these means, we introduce an evidence-based cut-off for tubules containing ES to distinguish HS from complete spermatogenesis.

We conclude that once this framework is incorporated in the HPO, the standardized vocabulary will facilitate communication in clinical routine as well as in research between collaborating institutions and inform gene discovery.

P 5.2

Do WWC proteins have an impact on germ cell differentiation and male fertility?V. Höffken¹, N. Neuhaus², F. Tüttelmann³, H. Pavenstädt¹, J. Kremerskothen¹¹University Hospital Münster, Molecular Nephrology, Internal Medicine D, Münster, Germany; ²University Hospital Münster, Centre of Reproductive Medicine and Andrology, Münster, Germany; ³University Hospital Münster, Institute of Reproductive Genetics, Münster, Germany

Over 70% of male infertility cases remain unexplained. At least 4% of diagnosed cases underlie genetic reasons, hence research on genes affecting male fertility is needed. WWC proteins are known to regulate cell proliferation, differentiation, and organ growth, thus being interesting candidates in context of male fertility. Our project aims to elucidate the role of WWC proteins in spermatogenesis and male germ cell differentiation. qPCR analysis of murine testis revealed low *Wwc1* but high *Wwc2* mRNA expression. The *Wwc2* level increases during murine postnatal development, similar to expression patterns of germ cell markers (e.g. *Ddx4*). Based on

published 10X genomics data, analysis of single cell gene expression of human testicular WWC proteins revealed low *WWC1* and *WWC3* levels. In contrast, *WWC2* mRNA was highly expressed in various cellular subpopulations and its expression pattern overlapped with zygotene and pachytene spermatocyte clusters. *WWC1* and *WWC2* mRNA levels of testis tissue were compared between patients with complete spermatogenesis (CS, n = 5) and those with Sertoli cell only (SCO, n = 5) or Klinefelter syndrome (KS, n = 3), both lacking spermatogenesis. *WWC1* was uniformly expressed at low levels, while *WWC2* was highly expressed in testes with CS but significantly reduced in SCO or KS tissues. Exome screening of patients with severe spermatogenic failure (n = 735) revealed 20 rare heterozygous missense and 3 rare heterozygous splice variants (minor allele frequency < 0.01) in the *WWC2* gene. Our data indicate specific expression patterns of WWC genes in testis. *Wwc2* expression increases during murine postnatal development. Human *WWC2* is expressed heterogeneously in spermatogenic cell types. Testis tissues of patients lacking spermatogenesis showed decreased *WWC2* expression and several heterozygous *WWC2* variants were identified in exomes of patients with unexplained infertility. Thus, *WWC2* could be a potential regulator for germ cell differentiation and male fertility.

P 5.3

Swine spermatozoa trigger neutrophil extracellular traps (nets) leading to adverse effects on sperm functionR. Sánchez¹, F. Zambrano¹, C. Namuncura¹, F. Pezo¹, P. Uribe¹, M. Schulz², R. A. Burgos², A. Taubert³, C. Hermosilla³¹Universidad de La Frontera, Temuco, Chile; ²Universidad Austral de Chile, Institute of Pharmacology and Morphophysiology, Valdivia, Chile; ³JL University of Giessen, Institute of Parasitology, Giessen, Germany

Introduction In pigs, the number of PMN in uterus lumen increases within few hours after natural or artificial insemination resulting in early PMN-derived innate immune reactions. Sperm-NETs formation was recently reported to occur in various mammalian species. The objective was to evaluate the direct interactions of boar spermatozoa with swine PMN, the release of sperm-mediated NETs, and to assess NET-derived effects on sperm functionality.

Material and Method PMN/spermatozoa were co-cultured in an incubator at 37° C. The kinetic studies were evaluated for 1, 3 and 5 h of exposure and with a cell ratio of 1:3 (PMN [2.5 × 10⁵] and spermatozoa [7.5 × 10⁵]). Sperm-triggered NETs were visualized by SEM- and immunofluorescence analyses. Sperm-mediated NETosis was confirmed by presence of extruded DNA with global histones and NE.

Results Sperm-mediated NETosis was confirmed by presence of extruded DNA

with global histones and NE. Largest sizes of sperm-mediated *aggNETs* were detected after 5 h thereby resulting in effective massive sperm entrapment. The number of *aggNETs* increased from 3 h onwards. Kinetic studies of swine sperm-mediated NETosis showed to be a time-dependent cellular process. In addition, number of NETs-entrapped spermatozoa increased at 3 h of exposure whilst few free spermatozoa were detected after 3 h. Anchored NETs also increased from 3 h onwards. The cytotoxicity of NETs was confirmed by diminution of the total motility and the progressive motility. Spermatozoa membrane integrity and function loss exposed to NETs was confirmed from 3 h.

Conclusion NETs-derived induced damaging effects on swine spermatozoa in membrane integrity, -motility and functionality. We hypothesize that swine sperm-triggered *aggNETs* might play a critical role in reduced fertility potential in swine reproductive technique.

Acknowledgements Supported by FONDECYT REGULAR Project N° 1180912, ANID, Chilean Government and Post-Doctoral Grant, VRIP, Universidad de La Frontera, Chile

P 5.4

Pharmacology of the steroid- and prostaglandin-binding site controlling the activity of CatSper channels in human sperm

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Introduction In human sperm, the Ca²⁺ channel CatSper translates changes in the chemical microenvironment into changes in swimming behavior. As sperm pass through the male and female reproductive tract, they are exposed to multiple chemical cues, such as those present in seminal (SF) and follicular fluid (FF), which contain a complex mixture of prostaglandins and steroids, respectively. It is well-established that CatSper is activated by steroids and prostaglandins via two distinct, extracellular binding sites. However, the pharmacology of the steroid and prostaglandin binding site in human sperm is ill-defined. In particular, it is largely unknown how the different steroids and prostaglandins present in SF and FF affect the activity of CatSper.

Methods We studied the action of FF, SF and a set of different steroids and prostaglandins identified in these fluids on CatSper in human sperm using Ca²⁺ fluorimetry.

Results We show that, with no exception, the steroids and prostaglandins identified in FF and SF, respectively, serve as agonists that activate CatSper via their respective binding sites; yet, with different potency and efficacy. Moreover, we show that many of these steroids and prostaglandins are present in FF or SF at concentrations that readily activate CatSper. This suggests that they act as chemical cues orchestrating the fertilization process. Finally, we found that prostaglandin-, but not steroid-activation of CatSper is suppressed by Zinc ions present in SF. This might suppress premature prostaglandin-induced hyperactivation of sperm right after ejaculation.

Conclusion Our results reveal the promiscuous activation of CatSper by SF- and FF-derived prostaglandins and steroids, respectively, highlighting the physiological significance of these ligands. Furthermore, we propose that the interplay of prostaglandins and Zinc in the ejaculate serves as a dilution sensor that is crucial for the timing of motility responses in the female genital tract.

P 5.5

Exposure to polycyclic aromatic hydrocarbons and nicotine and sperm DNA strand breaks

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Introduction Smoking has been suggested to cause mutations in sperm cells, and potentially to be due to exposure to polycyclic aromatic hydrocarbons (PAH). Smoking has also been reported to be associated with sperm DNA fragmentation, whereas DNA fragmentation more generally has been associated with the mutagenic potential of a chemical. Accordingly, the aim of this study was to study whether metabolites of PAHs and of nicotine, as biomarkers of exposure, were associated with sperm DNA fragmentation as a potential marker of mutations.

Patients and Methods From two cohorts of young men recruited from the general Swedish population, the PAH metabolites 1-hydroxypyrene and 2-hydroxyphenanthrene, as well as cotinine, were measured in urine from 381 men. Sperm DNA fragmentation index (DFI) was analysed by the Sperm Chromatin Structure Assay. Associations between metabolites as continuous variables, as well as in quartiles, and DFI were studied by general linear models, adjusted for abstinence time. A similar analysis was done for cotinine categorised as “non-smoker” (31 men), “environmentally exposed” (141 men) and “smoker” (209 men).

Results No association was found between any of the three biomarkers as continuous variables and DFI ($p = 0.35$ to 0.99), and no difference found in DFI between the lowest and highest quartile of the levels of the three different biomarkers ($p = 0.11$ to 0.61), nor between men with levels of cotinine classi-

fied as non-smoking and men with levels classified as smoking (DFI 13% vs 12 %, $p = 0.48$).

Conclusion In the exposure situations of these men, smoking and PAH exposure did not seem associated with DFI and potential germ cell mutations, to the extent that DFI is an appropriate marker for the latter.

P 5.6

Indication of novel alternate pathway of testosterone action in spermatogenic germ cells of male sprague dawley rats

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Introduction Leydig cell toxicant ethane dimethane sulfonate (EDS) triggers germ cell apoptosis depleting testosterone (T) and interfering spermatogenesis. Testosterone acts via both canonical and non-canonical pathways primarily inducing nuclear androgen receptors (nARs) canonically. We investigated existence of a functional non canonical pathway novel for testosterone in testicular germ cells. 10 days post-EDS treated male rats were compared with testosterone-replaced (EDS+extrinsic-T) and testosterone-supplemented rats. Testosterone and progesterone have similar receptor binding site hence presence of germ cell specific membrane progesterone receptors (mPRs) were investigated. mPRs in other tissues change ion influx via Progesterone Receptor Membrane Component 1 (PGRMC1). We further investigated whether testosterone binds mPRs in presence of low level progesterone, mimicking normal testicular environment.

Materials and Methods RT-qPCR, western blot, binding assay and IHC.

Results EDS treatment caused significant reduction of *3βHSD2* and *Ins3* transcription in Leydig cells along with testosterone depletion in serum and testes. The resultant testosterone loss increased germ cell apoptosis significantly reducing testicular weight compromising blood-testes-barrier (BTB) integrity. nARs were present in nuclei of Sertoli & myoid cells and stripped cytoplasm from spermatids but absent in germ cells. Transcription of five mPRs (α , β , γ , δ and ϵ) occurred in male rat testes among which α and β showed successful protein expression. Our novel results show successful identification of mPRs on isolated germ cells via IHC and competitive binding assay shows successful binding of testosterone with mPRs even in presence of low level progesterone.

Conclusion Novel results imply presence of an alternate pathway of testosterone action in testes opening novel avenues to further investigate for alternate therapeutic targets in the future for solving male reproductive issues.

P 5.7

Identifying novel activin A targets in mouse gonocytes

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Disruptions to fetal male germ cell (gonocyte) development underpin infertility and reproductive disorders including testicular germ cell tumours. We showed the TGF β superfamily member activin A (encoded by *Inhba*) directly affects germline differentiation transcripts in a human gonocyte-like cell. To reveal how testicular somatic cells support normal gonocyte development, this study used mouse models to test the hypothesis that activin A acts directly on gonocytes to promote differentiation. The effects of chronic activin A absence were assessed by RNA-Seq of gonocytes isolated by FACS from embryonic day (E) 13.5 and E15.5 testes from *Inhba* wildtype (WT) and knockout (KO) mice expressing the germline-specific *Oct4-Gfp* transgene (n = 3–4). Also, whole testis transcripts from E13.5 and E15.5 mice lacking activin A (*Inhba*KO) and with elevated activin A (*Inha*KO) were compared with WT littermates (n = 3). To discern acute and direct effects of activin A and its inhibition, isolated E13.5 gonocytes were cultured 24h in media with either 5 ng/mL activin A, 10 μ M SB431542 (activin/Nodal/TGF β inhibitor) or vehicle for transcript analysis (n = 5–6). RNA-Seq revealed 27 and 92 differentially expressed genes in KO vs WT gonocytes at E13.5 and E15.5, respectively. Interestingly, *Musashi-1* was significantly lower in both KO gonocytes at E15.5 and in SB431542-treated E13.5 gonocytes. *Slc38a5* was lower in E15.5 KO gonocytes, *Inhba*KO testes at E13.5 and E15.5, and increased in both *Inha*KO testes and activin A-treated E13.5 gonocytes. Further, in gonocyte cultures, activin A increased differentiation markers (*Piwil4*, *Mov10l1*, *Nanos2*), while SB431542 decreased these and increased an early marker, *Kit*. This multifaceted approach revealed novel activin A targets in the male germline and demonstrated direct effects of activin A that promote a differentiated phenotype. This platform can be used to investigate how disruptions to activin A signalling may underpin male reproductive pathologies.

P 5.8

The effect of sexual activity on mice spermatozoa mitochondrial functionality

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Recent studies on sperm physiology suggest the mitochondrial function as a biomarker of sperm health and fertility. Targeting mitochondrial bioenergetics would provide a

strong rationale for the novel approaches in conservation and fertilization techniques. The aim of the present study was to evaluate the physiological response of sperm mitochondria after sexual activity.

The tissue samples from 29 weeks old male Swiss Webster mice were collected on the next day after mating or from animals without sexual activity. The mitochondrial functionality of the spermatozoa was evaluated using high-resolution respirometry. Flux control factors (FCF, specific contribution of each substrate to mitochondrial oxygen consumption) were calculated.

After sexual activity, the spermatozoa ROUTINE respiration was significantly higher. Moreover, mitochondrial complex I-linked respiration at LEAK and oxidative phosphorylation (OXPHOS)-dependent states were significantly higher after sexual activity. However, there was no difference in OXPHOS coupling efficiency between groups, indicating on the proton leak after sexual activity, that was followed by increased H₂O₂ (reactive oxygen species) production rate in the sexually active group. In comparison to mice without sexual activity, the FCF of glycerol 3-phosphate was decreased, while FCF of succinate, complex II substrate, was increased in spermatozoa mitochondria of sexually active mice, indicating on energetic pathway switch after sexual activity. Besides, spermatozoa count was decreased in mice after mating.

Our data show that spermatozoa mitochondrial functionality is altered after sexual activity. The altered mitochondrial bioenergetics could be associated with increase in immature spermatozoa fraction in sperm samples from sexually active mice. Therefore, for the cryopreservation of the semen, samples should not be collected on the next day after the mating.

P 5.9

Detection of oxidative stress in native semen samples: a new flow cytometric technique

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Oxidative stress (OS) has been largely associated to male reproductive dysfunction and detection of ROS (Reactive Oxygen Species) in semen is important for the assessment of male infertility. Here, the percentage of live spermatozoa with oxidative stress (LOS) in native semen samples was detected using a new flow cytometric technique, coupling the staining of sperm mitochondrial ROS with MitoSOXTM to that of dead cells using the LIVE/DEAD Fixable Dead Cell Stain kit (LD). As expected, the technique is able to detect the increase of OS after challenging spermatozoa with hydrogen peroxide (1 mM), menadione (50 μ M) and mercapto-succinic acid (2 mM). In addition, after sorting live spermatozoa with and without OS

using a BD FACSMelody, we found higher levels of sperm DNA fragmentation (sDF) in the former, as assessed with Sperm Chromatin Dispersion test (mean, SD: 36.2, 13.0 vs 11.5, 5.8; n = 4). This result is consistent with the well-known link between sDF and OS and further validated the reliability of the new technique. We evaluated LOS in 69 subfertile men (SM, consecutively collected among male partners of infertile couples) and 10 donors (DM, recruited after excluding any condition, due to life style or medical history, known to increase OS). We did not find any difference between SM and DM (median [IQR]: LOS = 11.2 [8.1–18.9] and 12.6 [10.3–13.8] in SM and DM, respectively), however 28 out 69 SM (40%) showed a LOS value higher than the 75th percentile of DM. We noticed that LOS was particularly high in SM with presence of bacteria in semen (4 out of 69). This result was confirmed by recruiting further subjects with bacteria in semen, where median LOS was 32.1 [12.8–37.4] (n = 18), much higher than the values found both in SM and in DM (p < 0.0001). In conclusion, this study presents a new flow cytometric technique to detect OS in live spermatozoa of native semen samples resulting sharply linked to sDF. This technique could be used to identify SM with high levels of semen OS.

P 5.10

Optical recording of rapid non-genomic progesterone signaling events in human sperm

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Introduction To navigate the oviduct and fertilize the egg, human sperm rely on chemical cues provided by the female. Chemosensory signaling in sperm involves changes in the membrane potential (V_m) and intracellular Ca^{2+} concentration [Ca^{2+}]_i. The sperm-specific Ca^{2+} channel CatSper controls [Ca^{2+}]_i, while Slo3, the principle K^+ channel in sperm, sets V_m in a Ca^{2+} -dependent manner. Both CatSper and Slo3 are controlled by the female steroid progesterone. We proposed that the complex [Ca^{2+}]_i responses triggered by progesterone in human sperm rest on a CatSper-Slo3 interplay: progesterone-induced Ca^{2+} influx via CatSper activates Slo3 and the ensuing hyperpolarization deactivates CatSper, thereby curtailing Ca^{2+} influx.

Materials and Methods To simultaneously monitor V_m and [Ca^{2+}]_i signals in sperm, we developed an optical multiplexing technique called Frequency- and Spectrally-Tuned Multiplexing (FASTTM). Moreover, we established a method to convert V_m -indicator fluorescence recorded using FASTTM into absolute changes in V_m (in millivolts). This enabled us

to study progesterone-induced V_m signals in a quantitative fashion.

Results and Conclusion We show that progesterone evokes a rapid, transient depolarization followed by an extended hyperpolarization. Signals recorded from CatSper-deficient sperm confirm that the V_m responses are initiated by progesterone-activation of CatSper. Moreover, we found that buffering of $[Ca^{2+}]_i$ by intracellular Ca^{2+} chelators perturbs the V_m response, indicating that it is shaped by intracellular Ca^{2+} dynamics. Taken together, these results support the model that non-genomic progesterone signaling in human sperm involves an interplay of $[Ca^{2+}]_i$ and V_m .

P 5.11

Emerging roles of melanoma antigens in male germline stress-resistance

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Genetically modified animals with loss-of-function of genes that encode melanoma antigens (*Mages*) provided important insights into the enigmatic role of this protein family in the male germline. MAGEs are proteins that are restrictively expressed in testis but often aberrantly activated in cancer. Although they have been intensively investigated for cancer therapy, the more fundamental physiological role in male germline remained overshadowed. The expression of MAGEs at discrete stages of male germ cell differentiation suggested their functions throughout spermatogenesis. Intriguingly, knock out mouse models of different *Mage* genes suggested that they are dispensable for mouse fertility. Intriguing, when males were exposed to diverse stressors, including DNA damaging busulfan, testis over-heating, or calorie restriction, *Mages* provided stress protection to germ cells and reproductive advantage to animals. Given the recent evolutionary expansion of the MAGE gene family in placental mammals, the phenotypes of *Mage-a* and *Mage-b4* KO mice imply that distinct MAGE genes evolved as they provided protection to a specific male germ cell population against a particular type of stress, including *Mage-a* against metabolic stress in differentiating spermatogonia and *Mage-b4* against heat in undifferentiated spermatogonia. In addition to germ cells stress protection, *Mages* also regulate stemness of spermatogonial stem cells and enable faster regeneration of spermatogenesis after stress-induced damage. Together, *Mage* KO mice exposed the importance of the stress-protective pathways unique to mammalian male germ cells, understanding of which may catalyze novel strategies for male fertility preservation and cancer therapy.

P 6.1

Novel Indices for the evaluation of obesity in erectile dysfunction patients

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Introduction The association between Metabolic Syndrome (MetS) and erectile dysfunction (ED) is bidirectional. Inflammatory mediators from the visceral fat induce oxidative damages in the penile microvasculature resulting in ED. Waist circumference (WC) is not a reliable indicator of visceral fat as it includes subcutaneous fat also. Greater prevalence of MetS in Asian men compared to African-American men with the same WC is due to the relatively higher levels of visceral fat in them. Visceral Adiposity Index (VAI) and Lipid Accumulation Product (LAP) are novel indices that include functional parameters along with anthropometric parameters and gives a better assessment of visceral adipose dysfunction. The purpose of the study is to investigate the potential link between these novel indices and ED severity.

Material and Methods In this observational cross-sectional study, ED patients were divided into mild (score > 11) and severe (score ≤ 11) based on International Index of Erectile Function-5 scores. WC, Body Mass Index (BMI) and lipid profile were obtained and VAI, LAP were calculated using formulas, $VAI = WC/[39.68 + (1.88 \times BMI) \times \text{triglycerides}/1.03 \times 1.31/\text{high density lipoprotein}]$ and $LAP = [WC - 65] \times \text{triglycerides}$.

Result Of 116 men included in the study, 60 had mild ED and 56 had severe ED. The mean age was 51.83 ± 6.2 for mild ED group and 52.16 ± 5.8 for severe ED group. Mean VAI was statistically significantly higher in severe ED group compared to mild ED group (8.452 ± 2.33 vs 4.753 ± 1.52 ; $p < 0.001$). Mean LAP was also significantly higher in severe ED group (89.427 ± 31.48 vs 52.21 ± 29.96 ; $p < 0.001$). Interesting, difference

in WC (95.5 ± 9.5 vs 95 ± 8.5 ; $p = 0.146$) and BMI (26.48 ± 3.72 vs 24.67 ± 4.15 ; $p = 0.363$) was not statistically significant among two groups.

Conclusion VAI and LAP have a stronger correlation with ED severity than single anthropometric tools. Considering the simplicity and reliability, these novel indices should be included in the evaluation of obese ED patients.

P 6.25

Stereological properties of seminiferous tubules in infertile men with chromosomal and genetic abnormalities

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Chromosomal and genetic abnormalities are responsible for 15%–30% of male-factor infertility. This study aimed at determining stereological properties of seminiferous tubules in infertile men with Klinefelter syndrome (KS, 9 patients), Y chromosome microdeletions (MYC, 14 patients) and CFTR gene mutation (CFTR, 3 patients), and to compare them with obstructive azoospermia of non-genetic origin (control group, 18 patients). The total volume, surface and length of seminiferous tubules were determined at the microscope magnification of 100×, using the multipurpose Weibel test system with 42 test points. The surface area (A_t) of the test system was 1,245 mm², individual test line length (d) 0,185 mm, and the total length of test lines was (L_t) 3,885 mm. All investigated stereological parameters were significantly smaller in KS and MYC, respectively, compared with CFTR and control group. There were no differences in the results of stereological analysis when comparing histologi-

Table 1. Multiple comparisons of the total volume, total surface area, and total length of seminiferous tubules between individual examined groups (Kruskal-Wallis ANOVA, *post-hoc* analysis).

Group		Vst				Sst				Lst			
		KS	MYK	CFTR	OA	KS	MYK	CFTR	OA	KS	MYK	CFTR	OA
OA	z	25,224	19,710	0,075		19,683	13,162	0,077		23,391	13,714	0,525	
	p	<0,001	<0,001	1,000		<0,001	<0,001	1,000		<0,001	<0,001	1,000	
KS	z		9,848	16,926	25,224		9,339	13,328	19,683		12,535	15,292	23,391
	p		<0,001	<0,001	<0,001		<0,001	<0,001	<0,001		<0,001	<0,001	<0,001
MYK	z	9,848		11,213	19,710	9,339		7,612	13,162	12,535		7,336	13,714
	p	<0,001		<0,001	<0,001	<0,001		<0,001	<0,001	<0,001		<0,001	<0,001
CFTR	z	16,926	11,213		0,075	13,328	7,612		0,077	15,292	7,336		0,525
	p	<0,001	<0,001		1,000	<0,001	<0,001		1,000	<0,001	<0,001		1,000

Legend: OA, obstructive azoospermia; KS, Klinefelter's syndrome; MYK: microdeletion of Y chromosome; CFTR: CFTR gene mutation; Vst: total volume of seminiferous tubules; Sst: total surface area of seminiferous tubules; Lst: total length of seminiferous tubules; p: p-value; z: z-value.

Figure 7. D. Ježek, et al.

cal subgroups (Sertoli cell-only syndrome; mixed atrophy) inside KS and MYC groups, respectively. Qualitative histological analysis revealed significant impairment of spermatogenesis in KS and MYC, whereas testicular parenchyma was fully maintained in CFTR and control group. Spermatogenesis was most seriously impaired in KS. In conclusion, morphometric properties of seminiferous tubules are linked to certain inherited factors (Fig. 7)

Grants This study was supported by the Scientific Center of Excellence for Reproductive and Regenerative Medicine, Republic of Croatia, and by the European Union through the European Regional Development Fund, under grant agreement No. KK.01.1.1.01.0008, project “Reproductive and Regenerative Medicine – Exploring New Platforms and Potentials”.

P 6.3

Prostate volume in men with chronic spinal cord injury: a systematic review and meta-analysis of case-control studies

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Introduction Denervation and androgen deficiency, peculiar to individuals with chronic spinal cord injury (SCI), could hinder to some extent both prostate growth and activity. Although most of the available scanty case-control studies revealed a smaller prostate volume in men with SCI than in age-matched able-bodied controls, some authors found no statistically significant differences. In order to assess the relationship of SCI with prostate volume comprehensively, we carried out a meta-analysis of the available case-control studies.

Methods A thorough search of MEDLINE, Scopus and Web of Science databases was carried out to identify studies comparing prostate size in men with and without SCI. Quality of the studies was assessed using the Newcastle-Ottawa Scale. In presence of significant heterogeneity, data were combined using random effects models. Funnel plots and trim-and-fill analysis were used to assess publication bias.

Results Four studies met the strict inclusion criteria and provided information on 278 men with chronic SCI and 1385 able-bodied controls. The overall difference between the two groups was statistically significant (combined standardized mean difference: -1.31 , 95%CI: -2.59 to -0.03 , $p = 0.04$) even in the presence of high heterogeneity ($P < 0.00001$, $I^2 = 98\%$). The trim and fill analysis did not identify possible missing studies in funnel plot distribution, thus suggesting the absence of obvious publication bias.

Conclusion In men with chronic SCI prostate volume is on average smaller than in

age-matched able-bodied men. Prospective studies are warranted to clarify whether this condition is associated with a lower risk of age-related prostate proliferative diseases despite many factors, peculiar to this population, with a possible role in promoting prostate growth.

P 6.4

Association of erectile dysfunction and type II diabetes mellitus at a tertiary care centre of South India

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Introduction Erectile Dysfunction (ED) is more common in diabetic men and, unfortunately, occurs at an earlier age in diabetic patients when compared with the general population. The study aims to evaluate the independent predictors of ED in adult men with type 2 diabetes mellitus (DM) at a tertiary care center of South India.

Materials and Methods A total of 720 men aged 30–70 years who had been diagnosed with type 2 DM were enrolled for the study from January 2017 to January 2020 from the outpatient diabetes clinic of the Hospital. All patients completed the abridged version of the International Index of Erectile Function (IIEF-5) questionnaire.

Results The mean age of the patients was (58.4 ± 7.8 years). 68.6% of subjects had varying degrees of erectile dysfunction, of which 54.6% had moderate to severe ED. 55.8% had poor glycemic control ($HbA_{1c} \geq 7\%$). Subjects with ED had a longer duration of DM than those without ED (mean DM duration was 8.1 ± 4.9 years versus 4.4 ± 3.5 years; $p < 0.001$). Longer duration of DM, poor glycemic control, hypertension, peripheral arterial disease, testosterone deficiency were all independent predictors ED ($p < 0.05$).

Conclusions A high incidence of erectile dysfunction was observed in type 2 DM patients attending the diabetic clinic, and over half of the people affected were of moderate-to-severe in intensity. Poor glycemic control, testosterone deficiency, peripheral arterial disease were the modifiable risk factors for ED in diabetic subjects. At the same time, a longer duration of type 2 DM was noticed as a glaring non-modifiable risk factor, according to our study

P 6.5

Testosterone treatment in male patients with Klinefelter syndrome: a systematic review and meta-analysis

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Introduction Low testosterone (T) in Klinefelter's syndrome (KS) can contribute to typical features of the syndrome such as reduced bone mineral density, obesity, metabolic disturbances and increased cardiovascular risk. The aim of the present study is to review and meta-analyze all available information regarding possible differences in metabolic and bone homeostasis profile between T treated (TRT) or untreated KS and age-matched controls.

Material and Methods We conducted a random effect meta-analysis considering all the available data from observational or randomized controlled studies comparing TRT-treated and untreated KS and age-matched controls. Data were derived from an extensive MEDLINE, Embase, and Cochrane search.

Results Out of 799 retrieved articles, 21 observational and 22 interventional studies were included in the study. Retrieved trials included 1144 KS subjects and 1284 healthy controls. Not-treated KS patients showed worse metabolic profiles (including higher fasting glycemia and HOMA index as well as reduced HDL-cholesterol and higher LDL-cholesterol) and body composition (higher body mass index and waist circumference) and reduced bone mineral density (BMD) when compared to age-matched controls. TRT in hypogonadal KS subjects was able to improve body composition and BMD at spinal levels but it was ineffective in ameliorating lipid and glycemic profile. Accordingly, TRT-treated KS subjects still present worse metabolic parameters when compared to age-matched controls.

Conclusion TRT outcomes observed in KS regarding BMD, body composition and glyco-metabolic control, are similar to those observed in male with hypogonadism not related to KS. Moreover, body composition and BMD are better in treated than untreated hypogonadal KS. Larger and longer randomized placebo-controlled trials are advisable to better confirm the present data, mainly derived from observational studies.

P 6.6

Safety of injected testosterone undecanoate in the elderly male

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Introduction Quarterly intramuscular depot injections with testosterone (T) undecanoate (TU; Nebido®, Bayer) is considered more convenient by most patients, and prevents daily fluctuations in serum T. However, use of TU in elderly patients is limited due to lack of safety and feasibility studies.

Here, we describe the results from our single-center longitudinal study.

Methods Patients who initiated TU treatment between 2005–2010 were included in the study. Elderly patients were defined as born before 1955 and young patients between 1965–1990.

T dose was adjusted through yearly visits in the out-patient clinic. Standard treatment was 1000 mg TU/12 weeks. Treatment adjustments were performed based on 1) free T NADIR levels, 2) symptoms of T deficiency. Supraphysiological hematocrit led to dose adjustments independent of serum T levels.

Follow up was until April 1st, 2020 or discontinuation of treatment.

Results Median age at time of TU initiation was 61 years (n = 63) in the elderly- and 30 years (n = 63) in the young patients. Median duration of treatment was 13 years for both groups.

Risk of polycythemia did not differ between elderly (n=22 [35 %]) and young patients (n = 17 [27 %]), (RR = 1.32, 95%-CI: 0.70–2.48). Adjustments due to polycythemia was performed 43 times in the elderly vs. 45 times in the young (p = 0.74), due to increased free T: 51 times in the elderly vs 34 in the young (p = 0.07) and due to a combination of the two, 117 times in the elderly vs. 66 times in the young (p < 0.01).

Time between injections were prolonged in elderly patients (median: 1000 mg/15 weeks vs 1000 mg/13 weeks after 10 years of TU, p < 0.01) and accumulated dose of TU at time of polycythemia was lower in elderly – compared to young patients (p < 0.05).

Conclusion TU injections in the elderly patient does not increase risk of polycythemia, however, accumulated dose of TU at time of polycythemia is lower in the elderly male. Thus, frequent treatment adjustments are needed at all ages.

P 6.7

Testosterone deficiency and erectile dysfunction in middle-aged type 2 diabetics

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Introduction To study the prevalence of testosterone deficiency in middle-aged type

2 diabetics (T2DM) and its relationship with metabolic and clinical characteristics.

Materials and Methods A random cross-sectional study of men aged 45–60 years with a previously established diagnosis of type 2 diabetes mellitus. The diagnosis of testosterone deficiency (hypogonadism) was established according to the recommendations of the EUA. Erectile dysfunction (ED) was calculated by scale IIEF –5. A total of 30 patients were examined (mean age – 52.9 ± 0.62 years, BMI –30.7 ± 1.2 kg/m², waist circumference (WC) –102.7 ± 2.9 cm, duration of T2DM –7, 1 ± 1.23 years, HbA_{1c} –7.4 ± 0.31%). According to the protocol, clinical and laboratory data were studied, including the presence of chronic non-infectious diseases, the severity of age-related androgen deficiency according to the AMS scale. The content of total testosterone, luteinizing hormone, sex steroid binding globulin, and glycated hemoglobin in the blood was determined by standard methods. Free testosterone levels were determined by calculation.

Results The prevalence of hypogonadism was 46.7%, while the total testosterone level of less than 12 nmol/l was observed in 63.3% of the surveyed, and the level of less than 8 nmol/l in 30% of the surveyed persons. Moreover, impairment of libido was noted by 70% of the surveyed, and impaired erectile function –63.3%. The level of total testosterone in the blood significantly positively correlated with the level of SHBG in the blood (r = +0.368).

P 6.8

Male infertility with empty sella syndrome: a case report

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Introduction Empty sella syndrome, also known as arachnoidocele, is a rare disorder characterized by herniation of the arachnoid space within the sella which is often associated with variable degrees of flattening of the pituitary gland. This condition is more common in women and associated with obesity.

Patient A 48-year-old man presented to the andrology clinic with 4 years of primary infertility. He also complained erectile dysfunction and low desire. He revealed any history of puberty at the age of 13 but he seldom showed interest in sex since puberty. He was suffered from sinusitis for a long time. He denied any sign and symptoms of cranial mass such as headache and visual disturbance.

He has been diagnosed with empty sella syndrome (PES) since 2018 and had been treated with testosterone gel and got any improvement in his sexual health. But the treatment had been discontinued due to Covid-19 pandemic.

He is extremely obese, 182 cm tall and 182 kg weight and his waist circumference is more

than 150 cm. Further on physical examination, there were sparse facial, axillary and pubic hairs, bilateral gynaecomastia, stretch penile length of 4 cm and bilateral testicular volume of 3 mL.

Result MRI of pituitary fossa demonstrated empty sella with no pituitary mass lesion. Laboratory investigations at the beginning showed low FSH (0.05 mIU/mL), low LH (0 mIU/mL) and low testosterone (30.1 ng/dL). After 11 month of testosterone gel administration, testosterone level rose into 497 ng/dL but there were no improvement in FSH and LH levels. After treatment discontinuation, testosterone level dropped into 25 ng/dL.

Conclusion Regarding his hypogonadal status, he was advised to continue hormonal treatment and we are still doing challenge test to estimate his probability of having offspring, including any chance of gonadotrophin therapy to initiate spermatogenesis in this patient.

P 6.9

Male osteoporosis, a still overlooked issue: an identikit of patients seeking bone health evaluation at a tertiary academic medical centre

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Background Male osteoporosis is under-managed. The characteristics of men referring to health system for bone evaluation remains partially unknown.

Aim To characterize from real-life data male patients seeking the first bone health evaluation at a tertiary academic medical center, referral for both andrological and bone diseases, over a 13-year observation period.

Methods Restrospective, cross-sectional study, including men (> 18 years) referring to our Center from 2007 to 2020 for bone health evaluation. Osteoporosis and osteopenia were defined considering DXA outcomes, according to WHO criteria.

Results A total of 455 men (mean age 62.5 ± 15.1 years) were included: 42 aged 18–40 years, 57 aged 40–50, 79 aged 50–60, 109 aged 60–70, 122 aged 70–80, and 46 aged > 80. Overall, 125 patients (27.4%) were already followed at our Centre due to endocrinological/andrological diseases that are known to increase fracture risk (94 men) or not (31 men); general practitioners and other specialists asked for bone evaluation for 226 (49.6%) and 101 (22.1%) men. DXA has been performed for 354 patients. Prevalence of osteoporosis, osteopenia, and low bone mineral density for age were 25.9%, 26.4%

and 13.2%, respectively. At least one fragility fracture has already occurred in 213 patients (46.8%), with higher prevalence in non-endocrinological than endocrinological patients (56% vs 24%, $p < 0.001$). Considering fractured patients, 49 of them (23%) have never been treated with any anti-osteoporotic therapy, including calcium and vitamin D supplementation.

Conclusions Male osteoporosis presents with a high rate of fragility fractures (about 50%) among patients referring to a tertiary academic medical center for bone health evaluation. Most of fractured patients have not previously been evaluated by a clinician experienced in bone diseases or properly treated, suggesting that consideration for male osteoporosis should be reinforced in primary healthcare setting in order to prevent fractures.

P 6.10

High prevalence of sexual dysfunction among patients with Growth Hormone Deficiency (GHD): results from the Management of Adult GHD (MAGHD) Study.

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Introduction Adult growth hormone deficiency (AGHD) affects quality of life (QoL). Sexuality contributes considerably to well-being and QoL, but studies on sexual function in AGHD patients are lacking.

Aim To investigate the prevalence of sexual dysfunction in AGHD patients referring to a single endocrinological center and grouped according to r-hGH therapy and to assess the correlation between sexual function and QoL scores, r-hGH treatment, clinical and hormonal parameters.

Patients and Methods A prospective, real-life, clinical trial involving AGHD patients was performed in a tertiary, endocrinological center. The 83 enrolled patients were divided in 2 groups according to AGHD treatment: on long-term r-hGH therapy (Group 1, $n = 32$) and not treated with r-hGH (Group 2, $n = 51$). Clinical data and medical history were collected. IGF-1, IGFBP-3, sex steroids and pituitary hormones were assayed. QoL was assessed by Quality of Life Satisfaction in Hypopituitarism (QLS-H) and QoL Assessment of GHD in Adults (QoL-AGHDA) questionnaires. Index for Erectile Function-15 (IIEF-15) and Female Sexual Function Index (FSFI) were employed to evaluate sexual function in males and females, respectively.

Results 83 AGHD patients (31 females, 52 males, mean age 56.3 ± 14.7 years) were evaluated. The prevalence of sexual dysfunction

in AGHD males was 60%, with a higher erectile dysfunction (ED) prevalence in Group 2 (70%). A significant correlation was documented between QLS-H ($R^2 = 0.533$, $p < 0.005$) and QoL-AGHDA ($R^2 = 0.221$, $p = 0.001$) results and erectile function domain, moreover serum IGF-1 resulted directly correlated to all IIEF-15 domain scores, with the most significant relation with EF ($R^2 = 0.123$, $p = 0.019$).

Conclusions This study demonstrates a high prevalence of sexual dysfunction in AGHD patients and that r-hGH treatment seems to be associated to better sexual outcomes. Consequently, evaluation of sexual function should be integrated in the global assessment of AGHD patients.

P 6.11

Family history for cardio-metabolic diseases: a predictor of major adverse cardiovascular events in men with erectile dysfunction

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Introduction Family history (FH) of cardiovascular (CV) disease is a known CV risk factor. However, it is rarely considered for CV risk stratification. Furthermore, FH for metabolic diseases is generally overlooked. To evaluate, in a population of men with erectile dysfunction (ED), whether FH for cardio-metabolic diseases could provide insights in metabolic and sexual features and predict the occurrence of forthcoming major adverse CV events (MACE).

Methods A consecutive series of 4,693 individuals (aged 51.3 ± 13.3 years) attending an Andrology outpatient clinic for ED was studied. A subset of these ($n = 1595$) was evaluated retrospectively for MACE occurrence. Several metabolic and sexual function-related parameters were studied. For the retrospective study, information on incident MACE was collected over a mean follow-up of 4.2 ± 2.5 years.

Results A greater number of cardio-metabolic FH factors was associated with a worse metabolic profile, including higher waist circumference, triglycerides, glucose, glycosylated hemoglobin, and diastolic blood pressure, as well as lower high-density lipoprotein (HDL) cholesterol. An increased number of FH factors was associated with a worse erectile function, impaired penile dynamic peak systolic velocity and lower testosterone levels. In the retrospective study, a positive cardio-metabolic FH was associated with a significantly higher incidence of MACE, even after adjusting for age and comorbidities. Interestingly, when dividing the sample into high- and low-risk categories according to several CV risk factors (age, previous MACE, HDL-cholesterol and comorbidities), FH was confirmed as a predictor of incident MACE only among the low-risk individuals.

Conclusion Family history is simple and inexpensive information that should be part of the CV risk assessment in all men with ED because it helps in the identification of those who need lifestyle and risk factors modifications and whose risk would otherwise be overlooked.

P 6.12

Insight on the intracrinology of menopause: androgen production within human vagina

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Introduction In this study, we investigated the steroidogenic gene expression in human vagina and verified the ability of human vagina smooth muscle cells (hvSMCs) to synthesize androgens from the upstream precursor dehydroepiandrosterone (DHEA). As readout for androgen receptor (AR) activation, we evaluated the expression of the androgen-dependent gene STAMP2.

Materials and Methods hvSMCs were isolated from vagina tissues of women undergoing surgery for benign gynecological diseases. In these cells, by real time RT-PCR, we evaluated mRNA expression of several steroidogenic enzymes and sex steroid receptors. Androgen production was quantified by liquid chromatography tandem-mass spectrometry (LC-MS/MS).

Results In vaginal tissues, AR resulted significantly less expressed than estrogen receptor α (ER α), while, in hvSMCs, it was higher than progesterin and both estrogen receptors. In hvSMCs and in vaginal tissue, when compared to ovary, pro-androgenic steroidogenic enzymes (HSD3 β 1/ β 2, HSD17 β 3/ β 5), along with 5 α -reductase isoforms and sulfo-transferase resulted more abundant. In addition, enzymes involved in androgens inactivation were less expressed than in the ovary. The LC-MS/MS analysis revealed that, in hvSMCs, DHEA supplementation increased androstenedione levels after short term in spent medium, while increased testosterone (T) and dihydrotestosterone (DHT) secretion were detected after longer incubation. Finally, androgenic signaling activation was evaluated through STAMP2 mRNA expression after DHEA and T stimulation.

Conclusion This study confirmed that human vagina is an androgen-target organ with the ability to synthesize androgens, thus providing support for the use of androgens for local symptoms of genito-urinary syndrome of menopause.

P 6.13

Self-reported premature ejaculation and related distress – occurrence and clinical correlates: Results from the European Male Ageing Study

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Introduction There are few data which have looked at the occurrence and clinical correlates of self-reported premature ejaculation (PE) and its related distress in the general population. To determine the prevalence and clinical correlates of self-reported PE and PE-Related Distress in middle age and older European men.

Methods Subjects were recruited from population samples of men aged 40–79 years across 8 European centres. Self reported PE and its related distress were derived from the EMAS sexual function questionnaire (EMAS-SFQ). Becks depression Inventory (BDI) was used for the quantification of depressive symptoms, the Short Form 36 health survey (SF-36) for the assessment of the quality of life, the International Prostate Symptom Score (IPSS) for the evaluation of lower urinary tract symptoms.

Results 2,888 community dwelling men aged 40–79 years old (mean 58.9 ± 10.8 years) were included in the analysis. Among the subjects included, 889 (30.8%) self-reported PE. Among them, 211 (7.3%) claimed to be distressed (5.9% and 1.4% reported mild or moderate-severe distress, respectively). Increasing levels of PE-related distress were associated with a progressive worse sexual functioning and with couple impairment, along with a higher prevalence of depressive symptoms (all $p < 0.05$). Furthermore, a worse quality of life and higher IPSS score were associated with PE-related distress (all $p < 0.05$). The aforementioned results were confirmed even when patients using drugs possibly interfering with ejaculation were excluded from the analysis.

Conclusions Self-reported PE is relatively common in European men aged more than 40 years; however, its related distress is relatively modest. Nonetheless, men with any degree of self-reported PE show increasing levels of depression, worse quality of life and worse couple satisfaction. The reported limited PE-related distress may explain the relatively low number of medical consultations for PE.

P 6.14

Hyperhomocysteinemia and velocimetric parameters in patients with erectile dysfunction undergoing penile echo-color doppler

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Introduction Erectile dysfunction (ED) can be influenced by the presence of atherosclerosis, a pathology of the vessels that also affects the cavernous arteries and in which hyperhomocysteinemia plays a central role.

Objective We aimed to evaluate the association between homocysteine and velocimetric parameters detected by basal penile ECD (peak systolic speed, PSV; acceleration time, AT) in patients with ED.

Methods We conducted a cross-sectional study on ED patients who were studied from a clinical (medical history, BMI, blood pressure, smoking, antihypertensive therapy), biochemical (glycemia, HbA_{1c}, lipid panel, homocysteine, LH, total testosterone, SHBG and PSA) and instrumental (basal penile ECD) point of view.

Results 126 subjects (mean age 52.1 ± 12.6 years; mean BMI 25.6 ± 4.0 kg/m²) were enrolled. We detected mean PSV values of 13.1 ± 2.9 cm/s and mean AT of 2.28 ± 0.70 m/s². These parameters showed a strong correlation among them ($r = 0.690$; $p < 0.001$), with frankly pathological values of PSV and AT in 5% and 59% of the subjects examined, respectively. Mean homocysteine levels were 14.9 ± 9.5 μmol/l, with pathological values present in 26% of the subjects examined. We found an inverse correlation between homocysteine and PSV levels ($r = -0.213$; $p = 0.03$) and between homocysteine and AT ($r = -0.199$, $p = 0.05$). Furthermore, we found an inverse correlation between velocimetric parameters and age, BMI, blood glucose, HbA_{1c}, LDL cholesterol and triglycerides and a direct correlation between homocysteine and HDL cholesterol levels. Age showed a direct correlation with homocysteine levels ($r = 0.287$; $p = 0.003$), while no significant correlations emerged between homocysteine and metabolic parameters.

Conclusions Hyperhomocysteinemia is a frequent condition in ED patients and its prevalence increases with age. The data from our study confirm the effect of hyperhomocysteinemia in the genesis of ED of arterial origin regardless of other metabolic factors.

P 7.1

Testosterone, level of the lesion and age are independently associated with prostate volume in men with chronic spinal cord injury

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Introduction Although men with spinal cord injury (SCI) exhibit a prostate volume significantly smaller compared to age-matched able-bodied men, the independent association of lower prostate volume with its putative determinants has never been analyzed in this population. This study was designed to identify variables independently associated with prostate volume in men with chronic SCI.

Materials and Methods In this cross-sectional study, prostate volume of 138 men with chronic (> 1 years) SCI, aged 54.5 ($25-75^{\text{th}}$: $36.0-66.0$) years, was evaluated with transrectal ultrasonography. All patients underwent a complete neurological exam, as well as biochemical and hormonal assessment, including measurement of total testosterone (TT) levels. Free testosterone levels were calculated (cFT) by the Vermeulen formula.

Results The median prostate volume was 23.4 mL. At the univariate analysis, a larger prostate volume was significantly associated with higher TT ($p < 0.001$) and cFT ($p = 0.001$), SCI level below T12 ($p = 0.007$), more advanced age ($p = 0.04$) and lower body mass index ($p = 0.04$). However, at the multiple regression analyses, an independent positive association only persisted between the prostate volume with either TT or cFT levels, and, to a lesser extent, with age and a level of spinal lesion below T12. A prostate volume below the median value was observed in 91.4% of patients with both biochemical androgen deficiency (TT < 264 ng/dL) and SCI level $\geq$ T12, but only in 16.5% of patients with both normal androgen levels and SCI level below T12 ($p < 0.001$).

Conclusions Low testosterone levels and, to a lesser extent, a younger age, and a spinal lesion level $\geq$ T12 represent the only variables exhibiting an independent association with a smaller prostate volume in men with SCI.

P 7.2

Specific function of the neonatal epididymis is reflected in its contractile structures

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In adult epididymis throughout the whole duct histological structures of the smooth muscle layer (SML) reflect the specific functions of the organ. SML differs between the

proximal transporting parts and the distal sperm-storing part. However, in the neonatal epididymis only one function exists: waste disposal, i.e. the transport of exfoliated epididymal epithelial cells out of the ductal lumen [Weiser et al. 2020].

In this study we analysed the contractile structures of the neonatal epididymal duct in regard to its function by immunohistochemistry, life imaging and compared the data with adult rats. In all regions of the neonatal epididymal duct, SML showed a uniform thickness and consistent spontaneous contractions. In the adult rats, SMLs significantly increased from caput to cauda different to spontaneous contractions, which decreased from caput to cauda.

These contractile peculiarities of neonatal epididymis reflect the underestimated epididymal function of waste disposal during development to prevent obstruction-induced infertility.

P 7.3

Natriuretic peptides as a future alternative for tamsulosin in BPH treatment

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While the exact mechanisms for the development of benign prostatic hyperplasia (BPH) remain unknown, it is suggested that hormonal regulation plays a critical role. In BPH smooth muscle cells (SMCs) show an increase of muscle tone and cell proliferation. Cyclic GMP (cGMP) signaling pathways regulate a variety of physiological functions, such as SMC relaxation or cell proliferation. Activators of this pathway are the natriuretic peptides (NPs) ANP, BNP and CNP. High concentrations of CNP were found in the prostate and seminal plasma. However, the function of CNP in prostatic SMCs is not clear. Since existing drugs such as tamsulosin lead to side effects like abnormal ejaculation and even lead to discontinuation of therapy, research is focusing on new therapies. For this, the hormonal influence of CNP on the cGMP signaling pathway and its significance for the development of BPH needs to be investigated. We performed RT-qPCRs to investigate the influence of NPs on the expression of cGMP pathway components in cultured primary SMCs from human prostate. cGMP-ELISAs were used to show whether NPs activate this cGMP pathway. The effects of natriuretic peptides and the synthetic natriuretic peptide Vasopressin (VNP) on spontaneous contractility of prostate glands were investigated by Live-Imaging. We found that CNP, more than ANP, activates cGMP production in primary prostatic SMC cultures in a dose-dependent manner. Interestingly, the expression of GC-B was higher than that of GC-A in cell cultures, which may explain the higher production of cGMP after CNP treatment. Furthermore, we showed

that ANP, CNP and also VNP significantly reduced contraction frequency. Long-term effects of CNP and VNP were analyzed in primary SMCs. We found that the cell number was reduced after CNP or VNP treatment for 3 days. Thus, NPs affecting muscle tone and proliferation could be developed to promising drugs for BPH treatment as an alternative to tamsulosin with its side effects on ejaculation.

P 7.4

Does reduced lipid substrate mean impaired testicular function? Androgen and sperm production in men with heterozygous familial hypobetalipoproteinemia

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Heterozygous familial hypobetalipoproteinemia (FHBL) is a rare genetic condition mainly caused by truncating mutations in apolipoprotein B (apoB) gene. The truncated apoB proteins determine extremely low plasma levels of apoB-containing lipoprotein particles, which represent a fundamental substrate for steroidogenesis. The hypothesis that a reduction of plasma lipoproteins could interfere with steroid hormone production was investigated by few studies dating back to the 80s considering only adrenal steroidogenesis and enrolling maximum 4 patients. However, the testicular steroidogenesis has never been investigated in FHBL. Additionally, male mouse models of heterozygous FHBL exhibited impaired sperm count, motility and survival time. With this in mind, we enrolled 5 male patients (median age 41.6 [23.5–63.5] years) with genetic-confirmed apoB-related heterozygous FHBL to assess the testicular function. At the clinical evaluation, they presented normal testicular volume (right 16.9 [7.3–26.2] ml, left 16.6 [2.7–24.6] ml), while in 2 cases (40%) testicular ultrasound inhomogeneity was recorded. Androgen production resulted within reference ranges for all patients (median testosterone 4.5 [3.7–6.8] ng/ml), accompanied by normal levels of luteinizing hormone 2.6 [1.2–6.0] mIU/ml. On the contrary, 2 men resulted azoospermic, whereas seminal parameters were within the reference range in other cases (sperm number 206.4 [68–360] millions, progressive motility 50 [41–70]%). Thus, the testicular steroidogenic functionality seems not impaired in heterozygous FHBL men. However, a high incidence of azoospermia (40%) was detected, suggesting a potential association between heterozygous FHBL and altered sper-

matogenesis. Future studies, using the liquid chromatography-tandem mass spectrometry, could clarify the real steroidogenic potential of both adrenal and testicular tissue in such patients, representing an *in vivo* model of impaired lipid substrate for steroidogenesis.

P 7.5

Generation of three-dimensional extracellular matrix (ECM-) derived scaffolds to reorganize human testicular cells in vitro

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Fertility preservation is important for patients threatened by infertility like prepubertal boys suffering from malignant diseases unable to cryopreserve sperm. Thus, immature testicular tissues likely containing germ cells are preserved, but systems for *in vitro* differentiation are missing so far. Our study aimed at generating three-dimensional ECM-based scaffolds derived from adult human testis of patients undergoing male to female gender confirming surgery as those natural matrices should provide factors supporting cell reorganization and differentiation.

To decellularize adult testicular tissues, two protocols were tested regarding ECM structural maintenance and cell removal efficiency. Isolated human testicular cells were used for recolonization of generated scaffolds and collagen sponges. Immunohistochemistry showed a protocol containing triton treatment to be superior in ECM preservation, while testicular cell removal was more effective by another using 1%SDS. Flow cytometry analysis confirmed numbers of remaining cells after treatments to be lower using 1%SDS (370 cells) than triton (22000 cells). Isolated testicular cells successfully recolonized and organized in artificial collagen sponges but were not able to do so in ECM scaffolds. Additionally, hydrogel of homogenized ECM was used as coating for conventional testicular cell culture to assess molecular cytocompatibility. The hydrogel promoted cellular survival, structure and reaggregation more efficient than common matrix coatings. Cell aggregates contained several germ cell types and somatic cells. Thus, supportive factors for cellular reassembly were still present in the matrix derived hydrogel after processing.

Our data show that human testicular ECM contains regulatory factors potentially promoting germ cell differentiation *in vitro*. A combination of collagen sponges and testicular ECM components could be beneficial for development of *in vitro* system development for male germ cell differentiation.

P 7.6

Repair of penile fracture: how long can it be delayed?P. Patel¹, R. Neril², S. Ghagane²¹Jawaharlal Nehru Medical College, Belagavi, Urology, Belagavi, India; ²KLES Dr. Prabhakar Kore Hospital and research centre, Urology, Belagavi, India

Introduction “Penile fracture” is defined as the traumatic rupture of the tunica albuginea of an erect penis. Patients typically describe immediate detumescence, severe pain, and swelling as a result of the injury. The majority of patients can be diagnosed from the history and physical examination alone. Radiographic imaging studies, including ultrasonography, magnetic resonance imaging, and in some cases retrograde urethrography can aid in the diagnosis of unusual cases. Prompt surgical exploration and corporal repair is the most efficacious therapy.

Materials and Methods We retrospectively reviewed the hospital inpatient and outpatient records of all patients with penile fractures, who underwent repair 8 days after injury. This study was carried out following permission obtained from the Institutional/University ethical committee. The presenting symptoms, details of clinical examination, imaging records, and treatment plans were recorded and analyzed.

Results During a 10 year period, five patients undergoing repair of penile fracture 8 days after injury were identified. The mean age was 28.6 and the mean period taken to present to us was 16.89.7 days. The duration of time to repair was 189.7 days. The patients were evaluated at 12 weeks, 6 months, and 12 months after repair. There were no obvious complications noted. All 5 patients experienced spontaneous erections within 12 weeks.

Conclusions Surgical treatment/repair should always be offered to patients with penile fracture, irrespective of delayed presentation. Long term complications are rare and the patients in our series reported no erectile dysfunction at a minimum follow-up of 12 months.

P 7.7

Epididymosomes participate in the transfer of a subset of epididymis-specific proteins to spermF. Barrachina^{1,2}, M. A. Battistone¹, J. Castillo², C. Mallofré³, M. Jodar^{2,4}, S. Breton^{1,5}, R. Oliva^{2,4}

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Introduction Testicular sperm are immature cells unable to fertilize an oocyte. After leaving the testis, sperm transit along the epididymis to acquire motility and fertilizing abilities. It has been proposed that the transfer of information (proteins and RNAs) between epididymis and sperm is mediated by extracellular vesicles, named epididymosomes, released by epididymal principal cells (PCs). However, direct protein transfer to sperm through epididymosomes is still poorly characterized.

Materials and Methods An *in silico* analysis using human and mouse proteomics and transcriptomics data was carried out to infer proteins with a putative epididymis-origin that are potentially transferred to sperm by epididymosomes. High-resolution confocal microscopy of epididymis and testis tissue biopsies of human and mouse, and CFSE-fluorescently labeled epididymosomes were used for experimental validation.

Results Derived from the *in silico* analysis, we identified 25 sperm proteins with a putative epididymis-specific origin conserved in both human and mouse species. The epididymal origin of 4 of these putative epididymis-specific sperm proteins was validated by confocal microscopy. Specific protein expression patterns were observed in the different regions of the epididymis and, interestingly, 1 of the 4 studied proteins was exclusively expressed in epididymal clear cells (CCs), while the others were expressed in both PCs and CCs. Finally, we showed that fluorescently labeled epididymosomes interacting with sperm *in vitro* contain the 4 targeted sperm proteins with an epididymal origin.

Conclusion Our findings indicate that epididymosomes are capable of providing spermatozoa with a set of epididymis-specific proteins that could be essential for the post-testicular maturation of sperm cells, and support the novel role of CCs in the transfer of proteins to sperm and in epididymosome production.

Grants Supported by PI16/00346 to RO; FPU15/02306, ASBMB, Lalor Foundation to FB.

P 7.8

Altered secretory function of male accessory organs and oxidative stress may be involved in male infertility evoked by scrotal heat stressM. Fraczek¹, L. Wojnar², M. Kamieniczna¹, M. Piasecka³, K. Gill⁴, M. Kups⁵, V. Chopyak⁴, A. Havrylyuk⁴, J. Nakonechnyy⁶, A. Nakonechnyy⁶, M. Kurpisz¹

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Introduction This clinical retrospective study aimed to verify some current concepts about possible molecular pathways responsible for the onset of male infertility associated with scrotal hyperthermia.

Patients and Methods To perform the study, 226 men aged 22–40 were enrolled. The following research subgroups were distinguished: fertile men serving as control (n = 21), professional drivers (n = 52), infertile men with cryptorchidism in childhood (n = 50), infertile men with varicocele (n = 71), infertile men not exposed to prolonged genital heat stress (n = 32). Seminal biochemical (neutral alpha-glucosidase – NAG, fructose, citric acid) and oxidative stress (total antioxidant capacity – TAC, catalase activity, superoxide dismutase activity, malondialdehyde level) parameters were determined using commercially available kits. In addition, the sperm chromatin structure assay (SCSA), TUNEL assay and aniline blue staining were used to assess sperm chromatin integrity.

Results The NAG activity was significantly lower in cryptorchid men while drivers and infertile men with varicocele exhibited significantly lower TAC but higher catalase activity as well as sperm DNA fragmentation compared to the control group. The NAG activity was the main critical parameter correlated with basic sperm parameters in men exposed to active scrotal hyperthermia. Moreover, the greatest number of correlations of antioxidative parameters with sperm DNA integrity were observed in varicocele patients.

Conclusion The biochemical status of seminal plasma may be responsible for low sperm quality in men exposed to both external or internal genital heat stress. Dysregulation of seminal antioxidant components can be principally associated with the epididymal and prostate functions. Oxidative stress induced by local thermogenic factor can be one of the prominent mechanisms responsible for sperm DNA damage.

Grants The study was financed by the National Science Centre, Poland (grant No 2015/19/B/NZ5/02241).

P 7.9

TLR4/NFκB signaling pathway regulates epididymal embryonic developmentL. G. Ferreira¹, F. A. Nishino¹, S. G. Fernandes¹, C. M. Ribeiro¹, B. T. Hinton², M. C. W. Avellar¹

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The Wolffian duct (WD) is an embryonic tissue that undergoes androgen-induced coiling and elongation to become the adult epididymis. We showed a β-defensin (innate immunity component) as an androgen

target in the developing WD. Indeed, immune-/inflammatory mediators are required in developmental processes. β -defensins and extracellular matrix (ECM) components regulate cellular responses by acting as Toll-like receptor 4 (TLR4) endogenous ligands. TLR4 is a player in the maintenance of adult epididymis, however its expression and functionality during WD morphogenesis is unknown. Here we studied TLR4 expression and the regulation of TLR4-target genes during rat WD morphogenesis between embryonic days (e) 17.5 and 20.5. The functionality of TLR4/NF κ B signaling and the morphological consequences of its activation were assessed using WD organotypic cultures challenged with LPS from *E. coli* (TLR4 agonist; 25 ng/mL) and PDTC (NF κ B inhibitor; 100 μ M). TLR4 was constitutively expressed in the WDs whereas *Tlr4* mRNA levels increased between e17.5 (uncoiled WD) and e20.5 (coiled WD). A switch from a mesenchymal to a predominant epithelial TLR4 immunoreactivity also occurred in this period. Compared to controls, TLR4-target genes (inflammatory mediators) were differentially regulated and the IL1 β immunodistribution changed in WDs challenged with LPS and/or PDTC, confirming the functionality of TLR4/NF κ B signaling in this tissue. TLR4 activation by LPS also resulted in WDs with smaller length between bends and regions of ductal dilatation. These morphological disturbances were associated with laminin reassembly, implicating ECM as a participant in the regulation of WD morphogenesis by TLR4. Our results place TLR4/NF κ B signaling as player during epididymal development. Activation of this signaling pathway during maternal infectious and inflammatory conditions may impact reproductive outcomes of male offspring.

Funding FAPESP/CNPq/CAPES. Ethics approval: CEUA-Unifesp #1776201213.

P 7.10

Angiopoietin pathway in benign prostatic hyperplasia after pulsed electromagnetic field therapy: evaluation of a new serum inflammatory marker

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Introduction Chronic low-grade inflammation and endothelial activation seems to play an important role in Benign Prostatic Hyperplasia (BPH) etiology, due to the consequent hypoxia. We recently demonstrated that pulsed electromagnetic field therapy (PEMF) is effective to reduce prostate volume (PV) and lower urinary tract symptoms (LUTS) after 28 days of therapy, because of its anti-inflammatory and pro-angiogenic effect. In this study, no changes were found in classical inflammation markers. Angiopoietin (Ang) system regulates angiogenesis and is involved in the pathogenesis of inflammation:

Ang1 has an anti-inflammatory role, while Ang2 has a pro-inflammatory effect. The aim of this study was to evaluate Ang pathway as an effective serum marker in BPH patients who performed PEMF therapy. We provide preliminary data of this study.

Material and Methods This is a prospective interventional trial on 27 naïve patients with LUTS. At baseline (V_0) and after 28 days (V_1) of PEMF therapy, all patients had blood tests, Ang-1 and Ang-2 serum levels, transrectal ultrasound and questionnaires (IPSS). The PEMF device (Magcell® Micro-circ, Physiomed Elektromedizin) was applied

on perineal area 5 minutes twice daily. A Wilcoxon signed-rank test was performed to compare the effects of treatment at V_0 and V_1 .

Results No differences between V_0 and V_1 were found in Ang1 and Ang2 levels, even if a trend in reduction in Ang1 was found: Ang1 $_{V_0}$ 1.04 (0.3; 2.1) vs Ang1 $_{V_1}$ 1.65 (0.58; 3.56), $p = 0.054$. However, data show a significant reduction in Ang2/Ang1 ratio from V_0 to V_1 : Ang2/Ang1 $_{V_0}$ 2.1 (0.7; 5.0) vs Ang2/Ang1 $_{V_1}$ 1.2 (0.7; 2.9), $p = 0.036$.

Conclusion A deregulation of the balance between Ang2 and Ang1 may be associated with inflammation. We demonstrated that, af-

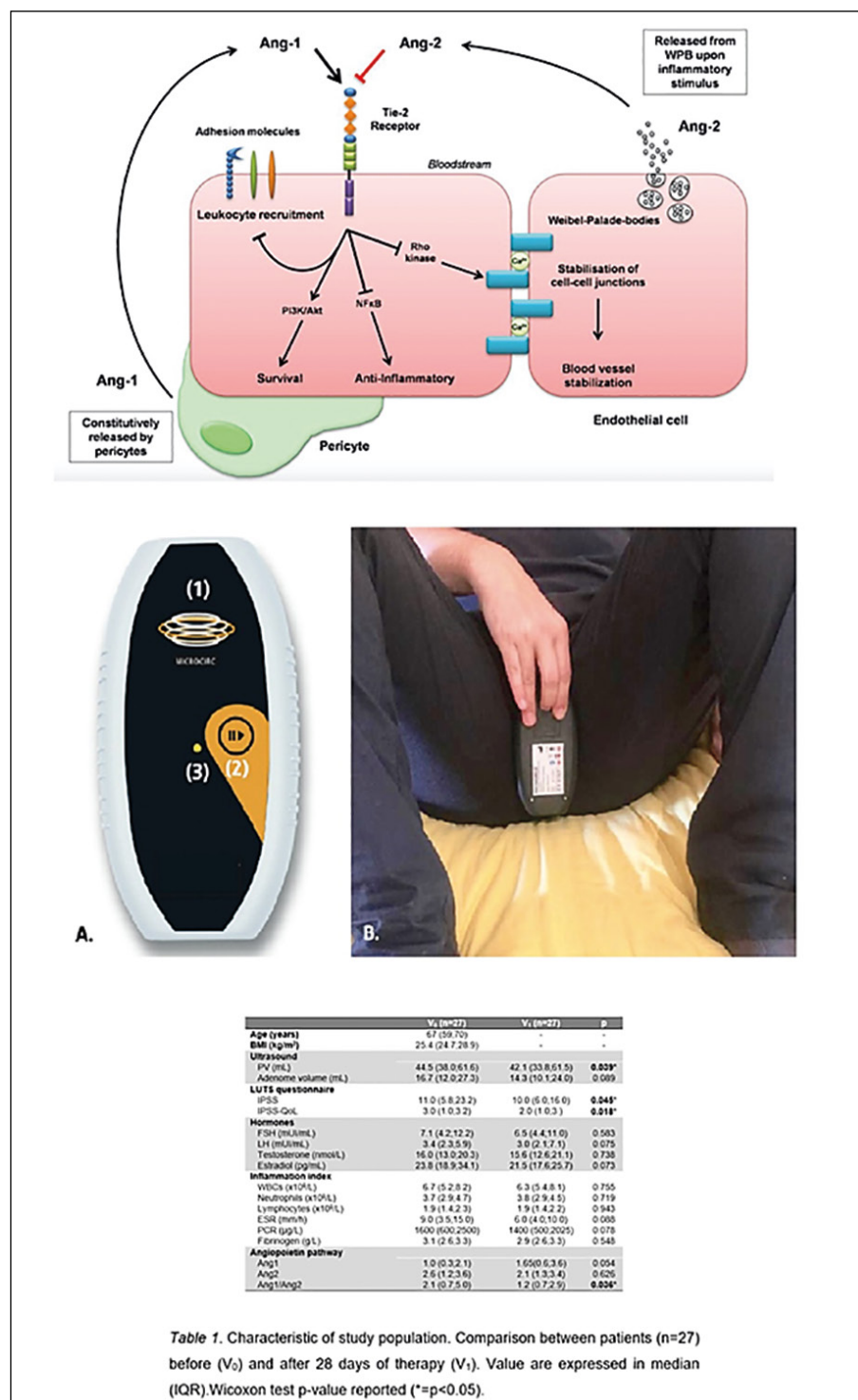


Table 1. Characteristic of study population. Comparison between patients (n=27) before (V_0) and after 28 days of therapy (V_1). Value are expressed in median (IQR). Wilcoxon test p-value reported (* $p < 0.05$).

Figure 8. M. G. Tarsitano, et al.

ter 28 days of PEMF treatment, Ang2/Ang1 reduces significantly, as PV and IPSS. Despite small sample size, these preliminary findings appear solid to identify Ang2/Ang1 as an effective serum marker of PEMF efficacy in BPH treatment (**Fig 8**: Angiopoietin pathway. Mod. from [van Meurs et al. Crit Care 2009; 13: 207]; Magcell® Microcirc, Physiomed Elektromedizin AG, Schnaittach, Germany set on frequency of 4–12 Hz and on an intensity of 1000 Gauss. A: (1) effective area; (2) start button; (3) status LED. B. Correct position with the marked active surface placed on the perineal region; Characteristic of study population. Comparison between patients (n = 27) before (V₀) and after 28 days of therapy (V₁). Value are expressed in median (IQR). Wilcoxon test p-value reported (* = p < 0.05).

P 7.11

Selection for female high fertility feedbacks on male reproductive performance

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The Dummerstorf high fertility mouse lines FL1 and FL2 are selected for increased litter size for more than 190 generations. Compared to the unselected control mouse line, which derived from the same genetic background, both fertility lines almost doubled the number of offspring per litter. Selection for a high litter size did not only increase the ovulation rate of FL1 and FL2, it is also an integral of other traits of female reproduction like folliculogenesis, uterine capacity and embryo survival. However, little is known about the effects of selection for exclusively female high fertility on the male side.

Many genes essential for reproduction performance are shared by both sexes in their reproductive organ. Hence, we assume that long-term selection (45 years) for female high fecundity also affected male reproductive performance. We found various alterations the concentration of sex steroids, sperm motility parameters and gene expression pattern in the testes. Our results indicate that FL1 and FL2 independently generated and justified their phenotype using different molecular strategies. In addition, FL1 and FL2 display some elevated male- as well as female-specific characteristics.

Therefore, these genetic heterogeneous mouse models are likely to provide novel signatures for increased reproductive performance and thus provide new insights into molecular and cellular complexity of highly fertile phenotypes.

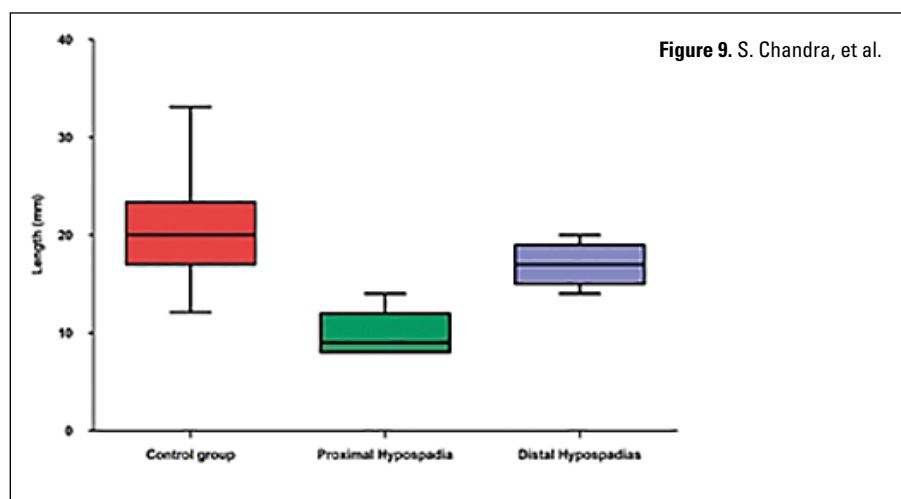


Figure 9. S. Chandra, et al.

P 7.12

Anogenital distance: a marker of severity of hypospadias and in-utero androgen production

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Introduction Hypospadias is a congenital anomaly in which the urethra opens onto the ventral aspect of the penis rather than at the tip. Anogenital distance (AGD) is an anthropometric measurement of genital development. Hypospadias, cryptorchidism, testicular germ cell tumor and low sperm counts constitute the Testicular Dysgenesis Syndrome (TDS) and have a common origin in early fetal life caused by an abnormality in the development of fetal testes. Genital development is programmed during a critical time period, “Male Programming Window” (MPW). Disruption of androgen action during this MPW results in hypospadias and reduced AGD. In this study, we intended to examine the association of AGD with proximal and distal hypospadias from all the newborn males.

Materials and Methods We examined all male newborns in our hospital from January 2015 to December 2019. The anogenital distance was recorded in millimeters (mm) with a sliding digital caliper, used for measuring the distance between the base of the scrotum to the center of the anus; defined as AGD.

Results During the above mentioned period, there were 28,426 (14,615 males and 13,811 females) full-term live births in our hospital. The mean AGD was 21.06 ± 5.57 mm in newborns without hypospadias, 9.92 ± 1.90 mm in newborns with proximal hypospadias and 17.03 ± 1.95 mm with distal hypospadias. The shorter AGD in newborn males with proximal hypospadias in comparison to distal hypospadias indicates AGD to be a marker of the severity of in-utero androgen production.

Conclusion Hypospadias is associated with reduced anogenital distance. AGD further

decreases with the severity of hypospadias and hence is a marker for in-utero androgen action. Since the TDS disorders originate from androgen disruption during the MPW, therefore an assessment of AGD is important in establishing testicular dysgenesis in newborns as these culminate in low fertility and testicular tumors in adulthood (**Fig. 9**).

P 8.1

Male oncofertility: Awareness, knowledge and barriers among oncologists in india

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Introduction Despite numerous developments in Oncofertility, there are barriers which hinder the successful establishment of a fertility preservation (FP) program. One of the barriers was found to be lack of knowledge in both healthcare providers and patient population worldwide, including India [Salama et al, JCO, Global Oncol 2020; 6: 379]. Hence, there is a need to assess the awareness and identify the barriers among healthcare providers to implement effective male FP program in the country.

Methods A nationwide survey was directed to oncologists attending various national conferences or academic meetings between May and November 2019 with questions related to male oncofertility services. Informed consent was taken at the beginning of the survey. The data was analysed by using descriptive statistics.

Results The response rate to the survey was 23% (49 of 214) from oncologists across various subspecialties. Pertaining to knowledge of male FP options, 52% of them reported adequate knowledge of sperm banking but only 25% of them had awareness regarding immature testicular tissue (ITT) freezing.

When asked about the time required for each of the procedures, 71% reported that it took < 1 week for sperm banking while 36% reported 2–4 weeks for TT freezing and 14–28% were not aware of the time taken for either of the procedures. The major barrier in oncofertility services was cited as, “financial burden” (82%) on the patient.

Conclusion The study demonstrated that majority of the oncologists agree on the importance of FP, however urged a need for oncofertility awareness programs. The survey also highlights the major barriers for effective utilization of oncofertility services in India. One of the limitations of the study is lack of details pertaining to the types of cancers treated by oncologists and the frequency of treating adolescent and young adult cancer patients.

P 8.2

MAGEC2 as a biomarker for testicular germ cell tumors

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Testicular germ cell tumors (TGCTs) are the most common malignancies among young men with increasing incidence leading to a need for biomarkers for early detection and diagnosis. TGCTs are divided into seminomas (SE) and nonseminomas (NS). *MAGEC2* expression is restricted to testis. However, moderate to high protein expression was seen in malignant neoplasms and was already reported as a sensitive marker for SE. Still, investigations of DNA methylation pattern has not been reported yet. We performed a comprehensive *in silico* bioinformatics analysis at the DNA methylation level and compared it with data of protein expression in SE and NS tissue.

In 108 TGCT samples, *MAGEC2* protein expression was analyzed by immunohistochemistry in healthy testicular seminiferous tubule tissue, GCNIS, TGCTs, SE, and all NS components. The immunoreactivity score (IRS) was calculated with a cutoff value of four for clinical diagnostic positivity. For the analysis of DNA methylation in TGCT, UALCAN was used.

MAGEC2 showed linear, decreasing trend of protein expression from healthy testicular tissue to GCNIS and to TGCTs, meaning it becomes suppressed with malignant progression. *MAGEC2* expression was significantly higher in SE than in NS. DNA methylation pattern was in line with *MAGEC2* protein expression, being significantly higher in NS than in SE.

Although significantly higher *MAGEC2* protein expression was detected in SE than in NS, according to our results *MAGEC2* expression was less sensitive for SE than previously reported. So, to consider *MAGEC2* as a TGCT diagnostic biomarker further investigation of expression and regulation at the DNA level is required.

Grants This study was supported by the Scientific Center of Excellence for Reproductive and Regenerative Medicine, Republic of Croatia and by the European Union through the European Regional Development Fund, under grant agreement No. KK.01.1.1.01.0008, project “Reproductive and Regenerative Medicine – Exploring New Platforms and Potentials

P 8.3

Immunopathology of testicular germ cell tumors – a clinical approach

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Despite the presence of complex immunosuppressive mechanisms contributing to immune privilege in the testis, recruitment of immune cells with pro-inflammatory properties is observed in testicular infections or testicular germ cell tumors (TGCT). Although seminoma (SE) and pre-invasive germ cell neoplasia in situ (GCNIS) are associated with immune cell infiltrates, infiltrate function and how they affect TGCT progression and prognosis, are unknown. As part of an ongoing prospective study, testicular tissue was obtained from 59 patients undergoing orchidectomy for TGCT (age 18–69 years, mean 25.7; SE n = 33; non-SE TGCT n = 7; mixed tumors n = 12; Leydig cell tumors n = 3; other pathologies n = 4). A comparative analysis of SE patients (n = 16) included Bouin-fixed and cryopreserved tissue from tumor-central, -adjacent and -distant sites. Histopathology showed considerable heterogeneity, displaying SE (n = 10), GCNIS (n = 7), preserved spermatogenesis/germ cell aplasia (HYP/SCO) (n = 13), or combinations thereof (SE+GCNIS n = 4, SE+GCNIS+HYP/SCO n = 2, SE+HYP/SCO n = 1, GCNIS+HYP/SCO n = 10). Using immunohistochemistry, cells positive for CD68, CD11c, CD3, CD4, CD8, and CD20, and markers for Treg and Tfh cells (CD25, FOXP3, CXCR5, BCL6) were mainly identified in GCNIS and SE. Semi-quantitative scoring revealed the highest numbers of immune cells, especially CD3+

T-cells, in tumor-central areas. Quantitative RT-PCR of selected cytokines/chemokines confirmed that TGCT is associated with a pro-inflammatory milieu. The clinical database of the study will allow tests for possible correlations between immunopathological patterns of TGCT and parameters of tumor stage and progression. By this comprehensive approach, we aim to decipher the role of “immune editing” during TGCT development and progression/metastatic behaviour. Results will help to identify novel prognostic factors and/or immune-therapeutic concepts for human TGCT.

Grant Supported by DFG GRK 1871/2.

P 8.4

Evidence of a dramatic alteration of the H4 acetylation pattern in testicular cancer patients prior to any treatment

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Introduction Histone H4 acetylation (H4ac) is a well-established epigenetic regulator of spermatogenesis in many species, particularly important during spermiogenesis for the histone-to-protamine transition. In this work, we aimed at gaining more understanding about this epigenetic mark through the study of its potential alterations in patients with testicular cancer.

Patients and Methods We performed immunohistochemistry in human testicular biopsies (n=24) in order to compare the H4ac pattern between patients diagnosed with 5 different types of testicular cancer displaying spermatogenic activity and control men with normal spermatogenesis.

Results We detected a drastic disruption of the H4ac pattern in all subgroups of testicular cancer patients assessed prior to any treatment as compared to controls. In particular, a global increase of the H4ac levels occurs in the seminiferous tubules adjacent to the tumour during the first stages of the spermatogenesis up to later-stages round spermatids, as well as in the somatic Sertoli cells. This pattern is markedly different to that found in controls, in which a gradual increase of H4ac with a maximum at elongating spermatids was detected consistent with previous reports.

Conclusion Our novel results suggest that, despite presenting spermatogenic activity, there is a global deregulation of H4ac in testicular cancer patients, which would potentially result in molecular alterations of the male gamete. The consequences of this epigenetic alteration might be relevant in testicular cancer patients expressing desire of fatherhood, since testicular cancer diagnosis is common during reproductive age. Future studies would reveal the actual risk and consequences towards the progeny underlying fertility preservation of epigenetically altered male germ cells, which is in many cases the only reproductive option available for testicular cancer patients.

Grants Supported by PI16/00346 to RO, CD17/00109 to JC, FI17/00224 to AI

P 8.5

miRNA in prostate cancer – (pre) analytical challenges

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Prostate cancer (PCa) is the most commonly diagnosed neoplasia among men. MicroRNAs (miRNAs) in liquid biopsies and tissue have emerged as potential biomarkers that could improve PCa diagnosis, prognosis, and management. With growing body of research, conflicting data was reported, and questions are being raised regarding diverse (pre)analytical factors influencing miRNA analysis, hindering its translation into clinical practice.

To analyze and address current problems in miRNA clinical research on PCa, a PubMed-based literature search was conducted. Diverse experimental designs and (pre)analytical factors influencing miRNA analysis were studied and compared across studies.

We observed that studies widely differ in design parameters such as control groups, serum and plasma comparison, sample storage, quality control steps and data normalization. There is an immense lack of data on methodology framing parameters used in published studies.

We propose following steps for improvement of future research: 1) control groups should be age-matched, preferably benign prostate hyperplasia patients; 2) sample collection and handling should be explained in detail: sample timing, centrifugation, hemolysis monitoring, storage conditions; 3) plasma and serum samples should be stored at -80°C up to 5 years before analysis, while FFPE blocks up to 10 years and data adjusted according to block's age; 5) both endogenous and exogenous miRNAs should be used for quality control; 6) miRNAs dysregulated in PCa should be avoided for data normalization, and MIQE guidelines adhered when publishing RT-qPCR results.

Grants This study was supported by the Scientific Center of Excellence for Reproductive and Regenerative Medicine,

Republic of Croatia, and by the European Union through the European Regional Development Fund, under grant agreement No. KK.01.1.1.01.0008, project “Reproductive and Regenerative Medicine – Exploring New Platforms and Potentials”).

P 8.6

The Persistent Müllerian Duct Syndrome (PMDS) in an adult – case report

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Introduction Paramesonephric (Müllerian) Ducts (MD) form uterus, fallopian tubes, upper 4/5 of the vagina. In the male fetus MD should disappear until the 9 week of pregnancy due to anti-müller hormone (MIS, müllerian inhibiting substance), produced by Sertoli cells of the fetal male gonads. The presence of MD is accompanied by the undescending testicles, inguinal hernia (*hernia uteri inguinalis*) with adequate developed penis, the presence of male gonads in the ovary position; sometimes accompanying external genital pseudohermaphroditism female form. Diagnosis is usually stated in the prepubertal period during the correction of hernias or cryptorchidism. Due to the risk of neoplasm of the dysgenetic testes and the MD should be removed.

Materials and Methods 45 yo patient with azoospermia, bilateral undescended testis, after bilateral inguinal hernia surgery; hypogonadism; glomerulonephritis; IgA nephropathy; chronic renal insufficiency. US: tumor of the right testis, mass in the left inguinal canal, no abnormalities in pelvis. Tumor were markers normal. Cryopreservation of testicular tissue was planned.

Results No evident tumor inside right testis, part of gonadal tissue was cryopreserved. In the left groin clinically oviduct with accompanying nodule (gonad in the oviductal position), uterus and vagina were found. Pathologist report: testis with the hypoplasia of the parenchyma (without spermatogenesis); Leydig cells hyperplasia; testicular ducts and vessels hypoplasia; the seminal cord tissue with a hamartomatic system; hypoplastic vagina and uterus, in the light covered with atrophic mucous membrane. A fragment of hypoplastic tissues of the uterine shaft with fallopian tube and a fragment of tissues with a hamartomatic system with a change of type: adenomatoid tumor. Diagnosis: The Persistent Müllerian Duct Syndrome.

Conclusions Every man with bilateral undescended testis should have diagnostics for PMDS due to the risk of neoplasia.

P 8.7

FXYD5/dysadherin is a new marker of prostate cancer progression and aggressiveness associated to epithelial-to-mesenchymal transition

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Prostate Cancer (PCa) is the 2nd most frequent cancer diagnosed in men. Among mechanisms involved, Epithelial to Mesenchymal Transition (EMT) stands up, and Epithelial Cadherin (CDH1) expression/function loss is an EMT hallmark. Decreased CDH1 was related to FXYD5/Dysadherin (FXYD5/Dys) expression, a poor prognosis and metastasis marker in other tumors. Expression of EMT markers and FXYD5/Dys in PCa and their association with tumor aggressiveness were evaluated using bioinformatics, cell models and tumor samples. Gene-disease association analysis (DisGeNET) identified CDH1 among PCa 50 top-genes, and mutation analysis (COSMIC) showed 2.54% CDH1 mutations (57/2243). EMT markers and FXYD5/Dys expression analysis done in human PCa cell lines (metastatic potential null/low: LNCaP/C4-2/22Rv1; medium/high: C4-2B/DU-145/PC3) revealed lower CDH1 mRNA levels and higher ZEB1, SLUG, N-cadherin and Vimentin in PCa with medium/high metastatic potential (P<0.05). PC3 cells showed higher (P<0.05) expression of Delta34-Ecadvar, an EMT-related novel CDH1 splice variant (2/57 mutations around splicing site). PC3 cells had higher (P<0.05) expression of FXYD5/Dys mRNA and a 50 kDa protein localized to the cell membrane/cytoplasm, and immunodetected in PC3 derived tumors in nude mice. PC3 cells treatment with a FXYD5/Dys siRNA resulted in reversed expression of EMT markers and higher cell-cell adhesiveness. A data set (GSE7930) analysis of low/high metastatic potential PC3 cell-derived tumors showed higher EMT markers and FXYD5/Dys expression in the more aggressive ones. A USCXena TCGA PCa data set analysis showed association between higher FXYD5/Dys expression and lower free disease survival, higher Gleason, Stage and Grade Group (P<0.05) and an EMT profile. A multiple regression analysis identified FXYD5/Dys as predictor of high Grade and Gleason score. These studies characterized FXYD5/Dys expression in PCa, and its relation with EMT markers, disease progression and aggressiveness.

P 8.8

Short anogenital distance is associated with testicular germ cell tumour development

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Introduction Testicular germ cell tumour (TGCT) is a multifactorial disease in which various genetic and environmental factors play a role. TGCT is part of the testicular dysgenesis syndrome (TDS) which includes also cryptorchidism, hypospadias, oligo/azoospermia and short anogenital distance (AGD). The relationship between AGD and different components of TDS has been assessed in a number of studies while information about short AGD and TGCT are not available in the literature. The role of AR (CAG)_n in the development of TDS disorders and in relationship with AGD are scarce and inconclusive. We aimed to evaluate AGD in relationship with TGCT and to assess the AR (CAG)_n polymorphism in relationship with AGD and TGCT.

Material and Methods 156 TGCT patients and 110 tumour-free normozoospermic controls of Spanish origin. All subjects underwent full andrological workup (including semen and hormone analysis) and genetic analysis (Androgen Receptor (CAG)_n). The main outcome measures were the anopenile distance (AGDap), the anoscrotal distance (AGDas) and AR(CAG)_n.

Results We observed significantly shorter AGD in the group of TGCT patients in respect to controls ($p < 0.001$) independently from sperm count and testis histology. Threshold values were calculated for AGD with the best sensitivity and specificity. Subjects with AGDap and AGDas below threshold showed a significantly increased risk for TGCT (OR = 4.97, 95%-CI = 2.01–12.33, $p = 0.001$ and OR = 4.11, 95%-CI = 1.89–8.92, $p \leq 0.001$, respectively). No significant correlation was observed between AR(CAG)_n polymorphism and AGDs. The median values of the AR(CAG)_n were similar between cases and controls.

Conclusions Ours is the first study focusing on AGD in TGCT patients. We identified short AGDs as a significant risk factor for this disease. After further validation of our preliminary data, AGD measurement could become part of TGCT screening in order to better define those individuals who would benefit from long-term active follow-up.

P 8.9

Leydig cell tumor presenting as azoospermia: Testis sparing surgery and micro TESE. A single center experience

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Introduction and Objectives Leydig cell tumor (LCT) is a relatively rare testicular tumor, consisting the most common non-germ cell testicular tumor, accounting for 1–3% of all testicular malignancies. Most LCTs are benign, but 10% of them are malignant. Typically, they present as a testicular mass, often associated with azoospermia. We present our single center experience on micro-TESE along with Testis Sparing Surgery (TSS).

Materials and Methods Three azoospermic patients underwent surgical exploration for testicular tumors and micro-TESE with TSS intent in our center during the last 9 months. One patient presented with a unilateral lesion and the other two bilateral lesions. All the patients had negative tumor markers, such as β -hCG, α FP, and LDH, elevated FSH and LH levels and borderline testosterone levels. Abdomen and thorax CT were normal. In all three cases an inguinal approach was used along with temporary cord occlusion and cold ischemia of the testicles. Under microscopic magnification and intraoperative ultrasound assistance all masses were enucleated. Frozen section (FS) analysis of the lesions and random biopsies were obtained, in order to exclude other pathologies of the testis, including ITNGCU. Benign findings allowed for TSS. In all three cases bilateral micro-TESE was performed, allowing spermatozoa extraction, confirmed by the embryologist in the operating room.

Results The enucleated lesions measured 0.7–1.2 cm in the largest diameter. All of the lesions were sex cord-stromal tumors, benign Leydig cell type with low Ki 67 index and TSS was accomplished in these cases. Complete concordance was observed between the results of FS and permanent sections. In all three cases spermatozoa were found and cryopreserved. Post-surgically none of the patients reported any complication, and their testosterone levels remain unaffected. One live birth has been reported after a successful ICISI.

Conclusions LCT consists 1–3% of all testicular tumors, with 90% of them being benign. Most of the cases appear unilateral, but 3–9% of them are bilateral. Since azoospermia can be associated with testicular malignancy in up to 15% of the cases it is crucial for the urologist not to overlook such a possibility, even if the tumor found is benign such as LCT. In well selected cases, organ sparing surgery can be performed simultaneously with micro-TESE, without compromising the oncological outcome.

P 8.10

Abnormalities in the sperm DNA methylome both pre- and post-treatment in men with Hodgkin's disease and testicular cancer

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Background Although combination chemotherapy has contributed to increased survival from Hodgkin's disease (HD) and testicular cancer (TC), questions about sperm quality after treatments have arisen. Studies have shown nuclear chromatin damage post-treatment, however, the sperm epigenome has received little attention. Our objectives were to determine the impact of HD and TC, as well as their treatments, on sperm DNA methylation.

Methods Sperm were collected from community controls (CC) and men with HD or TC (before and after chemotherapy treatments; $n = 6-7$). Sperm DNA methylation was assessed using genome-wide and locus-specific approaches.

Results Before treatment, using 450K arrays, a subset of probes distinguished sperm from TC, HD and CC subjects. On comparing altered sperm methylation between HD and TC patients versus CC men, twice as many sites were affected in TC than HD men; both groups demonstrated mostly hypomethylation. In TC patients, the promoter region of *GDF2* contained a large region of differential methylation. To assess alterations in DNA methylation over time/post-treatment, serial samples from individual patients were compared. With 450K arrays, following chemotherapy, patients showed increased alterations in DNA methylation, up to several years post-treatment, when compared to CC. Similarly, using a high resolution human sperm-specific assay, at includes assessment of environmentally-sensitive sites (dynamic sites), demonstrated altered sperm DNA methylation in patients post-treatment and suggested preferential susceptibility of dynamic CpGs.

Conclusions Distinct sperm DNA methylation signatures were present pre-treatment in men with HD and TC and may help explain increases in birth defects reported in clinical studies. Epigenetic defects in sperm of some cancer survivors are present up to two years post-treatment. Abnormalities in the sperm epigenome both pre- and post-chemotherapy may have detrimental effects on future health.

Grants Funded by CIHR

P 9.1

First night impotence dilemma in man: a Malaysian Study

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When the husband failed to do penetrative sex on the wedding night, the condition is known as first night impotence. If failure to perform penetrative sex persists, then the marriage is unconsummated. Considering the

husband's role in intimacy is vital, failure to do it might trigger marital crisis and eventually marital breakdown. The Men's Wellness Clinic handles male sexual and reproductive issues. Based on a one-year (2018) records, a total of 152 couples and husbands attended the Clinic. All the male partners registered as having difficulty in performing penetrative sex. Each male went through history taking, filling of (IIEF-5) questionnaire and scoring the penis hardness score scale. Majority of the males were virgins and they attempted penetrative sex with their spouses with book or internet sources about sex. Their usual experiences include rapid ejaculation before penetration, rapid flaccidity before penetration or loss of rigidity when the penis buckled on pressing a wall-like barrier at the entrance of the vagina. Further attempt at penetrative sex resulted in failure. Most of the male attendees had tried folklore and traditional medicines. Some even resorted to seeking help from local Samman believing they were charmed by evil spirits. Some have even attempted taking herbal capsules laced with PDE-5 inhibitors but failed in their sexual attempts. Based on the examinations, male attendees who were healthy and who got Grade 3–4 Penis Hardness score, with wives known to experience vaginal spasms, the wives were referred to the Gynaecologists. After self-vaginal dilatation and „reverse“ Kegel exercises, their sexual dilemma was overcome. Male attendees who were suffering from „performance anxiety“ were counselled and were given PDE-5 inhibitors. Male attendees who were found to have weak pelvic floor were taught Kegel's exercises to firm up the muscles and the penis during erection. For all these men first night impotence became a thing of the past.

P 9.2

Prevalence of self-reported sexual dysfunctions among male COVID-19 patients in Italy

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Introduction A growing body of evidence is supporting the notion that the novel coronavirus disease (COVID-19) due to SARS-CoV-2 might be an endothelial disease. Given the well-known role of endothelial dysregulation in the pathogenesis of male sexual dysfunctions, we aimed to investigate whether patients with a self-reported history of COVID-19 had worse erectile function than healthy controls, as measured by psychometric tools, once psychological symptoms have been ruled out.

Materials and Methods The Sex@Covid study was a case-control study was performed through a web-based survey between April 7 and May 4th, 2020 in Italy. 6821 questionnaires were collected (males: 2644, among

which sexually active: 985). By propensity score matching (PSM), we investigated whether prevalence of sexual dysfunction, as measured by IIEF score ≤ 21 , was different among COVID-19 patients once matched for age and prevalence of anxiety and depression (assessed by General Anxiety Disorder-7 [GAD-7] and Patient Health Questionnaire-9 [PHQ-9] validated questionnaires), using a 1:3 ratio.

Results Among the 985 sexually active men, 25 reported having contracted COVID-19. 100 men were therefore included in analysis following PSM (25 COVID-19 patients, 75 controls). No significant difference in age, BMI, GAD-7 and PHQ-9 scores were found following matching ($p = 0.39$, $p = 0.54$, $p = 0.41$ and $p = 0.21$, respectively). An overall 14% prevalence of erectile dysfunction (IIEF score ≤ 21) was found for the study population: COVID-19 patients had higher prevalence (7/25, 28%) than controls (7/75, 9.3%; $p = 0.0208$).

Conclusions Prevalence of sexual dysfunction is common in COVID-19 patients. As IIEF investigates erectile function, our findings corroborate the role of endothelial dysfunction due to COVID-19 in the pathogenesis of vasculogenic ED, even after matching for presence of psychological issues.

P 9.3

The physicians gender influences the results of the diagnostic workup for erectile dysfunction

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Background Despite the well-known influence of psychological and situational factors on erectile dysfunction (ED), the influence of the physician's gender on the andrological work-up has never been investigated so far. To investigate physician's gender influence on the erectile dysfunction (ED) diagnostic workup.

Methods Cross-sectional study with retrospective data collection. We evaluate a consecutive series of ED patients: 95 at the University of Modena and Reggio Emilia (UNIMORE) and 1808 at the University of Florence (UNIFI). In the UNIMORE cohort (Cohort 1), intracavernous injection (ICI) test was performed in case of suspected vascular pathogenic component. In the UNIFI cohort (Cohort 2), patients were evaluated by Structured Interview on Erectile Dysfunction (SIEDY) and ANDROTEST. Both cohorts were divided in 2 groups according to the gender of the physician who performed the ICI test or the structured interview.

Results In Cohort 1, patients who had the ICI test performed by a female physician had a significantly higher probability of ob-

taining a better ICI test response. In Cohort 2, patients interviewed by female physician more frequently reported to have a conflictual couple relationship and a reduced frequency of climax in their partners. However, they reported less difficulties in achieving and maintaining erection, higher frequency of autoeroticism, lower occurrence of ED during masturbation and lower impairment in morning erections.

Conclusions Physician's gender affects the results obtained during the ED diagnostic workup. Men interviewed by a female physician describe a less severe ED probably as attempt to defend their own virility. On the other hand, the presence of a male physician during ICI test is associated to a worse response suggesting a possible unconscious competition.

P 9.4

Shaeer's punch technique for trans-corporal excavation of Peyronie's plaques upon penile prosthesis implantation

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Introduction Cases with severe erectile dysfunction and severe Peyronie's deformity may require penile prosthesis implantation along with plaque surgery. The latter requires considerable additional operative time and adds to the possibility of infection. Shaeer's Punch Technique is a minimally invasive procedure for plaque de-bulking from within the corpora cavernosa, with low or no risk of urethral or nerve injury.

Patients and Methods The procedure was performed for 26 patients. Following corporotomy and dilatation, Peyronie's plaques were punched-out using the punch forceps. The punch forceps is a reusable metal instrument widely used to cut-out vertebral bony protrusions in neurosurgery. After Peyronie's plaques were cleared out, penile prosthesis implantation proceeded as usual.

Results Prior to excavation, deviation was evaluated in artificial erection and ranged from 40–80 degrees, average 68 ± 2 . Direction of curvature was dorsal in 11 patients, dorsolateral in 7 and ventral/ventrolateral in 8.

Following the Punch technique, all patients were clear of Peyronie's plaques as per palpation. Upon evaluation of deformity with the implant in the erect state, all patients had a straight penis. No adjuvant measures were required. Average additional operative time for Punch technique ranged from 5 to 10 minutes.

Twenty-five implants survived adequately, with no residual or recurrent curvature. No urethral complications or extrusions were noted. Penile sensitivity was preserved as per subjective reports.

Conclusions Shaeer's Punch Technique allows safe and expedited excavation of Peyro-

nie's plaques upon penile prosthesis implantation, obviating the need for mobilization of the neurovascular bundle or the spongiosum, and obviating the need for plaque excision and grafting.

P 9.6

Erectile dysfunction as an early marker of cardiovascular disease

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Background Cardiovascular diseases (CVD) have a very similar profile of risk factors with such a common reproductive disorder as erectile dysfunction (ED). Therefore, we performed several studies to define link between erectile dysfunction and social significant disease (CVD, diabetes, metabolic syndrome etc.).

Methods We present data from different studies performed in Research Institute of Urology from 2010. Totally more than 3000 patients were included. Patients filled the IPSS and IIEF-5 questionnaires. Anamnestic data, the results of anthropometry (height, weight, body mass index (BMI), waist circumference), and laboratory research methods: plasma cholesterol and glucose levels were recorded. Selected categories of patients underwent a comprehensive cardiologic evaluation. Sometimes we used methods of predictive analytics (CHAID algorithm, neural network) in addition to conventional statistics.

Results Prevalence of ED was 51.04%. In this case, a mild ED is observed in 38% of men, moderate and severe ED – in 10.5% and 2.5%, respectively. After applying the algorithm of CHAID decision tree, we found that the most significant risk factor for ED occurrence is the severity of LUTS (an importance factor of 0.64), then with a significant lag follow waist circumference (0.08), cholesterol level (0.05), hypertension (0.05). Accuracy of the ED prediction using a trained neural network was 81.9%. Predictors of arterial hypertension development were pulse frequency, severity of LUTS age and body weight. The use of predictive analytics methods allowed generating an algorithm for predicting the development of cardiovascular diseases with an accuracy of 80.3%.

Conclusion Using erectile dysfunction as an early marker of cardiovascular diseases, urologist can not only improve the quality of life of a patient with erectile dysfunction, but also save his life

P 9.7

Circumferential acquired macro-penis: definition and proposal of a geometrically-based reduction corporoplasty

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Introduction In the medical literature there are scattered but recurrent reports of penises too wide to allow effective penetration. Our purpose is both: to define a new medical entity, the “circumferential acquired macro-penis”: development over time of a penile girth enhancement that mechanically hampers penetration, and to propose a mathematical model to perform a geometrically-based reduction corporoplasty.

Patients and Methods A recent case of acquired penile circumferential widening prompted us to a Medline PubMed and a Google search literature review of similar cases.

Results We identified seven published cases of acquired penile circumferential widening; in five ones intercourse was hindered by the shaft deformation. In the majority of cases priapistic episodes were deemed the causal factor of the morphologic anomaly; in other cases, including ours, a clear etiology could not be identified. Surgically treated cases underwent bilateral elliptical albuginea excisions. Workup in our case included: dynamic infusion cavernosometry and cavernosography (figure) that ruled out vascular erectile problems, and dynamic Magnetic Resonance Imaging that depicted an aneurismatic dilatation of both corpora cavernosa. We developed a mathematical model to precisely define the amount of tunica albuginea to be resected. We performed accordingly a geometrically sound reduction corporoplasty. A thinned albuginea was present in the affected area; in order to prevent possible recurrences we applied a bovine pericardium patch above both corpora from one paraurethral margin to the other. Follow-up is ongoing; at two months patient resumed complete intercourse activity, with full patient/partner satisfaction at 11 months.

Conclusions Circumferential acquired macro-penis is a rare but defined medical entity that prevents affected men from sexual penetration. We propose a mathematical model to perform a sound geometric surgical correction. Follow-up is encouraging (Fig. 10).

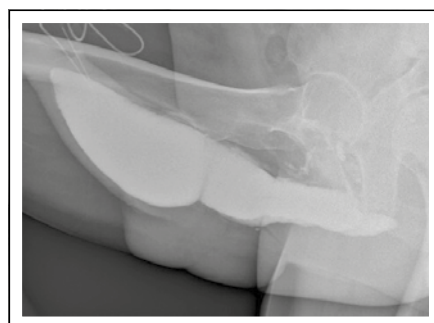


Figure 10. E. Pescatori, et al.

P 9.8

The role of family history of cardiovascular disease in andrological patients in the late ontogenesis

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Introduction Cardiovascular diseases (CVD) are the most common diseases in late ontogenesis and one of the most frequently associated comorbidities condition in andrological patients. A special role in the occurrence of CVD is given to family anamnesis. Several evidences established a link between erectile dysfunction (ED) and CVD. It is interesting to investigate the association of ED and family history of CVD.

Patients and Methods The study included 46 patients 44 to 86 years old (mean age $\pm$ SD 61 ± 10.1) who attended an andrology outpatient clinic complaining of ED. Men were classified as have a positive family history of CVD – group 1 ($n = 23$) (60 ± 8.3 years) or patients without family history of CVD – group 2 ($n = 23$) (60 ± 10.6 years). The clinical and laboratory surveys including International Prostate Symptom Score (IPSS), Aging Male Symptom (AMS) scale and International Index of Erectile Function (IIEF-5) score were investigated. Family history of CVD was defined as having a first-degree relative with a cardiovascular event such as coronary heart disease or ischemic stroke before the age of 60 years for female and 55 years for men.

Results 1.) The comparison of the andrological parameters show that, aging male symptoms were more pronounced in the sample of patients with positive family history of CVD ($p < 0.05$), where the mean of AMS score was 44.6 ± 11.8 , and 37.7 ± 7.3 in second group. As well there was a statistical difference in IPSS ($p < 0.05$) 10.4 ± 7.9 and 5.6 ± 4.1 , respectively. But there were no significant differences between total testosterone levels and the IIEF-5 score.

2.) While comparative anthropometric measures didn't show any significant differences: BMI in the first group was 26.5 ± 2.9 kg/m², and 27.9 ± 2.8 kg/m² in the other group, waist circumference was 102.2 ± 11.40 cm and 98 ± 7.7 cm, respectively.

Conclusion Family history of CVD a good predictor of the severity of clinical manifestations in andrological patients.

P 9.9

Long term penile perception scores and sexual health outcomes in patients treated for exstrophy bladder

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Introduction and Objectives Children born with bladder exstrophy (CBE) have achieved

a near normal life-expectancy over the past four decades. These patients undergo a number of reconstructive procedures to achieve closure of the exstrophy, gain continence and treat genital anomalies. All these procedures have a profound impact on the physical appearance, psychological and sexual well-being of the patients. As these patients grow, sexual function and fertility become an increasingly important aspect of their day-to-day lives. We prospectively assessed patients with CBE having undergone treatment at our centre previously (at-least 5 years prior) and were 18 years or more in relation to their sexual function and fertility status.

Materials and Methods Male patients 18 years of age and having undergone surgical treatment at our centre for CBE (at-least 5 years previously) were prospectively assessed for sexual function and fertility status. The patients were asked to answer validated questionnaires, including the Sexual Health Inventory for Men (SHIM), Penile Perception Score (PPS), and Male Sexual Health Questionnaire for assessing ejaculatory dysfunction.

Results and Observations During the period Jan. 2019 – Dec. 2019 a total of 28 male patients previously operated for exstrophy of bladder attended the Urological services. Penile perception scores ranged from very satisfied to very dissatisfied. Nineteen (67.85%) patients were sexually active and only three patients gave history of antegrade ejaculation.

Conclusions Patients operated for bladder exstrophy present with sexual and relationship concerns similar to the population at large. Though not unique, these concerns need to be addressed on a case to case basis.

P 9.10

Autophotography underestimates clinical extent of Peyronie's disease for penile angulation assessment

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Objectives Since Kelami introduced at home autophotography (AHP) for evaluation of penile curvature in 1983, standardized photography of the patients' phallus in full erection is considered a tool for objective penile deformity assessment. With several conservative and surgical treatment options available today, evaluation of precise degree of angulation is essential for deciding further treatment strategy. Our study evaluated AHP to intravaginal alprostadil injections (ICI) prior treatment decision.

Methods We performed a prospective study of 55 consecutive patients with Peyronie's disease prior receiving treatment. All patients provided standardized AHP from in front,

lateral and craniocaudal position of the fully erect phallus. Clinic based assessment ICI (10ug) with angulation measurement and an erectile function questionnaire (IIEF-15). A paired-samples t-test was calculated to compare the mean degree of curvature using photos to the mean degree of curvature using alprostadil injection.

Results The mean degree of curvature using AHP was 46.7° (sd = 12.6), and the mean degree of curvature using ICI was 54° (sd = 16.7). A significant increase in angulation from AHP to ICI was found ($p < 0.001$). A subgroup analysis was conducted on patients with a curvature using photos of less than 50° also showed significant increase in angulation from photo to alprostadil ($p < .001$). A multiple linear regression was calculated predicting the difference in the curvature based on patients' age and IIEF. The regression equation was not significant ($p > 0.05$).

Conclusions Homebased autophotography of the erect phallus underestimates the degree and extent of curvature. Neither IIEF nor age are a significant predictor of the difference in AHP and ICI induced erection. Clinic based alprostadil injection should be performed for assessment prior planning treatment strategy, especially prior surgical correction.

P 9.11

Collagenase Clostridium histolyticum for the treatment of Peyronie's disease: a prospective 24 month follow up study

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Objectives Collagenase Clostridium histolyticum (CCH) injections are the only licensed medical treatment for Peyronie's disease (PD). Only few studies are available assessing long term follow up in the current literature. Our study evaluated complications and functional outcomes during a 24 month follow up after therapy.

Patients and Methods Baseline, treatment and follow-up (FU) data of 34 consecutive patients treated with CCH using the London protocol (6 intralesional injections of CCH 0.9 mg at 4-weekly intervals in addition to home modelling) were assessed. International Index of Erectile Function (IIEF), PD questionnaires (PDQ), and autophoto-documentation were performed at baseline and at 3, 6, 9, 12 and 24 months to evaluate degree of curvature and functional outcomes.

Results Median age was 58 ys (53; 65.2). Median penile length was 13 cm (12.5; 14) and median degree of curvature was 55° (38.7°; 70°). PDQ score before treatment was 31 (23; 42.3), median IIEF prior treatment was 46.5 (34; 56). Follow up data at 3, 6,

9, 12 and 24 months revealed a statistically significant mean reduction in degrees of curvature (3 mo: -11.7, $p < 0.0001$; 6 mo: -14.1, $p < 0.0001$; 9 mo: -16.9, $p < 0.0001$; 12 mo: -20.5, $p < 0.0001$; 24 mo: -23.1, $p < 0.0001$), PDQ (-8.9, $p < 0.0001$; -10.7, $p < 0.0001$; -12.1, $p < 0.0001$; -13.3, $p < 0.0001$; -14.9, $p < 0.0001$) and IIEF (5.47, $p = 0.002$; 4.12, $p = 0.011$; 5.35, $p = 0.007$; 6.38, $p = 0.004$) from baseline. Common side effects included minor pain upon injection (59%) and minor local hematoma. No allergic reaction, rupture of tunica albuginea or other severe AE were observed.

Conclusions The treatment with CCH injections for PD has a lasting positive impact on degree of curvature, patient's erectile function and quality of sexual life scores over more than 24 months. Pain is a common adverse event of CCH injections and should be prevented by penile block prior CCH injection. CCH therapy can be considered a lasting, effective and safe for treatment of PD.

P 10.1

Adenosine is a pro-inflammatory molecule in human testicular peritubular cells

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Human testicular peritubular cells (HTPCs), together with extracellular matrix, form a compartment surrounding the seminiferous tubules of the human testis. HTPCs are involved in sperm transport, cell-cell communication and have immunological roles. In many cases of impaired spermatogenesis, the architecture of the wall and the phenotype of HTPCs change, implying a role of these cells in male (in)fertility. Extracellular ATP acts as a danger molecule and is involved in promoting a pro-inflammatory environment. A previous study in HTPCs indicated such actions after treatment with ATP [Walenta et al., Sci. Report, 2018]. ATP can, however, be degraded by the two ectonucleotidases ENTPD1 and NT5E, which are expressed by HTPCs in situ and in vitro. Their functionality was confirmed in vitro (Malachite Green assay) and inhibition of ENTPD1 (by POM-1) also reduced the increase of pro-inflammatory cytokine levels evoked by ATP treatment. Hence, metabolites of ATP (ADP, AMP and adenosine) are likely involved. We focused on adenosine (A), because it showed strong pro-inflammatory actions and elevated levels of several cytokines in HTPCs. It exerts its actions via four ADORA receptors (A1, A2A, A2B and A3). A2B is strongly expressed at mRNA level in HTPCs and we found expression of A2B in corresponding cells of human testicular sections, notably in fibrotically thickened peritubular walls. Activation of the A2B receptor in HTPCs, using the specific A2B agonist BAY60-6583,

resulted in significantly elevated transcript and protein levels of IL6 and CXCL8, a result also obtained with A. However, while A elevated levels of CCL7, BAY60-6583 did not. This indicates that in addition to A2B, other ADORA receptors are also involved. These results pinpoint A as a pro-inflammatory factor in HTPCs and indicate that interference with the generation of ATP-metabolites can reduce inflammatory effects in HTPCs.

Grants Supported by DFG MA1080/23-2.

P 10.2

Regulation of macrophage responses in bone marrow-derived and cell line macrophages by activin A: implications for testis immunology

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Regulation of the anti-inflammatory phenotype of the testicular macrophages is not well understood. The immunoregulatory cytokine, activin A, which is highly expressed by several testicular cell types, including the Sertoli cells, could potentially contribute to this regulation. However, activin has been reported to exert both pro- and anti-inflammatory effects in different studies. The possibility that the effects of activin are dependent on the origin and activation state of the macrophage was investigated by comparing primary CSF1-matured bone marrow-derived macrophages (BMDMs) with two macrophage cell lines: immortalised bone marrow-derived macrophages (iMACs) and RAW264.7 macrophages. In BMDM cultures treated with bacterial lipopolysaccharide (LPS), activin A enhanced expression of pro-inflammatory markers, such as *Tnf*, but decreased their expression in the cell lines. By contrast, activin inhibited the expression of the anti-inflammatory cytokine, *Il10*, in response to LPS, but increased expression of the anti-inflammatory macrophage marker *Arg1* in all three cell types. Furthermore, preliminary examination of immunometabolic changes of activated BMDMs and iMACs by Seahorse oxygen consumption analysis indicates that activin may regulate the metabolic switch from glycolysis (inflammatory) to oxidative phosphorylation (anti-inflammatory) in immortalised cells. In summary, these studies indicate that the effect of activin is downstream of inflammatory signalling induced by LPS, exerts complex effects on different gene responses, and that the effects of activin on inflammatory activity are dependent on the maturation and activation status of the macrophage under investigation. Further studies of the differences between the responses of

macrophages under different maturation and activation states will elucidate the role that activin A plays in regulating the functional phenotype of tissue-resident macrophages in the testis and other organs where activin is produced.

P 10.3

Do G protein-coupled estrogen receptor (GPER) and peroxisome proliferator-activated receptors (PPARs) regulate lipid metabolism and steroidogenesis in Leydig cell tumors?

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Introduction In recent years, a marked increase in the incidence of Leydig cell tumor (LCT; leydigioma) has been reported, however the molecular and biochemical characteristics of LCT is scarce [Gheorghisan-Galateanu, BMC Res Notes 2014; 7: 656]. Adequate hormonal balance within the testis plays a pivotal role for blocking hormone-secreting LCT development. The study aims to determine a potential link between GPER and PPARs (α , β , γ) in LCTs, and involvement of this link in lipid homeostasis.

Material and Methods Residual tissues from testicular biopsy were collected from azoospermic patients (31–45 year-old; n = 24). Scanning electron microscopy and routine hematoxylin-eosin (H-E) staining were carried out to analyze general structure and morphology of LCTs. Protein levels of GPER, PPAR α , PPAR β , PPAR γ and LHR (lutropin receptor), PKA (protein kinase A), PLIN1 (perilipin 1), HSL (hormone-sensitive lipase), StAR (steroidogenic acute regulatory protein), TSPO (translocator protein), HMGCS and HMGCR (HMG-CoA synthase and reductase) were detected by western blotting.

Results Leydigioma tumors were compact with tumor cells closely apposed and tightly adhering to one another. H-E staining revealed a mixture of four cell types. Most cells were polygonal with abundant cytoplasm, indistinct cell borders, and prominent nuclei. In LCTs, increased expression of GPER and decreased expression of PPARs (α , β , γ) were detected compared to controls. Moreover, expression levels of LHR, PKA, PLIN1, StAR, HMGCS, HMGCR were altered while no changes in HSL and TSPO levels were detected when compared to Leydig cells in control samples.

Conclusion Changes in the expression of steroidogenic- and lipid balance-regulating proteins indicate functional interplay between GPER and PPAR-mediated signaling

likely controlling and/or affecting lipid metabolism and steroidogenesis in LCT.

Grants Supported by a grant 2016/23/B/NZ4/01788 (OPUS12 from the National Science Centre, Poland

P 10.4

Indolamine deoxygenase-1 (*Ido-1*) expression and the effects of follistatin treatment in murine autoimmune epididymo-orchitis

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Epididymo-orchitis can cause chronic pain and infertility. The immunology underlying this condition is poorly studied. An important factor may be *Ido-1*, a tolerogenic enzyme highly expressed in the caput epididymis. *Ido-1* is regulated by activin A, a pro-inflammatory cytokine. Its binding protein, follistatin (FST), inhibits Activin A. We examined *Ido-1* expression and the effects of exogenous FST treatment in a murine model of experimental autoimmune epididymo-orchitis (EAEO).

Adult C57/Bl6 mice were immunised with mouse testicular homogenate in complete and incomplete Freund's adjuvant, along with *Bordetella pertussis* toxin. Controls received adjuvant only, or were untreated. Prior to immunisation, some mice were injected with a non-replicative recombinant adeno-associated viral vector carrying a gene cassette for FST, which raised peripheral FST levels 5-fold, or an empty vector as control. Tissues were analysed 30 and 50 days after the first immunisation.

The cauda epididymis is highly susceptible to inflammatory damage. *Ido-1*, which is expressed at relatively low levels in the normal testis and cauda epididymis, was not increased in orchitis, but increased in the cauda in proportion to the severity of inflammation. In the cauda, immune cell markers such as *CD45*, *F4/80* and *CD80* were increased with increasing severity of epididymitis. Exogenous FST, which has been shown to lower the severity of orchitis in this model, reduced the expression of *Ido1* and immune cell genes such as *Cx3cr1* and *CD80* in the cauda during epididymitis.

The data indicate that *Ido-1* expression is selectively increased in the cauda epididymis during EAEO. This may be due to differences in the immunological environment and functional role of *Ido-1* within the testis and different epididymal regions. Exogenous FST reduces inflammation and the subsequent increase in *Ido-1* expression in the cauda epididymis, indicating its therapeutic potential for treatment of this disease.

P 10.5

Testicular dysgenesis syndrome has a stem cell basis

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Incidence of infertility and testicular cancers has increased in young men in recent times with decreased sperm count. The underlying etiology leading to these defects remains to be understood. Testicular dysgenesis syndrome (TDS) is suggested to have a genetic basis and possibly arises due to adverse environmental influences. Being immortal, altered biology of testicular stem cells due to perinatal disturbances may result in adult onset of diseases including cancer.

Testes harbors two populations of stem cells including pluripotent very small embryonic-like stem cells (VSELs) and spermatogonial stem cells (SSCs). VSELs can be isolated from single cell suspension obtained after enzymatic digestion by first spinning at 200–300g which allows the majority of cells to pellet down. However, the stem cells remain buoyant at this time and can be enriched by further centrifuging the supernatant at 1000g. VSELs are 2–6 µm in size, express pluripotent markers (*Oct-4A*, *Sox2*, *Nanog*, *Stella*, *Fragilis*) and can be enumerated by flow cytometry as viable LIN-CD45-SCA1+ cells. Co-expression of OCT-4 with ERα and ERβ makes them directly vulnerable to endocrine disrupting chemicals.

Treatment effects of diethylstilbestrol (DES, 2 µg/pup/day on days 1–5) were studied in adult mice on D100. Spermatogenesis was disrupted associated with altered ploidy status, reduced sperm and infertility. This was associated with 7 folds increase in VSELs numbers and marked reduction of c-KIT positive spermatogonial cells by flow cytometry and confirmed by qRT-PCR. NP95 (chromatin remodeling agent) expression was disrupted suggesting altered epigenetic status of stem cells by endocrine disruption possibly results in their excessive self-renewal and blocked differentiation. Nine of ten mice treated with DES had signs of testicular tumor with markedly increased expression of embryonic markers (OCT-4, SSEA-1). To conclude, stem cell basis for TDS is delineated for first time and VSELs initiate cancers.

P 10.6

A journey into the testicular tissue of cryptozoospermic patients using single cell RNA sequencing analysisS. Di Persio¹, T. Tekath², L. M. Siebert-Kuss¹, J. F. Cremers³, J. Wistuba¹, X. Li⁴, G. Meyer zu Hörste⁴, H. Drexler^{4,5}, M. Dugas², S. Kliesch², S. Schlatt¹, S. Laurentino¹, N. Neuhaus¹¹University Hospital of Münster, Centre of Reproductive Medicine and Andrology, Münster, Germany;²University Hospital of Münster, Institute of Medical Informatics, Münster, Germany; ³University Hospitalof Münster, Centre of Reproductive Medicine and Andrology, Department of Clinical and Surgical Andrology, Münster, Germany; ⁴University Hospital of Münster, Department of Neurology, Institute of Translational Neurology, Münster, Germany; ⁵Max Planck Institute for Molecular Biomedicine, Bio-analytical Mass Spectrometry Unit, Münster, Germany

In the last years, single cell RNA sequencing (scRNA-seq) of human testicular cells has allowed to characterize different spermatogonial subpopulations, to identify molecular footprints of each meiotic step, to describe transcriptional changes during spermatid development, and to classify the spectrum of somatic cells in the human testis. Despite the important breakthroughs, this approach had not been applied to study the testicular tissue of patients with impaired spermatogenesis. In the current study, we performed scRNA-seq of about 30,000 cells from cryptozoospermic and obstructive azoospermic men (n = 3 each) undergoing therapeutic testicular sperm extraction. Using 52 published markers we identified 9 different germ cell stages and 6 testicular somatic cell subpopulations. Comparing the proportion of the different cell types in the two groups, we found no change in the number of spermatogonia and early spermatocytes. However, the cryptozoospermic samples displayed reduced numbers of the most advanced germ cells (from pachytene spermatocytes onwards) and increased numbers of somatic cells. Furthermore, differential gene expression and gene ontology analyses allowed the identification of two additional somatic cell types in the cryptozoospermic group, specifically the perivascular and immune cells. To unravel the molecular regulators of the different germ and somatic cells and the potential alterations in the cryptozoospermic group, we performed gene regulatory network analysis and identified 403 transcription factors regulating up to 2,450 genes each. Thirteen of these regulatory networks were differentially activated between the two datasets, with 11 specifically altered in the germ cells and two in the somatic cells. Overall the use of this new technology allowed us to highlight the cellular and molecular alterations in this patient cohort despite their heterogeneous testicular tissue composition.

P 10.7

Delta-like and Jagged proteins are regulated by androgens in mouse Sertoli-cells in vitroA. Hejmej, A. Kamińska, S. Marek, M. Brzoskwinia, B. Bilińska
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Introduction Notch pathway mediates contact-dependent intercellular signaling, which plays important role in spermatogenesis [Parekh, et al. FASEB J 2019; 33: 8423–35]. Delta-like (DLL) and Jagged (JAG) proteins are transmembrane ligands activating Notch pathway in neighboring cells [Bray. Nat Rev

Mol Cell Biol 2016; 17 722–35]. Our recent findings show that androgens control the expression of Notch pathway components in vivo [Kamińska, et al. Reprod Biol Endocrinol 2020; 18: 30], however molecular mechanisms of this regulation are not known. The study was aimed to explore the role of nuclear (AR) and membrane (ZIP9) androgen receptors in the effect of testosterone (T) on DLL1, DLL4 and JAG1 expression in Sertoli cells.

Material and Methods TM4 (AR+, ZIP9+) and 15P-1 (AR-, ZIP9+) Sertoli cell lines were treated with T or transfected with AR siRNA or ZIP9 siRNA to knockdown androgen receptors. Real-time RT-PCR, western blot and immunofluorescence were used for analyses of mRNA and protein expression of DLL1, DLL4 and JAG1.

Results DLL1 mRNA and protein expression was reduced by T only in TM4 cells (p < 0.001), whereas AR knockdown abolished this effect, which indicates that AR is involved in the regulation of DLL1 by androgens. Testosterone decreased JAG1 expression in both cell lines (p < 0.05; p < 0.001), but only ZIP9 knockdown abrogated T impact on JAG1. This points to a role of ZIP9 in the control of JAG1 expression. Testosterone increased DLL4 (p < 0.05; p < 0.01; p < 0.001) in TM4 and 15P-1, but this effect was independent on AR or ZIP9.

Conclusion In Sertoli cells androgens acting via various signaling pathways regulate the expression of DLL and JAG ligands. Our results demonstrated that activation of the AR is involved in the control of DLL1 expression, while ZIP9 signaling influences JAG1 expression. The mechanism of DLL4 regulation remains to be elucidated in further studies.

Grants Supported by a grant 2017/25/B/NZ4/01037 (OPUS13, National Science Centre, Poland)

P 10.8

Initiation of testicular differentiation in prepubertal marmoset (*Callithrix jacchus*) testicular tissue using an in vitro organ culture systemS. Sharma, R. Sandhowe-Klaverkamp, J. Wistuba, S. Schlatt
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The current study aims at achieving *in vitro* maintenance and differentiation of primate germ cells, using testicular tissue from prepubertal marmoset (*Callithrix jacchus*) by employing a gas-liquid interphase culture system. Ethical approval for the use of marmosets was obtained according to German federal law. Testis from three-prepubertal marmosets (co-placental triplet) were used. Monkeys received a deadly overdose of pentobarbital, their body weights were recorded and blood was collected for serum testosterone analysis. Left-testis from each monkey was de-capsulated and the parenchyme dissected. Tissue

fragments (approximate size 1–3 mm³) were cultured on polycarbonate trans-well membrane inserts (8 µm pore size) at 35°C and 5 % CO₂ for up to 12-days in two-different conditions: “basic-culture” and “complex-culture” (supplemented with FSH and hCG). Cultured fragments were harvested at day-12 and fixed in Bouin’s solution for histology. Both, pre-culture control and cultured samples were analysed using Periodic Acid-Schiff (PAS) and immunohistochemical staining for evaluating testicular maturation. Results from complex-culture fragments demonstrated improved maintenance of structural integrity, testicular organization, and epithelial arrangement compared to samples cultured under basic conditions. Immunohistochemical characterization demonstrated more advanced testicular maturation status in fragments cultured in complex media compared to basic media and controls, i.e., improved epithelial arrangement of Sertoli cells (SOX9+), encapsulation of cultured fragments with peritubular-myoid cells (αSMA+), localization of spermatogonial stem cells (MAGEA4+) and presence of pachytene spermatocytes (Boule+) indicating initiation of meiotic transition *in vitro*. This model represents a novel *ex vivo* approach to explore the functional activation of primate testis during crucial developmental phases.

P 10.9

Presence and distribution of mast-cells in the testis and epididymis of ten species

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Mast cells play a vital role in the intermediate type of hypersensitivity reaction, fibroblast activation and collagen synthesis in most human tissues and organs. They inhabit the interstitial tissue of humans. Testes from the fetal period onward and are also known to play a significant role in human testicular pathology, surpassing the blood-testis barrier. Several studies have reported the absence of mast cells in the mouse testis and the results on rat testes are strain-dependant. *Ex vivo* studies of mast cells in the testis are non-existent, so it is important to find an animal model. Our goal is to determine the presence and distribution of mast cells in the testes and find an animal species that could be used as an optimal experimental model for mast cell investigation. We included human, mouse (C3H), rat (Fischer), cat, serval, dog, stallion, fox, wild boar and bull archive FFPE samples. Testes and epididymides of ten species were immunohistochemically stained using antibodies against mast cell tryptase and chymase (sc-59587, sc-59586, respectively, Santa Cruz Biotechnolo-

gy, SA, CA, USA). Mast cell tryptase-positive cells were found in human, wild boar and stallion testis and human, rat, dog and wild boar epididymis. All species, except fox and cat had positively stained stereocilia of the ductal pseudostratified columnar epithelium in the epididymis. Chymase-positive cells were found only in human testis and epididymis. Additional animal species should be further analysed, including several rat strains, to determine the optimal experimental model for mast cell investigation.

Grants This study was supported by the Scientific Center of Excellence for Reproductive and Regenerative Medicine, Republic of Croatia, and by the European Union through the European Regional Development Fund, under grant agreement No. KK.01.1.1.01.0008, project “Reproductive and Regenerative Medicine – Exploring New Platforms and Potentials”.

P 10.10

Comparative analysis of apoptosis and proliferation in teratocarcinoma and the experimental teratocarcinoma *in vivo* model

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Teratocarcinoma (TCa) is a type of testicular germ cell tumor composed of teratoma and embryonal carcinoma (EC). Experimental mouse TCa are obtained by transplanting gastrulating mouse embryos underneath a kidney capsule of a syngeneic animal. While the model histologically corresponds to human TCa, their molecular signature remains obscure. The aim of present study is to compare proliferative and apoptotic activity on the protein level in human TCa and mouse experimental TCa in order to assess growth similarity.

In total, 20 experimental TCa obtained 4 weeks after transplantation and 28 human TCa were used. Three surrounding human non-tumor testicular tissue samples and five mouse testes were used as respective controls. We have semi-quantitatively assessed proliferation and apoptosis by immunohistochemistry (IHC) and Western blot (WB) using antibodies against proliferation marker PCNA and apoptotic marker caspase-3.

WB analysis found no statistically significant difference in PCNA and caspase-3 expression between TCa and their controls. In contrary to PCNA, IHC analysis for overall caspase-3 found statistically significant difference between human and mouse TCa. Human TCa exhibited higher caspase-3 staining, predomi-

nantly in EC component, while EC component in mouse TCa was mostly unstained.

Proliferative activity of human and mouse TCa correspond and exhibit the same tissue localization while their apoptotic activity significantly differs in the EC component. This discrepancy could be explained by different microenvironment and requires more detailed investigation of the models behavior.

Grants This study was supported by the Scientific Center of Excellence for Reproductive and Regenerative Medicine, Republic of Croatia, and by the European Union through the European Regional Development Fund, under grant agreement No. KK.01.1.1.01.0008, project “Reproductive and Regenerative Medicine – Exploring New Platforms and Potentials”.

P 10.11

Formation of the testicular immunological barrier through immune modulation by somatic cells

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The blood-testis-barrier (BTB), which is based upon Sertoli cells (SCs), divides the seminiferous epithelium into basal and adluminal compartments. The main role of the BTB is to form an immunological barrier in order to preserve the meiotic and post-meiotic stages of the germ cells from the immune system. The BTB is composed of a number of tight junction proteins, mainly the claudins family. Disorder of the BTB’s integrity caused by any internal or external factors might result in infertility in males. Even if the SCs are absent, only few immune cells enter the seminiferous tubules when peritubular cells (PTC) are present. This suggests that other cell types or factors contribute to the testicular immunological barrier (TIB).

Our study is aimed at elucidating the role of different cell combinations (mainly SCs and PTC) on the BTB integrity and to elucidate the contribution of each cell type to the TIB. Furthermore, we aimed to treat rat primary SCs with particular cytokines to address their effects on the BTB integrity and on the TJ proteins. Our experiments showed that SCs are the main constituent of the BTB. Co-culturing of both SCs and PTC on Matrigel only had a negligible effect on the BTB integrity. Treatment of 93RS2 rat SCs with bone morphogenetic protein2 (BMP2) demonstrated a negative effect on the BTB integrity. This effect was reversed by pretreatment with a BMPRI inhibitor. Our results also showed that the gene expression of the junctional adhesion molecule-3 (JAM-3), which is a well-known tight junction protein, was up-regulated after treatment of primary SCs with

testosterone via the classical or non-classical androgen pathway. Further research is needed to understand the physiological implications of the results obtained.

P 10.12

Peritubular myoid cells in men with normal and impaired spermatogenesis: CLEC3B as novel candidate for fibrotic remodeling

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The wall of the human seminiferous tubules is composed of layers of peritubular myoid cells (PMCs) and extracellular matrix proteins. A disturbance of these components is associated with male infertility, however a full characterization of the seminiferous tubule wall in infertile men is not available yet. This study aimed at extending the analysis of the PMC compartment in infertile men. We performed single cell RNA sequencing (scRNA-seq) of testicular cell suspensions from patients with obstructive azoospermia (normal, n = 3) and cryptozoospermia (crypto, n = 3). The results were validated using immunofluorescence stainings of markers for PMCs and fibrotic PMCs in an expanded patient cohort (n = 6 per group). ScRNAseq revealed that in normal patients only 6.4% and 1.3% of the cells are represented by PMC or fibrotic PMC, respectively. In the cryptozoospermic group, we found an increased proportion of PMCs and fibrotic PMCs accounting for 26.3% and 15.1% of all captured cells, respectively. Differential gene expression analysis between the fibrotic PMCs of the two patient cohorts resulted in identification of 188 differentially expressed genes, including CLEC3B. Co-localization analysis confirmed the expression of CLEC3B protein in decorin-positive (fibrotic PMC marker) cells in both normal and crypto patients. However, immunofluorescence analysis highlighted a heterogeneous distribution of CLEC3B in the tubular wall of cryptozoospermic patients. In conclusion, scRNA-seq revealed an increased proportion of PMCs and fibrotic PMCs in cryptozoospermic patients. We identified CLEC3B as a novel marker for fibrotic PMCs and demonstrated a distinct expression pattern for this protein in the cryptozoospermic group compared to the normal situation. Perspectives, linking the expression of CLEC3B to the spermatogenic state of the tubules and the proliferative activity of spermatogonia might reveal altered mechanisms associated with idiopathic infertility.

P 11.1

iTRAQ-based quantitative differential protein expression analysis revealed essential role of glycolytic pathway in flagellum energy balance in asthenozoospermic males

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Background The present study was carried to understand quantitative expression analysis of proteins in the tail fraction of the both motile and non-motile asthenozoospermic human spermatozoa samples. We demonstrated protein profiling to differentiate head and tail sections of human spermatozoa. We have also attempted to establish the relationship between the sperm motility and glycolytic pathway enzymes.

Material and Methods Semen samples of normal and asthenozoospermic males were used for the study. Electron microscopy evaluation was performed to understand ultrastructural abnormalities in non-motile spermatozoa such as mid piece, and principal piece. Sperm head and tail were separated by sucrose density gradient. The tail proteins were trypsin digested and subjected to iTRAQ labelling followed by identification by LC/MS. Proteomics result was validated with Immunohistochemistry using FITC conjugated antibodies.

Results Transmission electron microscopic examination of non-motile and fertile semen revealed major changes in the morphology of spermatozoa. Variable forms of structural abnormalities of spermatozoa were observed in non-motile sperms. Total 84 tail specific proteins were identified in the normal motile spermatozoa and 26 tail specific proteins in the non-motile (asthenozoospermic) individual.

The differential tail proteins identified belonged to cytoskeletal, flagellar and cell movement proteins and energy and metabolism proteins. Immunofluorescence study of semen samples revealed significantly lower levels of Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH), Enolase and Pyruvate kinase (PK) in tail and mid-piece region of non-motile spermatozoa to that of fertile males.

Conclusion Tail structural abnormalities and reduced expression of glycolytic pathway enzymes in the immotile sample could cause energy imbalance that potentially weakens sperm movement. These proteins may serve as diagnostic biomarkers of infertility and targets for male contraception.

P 11.2

Genetic architecture of severe spermatogenic failure and male infertility – time to advance standard of care

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Introduction Half of all infertility cases, affecting 10–15% of couples, arise from male factors like crypto- and azoospermia (very few or no sperm in the semen). Well-established genetic causes include Klinefelter syndrome (KS) and AZF microdeletions for quantitative spermatogenic failure (SPGF) and CFTR mutations for obstructive azoospermia (OA). However, although gene mutations are expected to explain most remaining cases, no respective analyses have yet informed clinical practice.

Patients and Methods Since 2017, we prospectively included crypto- and azoospermic men attending the Centre of Reproductive Medicine and Andrology (CeRA, Münster) into our Male Reproductive Genomics (MERGE) study. After excluding known conditions leading to SPGF (e.g., previous chemotherapy) and ruling out chromosomal aberrations and AZF deletions, we offered exome sequencing, analysed 48 genes with sufficient clinical validity, and strictly assessed variants according to clinical guidelines.

Results Chromosomal aberrations (mostly KS) and AZF deletions were identified in 15.9% and 2.8% of cases. In 512 men without such conditions, 11 patients (2.2%) with OA carried causal mutations in *CFTR/ADGRG2*. In 16 patients (3.1%) with SPGF, mutations in nine genes were identified as causal. Segregation analyses helped in assessing the pathogenicity of some variants, highlighted by a *de novo* mutation in *DMRT1*.

Conclusions The sequencing performed here offers a diagnostic yield comparable to that of current genetic tests and some findings are immediately relevant for infertility counselling/treatment, as they may indicate the success of treatments like testicular sperm extraction. Overall, panel/exome sequencing now provides strong enough evidence to be included in diagnostic workups of men with crypto- and azoospermia. Including more genes and functional assessments of variants of uncertain significance will quickly increase relevant diagnostic findings.

P 11.3

Clinical and seminal parameters associated with testis microlithiasis and its severity in males from infertile couples

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Introduction Testis microlithiasis (TM), representing an incidental finding in the scrotal ultrasonography (US), is associated with male infertility and a higher risk for testicular cancer as compared to that in infertile males without TM. This study aimed to evaluate whether TM and its severity were associated to a different degree of testicular dysfunction in men from infertile couples.

Patients and Methods This study was an observational, retrospective, case-control investigation involving males who underwent diagnostic work-up for couple infertility at an Andrology clinic, between January 2004 and December 2018. One hundred patients, out of the 2112 scored men, were found to have TM during the US evaluation. TM was defined as limited TM (LTM) or classical TM (CTM), when the maximum number of hypercogenic spots per sonogram was < 5 or ≥ 5, respectively. CTM, LTM, and control groups were compared for clinical variables, serum levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH), and total testosterone, as well for semen parameters and scrotal US features.

Results Males with CTM showed a lower mean testis volume ($p = 0.03$) and a lower sperm concentration ($p = 0.03$) as compared to the other two groups. A higher FSH level was observed in the CTM group as compared to that in the LTM group ($p = 0.02$) and in controls ($p = 0.009$). The multiple logistic regression analysis showed that only a smaller testicle volume exhibited an independent significant association with a higher odd of detecting CTM (OR = 0.84, 95%-CI: 0.75–0.94; $p = 0.02$). No significant differences were observed between groups in the prevalence of risk factors for testicular cancer, or in the prevalence of conditions associated with TM.

Conclusion Males from infertile couples who exhibit a reduced testicular volume should undergo scrotal US to exclude CTM. Significant evidence provided here is that the presence of a LTM has no clinical relevance in males from infertile couples.

P 11.4

Are bisphenols analogues safer than bisphenol A for human spermatozoa? An in vitro study

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Introduction The ubiquitous presence and environmental persistence of bisphenol A (BPA) along with its reputation of being an endocrine disruptor, have generated concerns about the possible links with a spectrum of human health disorders, including infertility. The increasing use of BPA analogs, such as bisphenol S (BPS) and bisphenol F (BPF), is attracting interest to these new compounds, which, however, could share chemical and biological properties similar to BPA. *In vitro* studies demonstrated that BPA induces sperm apoptotic/oxidative damages; however, effects of the human sperm exposure to BPS and BPF have not yet been investigated.

Materials and Methods Motile sperm suspensions were exposed for 4 h to scalar concentrations of BPS or BPF (10–400 μ M) or 400 μ M BPA, used as positive control. Mitochondrial membrane potential (MMP) and mitochondrial generation of reactive oxygen species (ROS) were assessed at flow cytometry using JC-1 dye and MitoSOX red, respectively. Sperm motility was analysed by CASA and vitality was assessed by eosin test.

Results MMP and ROS generation were not significantly affected by the exposure to scalar concentrations of BPS or BPF. Consistent with the lack of mitochondrial effects, no significant differences were observed for sperm motility and vitality in sperm treated with scalar concentrations of BPS or BPF. As expected, when compared to untreated samples, 400 μ M BPA produced a significant decrease in % of sperm with high MMP ($7.7 \pm 7.2\%$ vs $75.9 \pm 10.2\%$, $p < 0.0001$), which was accompanied by an increased % of sperm with mitochondrial ROS generation ($85.7 \pm 7.6\%$ vs $28.1 \pm 6.5\%$, $p < 0.0001$), complete sperm immobilization and loss of viability (viable sperm: $15.2 \pm 12.5\%$ vs $87.7 \pm 4.5\%$, $p < 0.0001$).

Conclusion When compared to BPA, the analogues BPS and BPF, at environmentally relevant concentrations, seem to be safer for sperm biology as they exert a neutral effect on sperm motility, viability and mitochondrial function.

P 11.5

Clinical and psychological characteristics of men with primary and secondary couple infertility

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Introduction No previous study compared clinical and psychological characteristics of men with primary and secondary infertility and fertile men and evaluated associations between psychological and clinical parameters in these groups.

Methods We evaluated clinical and psychological (Middlesex Hospital Questionnaire, MHQ) characteristics of 580 males of infertile couples (38.0 ± 5.8 years) and 115 fertile men (36.6 ± 5.3 years).

Results Among infertile men, 494 (85.2%) had primary infertility (group #1) and 86

(14.8%) secondary infertility (group #2). Group #3 was made of 115 fertile men. Groups #1 and #2 showed higher frequency of cryptorchidism and gonadotrophins and lower testis volume than group #3. Group #1 showed lower testis volume and higher FSH than group #2. Group #3 showed better semen parameters than groups #1 and #2, while group #1 showed lower sperm concentration and progressive motility than group #2. No difference in duration-of-infertility was found between groups #1 and #2. However, group #2-with-miscarriages showed higher duration-of-infertility than group #1. In group #1, duration-of-infertility was positively associated with male age ≥ 42 years. Regarding psychological traits, groups #1 and #2 did not differ and showed higher MHQ total and free-anxiety score than group #3. However, group #2-with-miscarriages had higher phobic-anxiety than group #1. In group #1, azoospermic men showed higher depression than the rest of the sample, duration-of-infertility ≥ 3 years was positively associated with phobic-anxiety, semen volume was negatively associated with somatization.

Conclusions Infertile men showed worse clinical and psychopathological characteristics than fertile men. Group #1 showed worse clinical characteristics than group #2, but group #2-with-miscarriages had higher duration-of-infertility and phobic-anxiety than group #1. In group #1, severe seminal features, duration-of-infertility ≥ 3 years, age ≥ 42 years were associated with psychopathological traits.

P 11.6

LIN28A and LIN28B expression in testicular fibrosis – a case study

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The mammalian genome encodes two Lin28 paralogs, Lin28A and Lin28B. LIN28A has been shown to be expressed in SSC of adult human testis, but also in malignant germ cells of testicular cancer. LIN28B has been implicated in the timing of onset of human puberty, while its expression was found in Leydig cells of animal models. Furthermore, the disturbance of their expression was found in a mouse model of hypogonadism. However, data about comparative distribution of LIN28A and LIN28B in human infertile testis are limited. Here we examined their protein expression profiles in testis samples of a patient with tubular fibrosis, with increased FSH levels (33 U/L) or with maturation arrest. FFPE samples of testicular biopsies of azoospermic men were obtained from KBC Zagreb Andrology clinic. The immunohistochemical labelling was performed in triplicate, with positive and negative controls. In patient with tubular fibrosis, LIN28A was surprisingly found to be strongly co-

expressed with LIN28B in interstitial tissue, with cytoplasmic signal for both markers in existing Leydig cell clusters (nodules). In testis of a patient with maturation arrest and no nodules, strong LIN28A/B staining was found in tubules, with cytoplasmic expression of LIN28A mainly in spermatogonial population, while LIN28B was presented with nuclear staining in most maturation stages. The interstitial cells were negative for LIN28A, but exhibited weak LIN28B expression. We conclude that LIN28A with LIN28B show an overlap in human testis with tubular fibrosis, but subcellular distribution of LIN28 proteins may be dependent on histological diagnosis of infertile testis. Further studies are ongoing to elucidate this assertion.

Grants This study was supported by the Scientific Center of Excellence for Reproductive and Regenerative Medicine, Croatia, and through the European Regional Development Fund, under grant agreement KK.01.1.1.01.0008. "Reproductive and Regenerative Medicine – Exploring New Platforms and Potentials".

P 11.7

The protein phosphatase with EF-hand domain 1, PPEF1, is a Ca^{2+} -dependent calmodulin binding protein present in mature spermatozoa

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Sperm capacitation is a post-ejaculatory maturational process that takes place in the female genital tract under the influence of calcium and its mediator, calmodulin. Although capacitation is associated with protein phosphorylation/dephosphorylation, the identity and contribution of sperm calmodulin binding proteins remain to be established. Having identified the **protein phosphatase with EF-hand domain 1 (PPEF1)** as a sperm calmodulin binding protein, we hypothesize that this Ser/Thr phosphatase could be involved in the capacitation process and the control of sperm hyperactivation. The main objectives of our study were to 1) identify and localize sperm PPEF1 isoforms by using biochemical and molecular approaches and, to 2) determine PPEF1 contribution to sperm capacitation by in vitro functional assays. Different **PPEF1** transcript variants encoding 4 isoforms were identified by PCR amplification of bull testis RNA. Western blot performed on sperm subcellular fractions indicated that PPEF1 was exclusively associated with sperm membranes. Indirect immunofluorescence staining revealed that PPEF1 is located in the neck, the flagellum and the acrosome of non-capacitated spermatozoa. Although a phosphatase activity was detected in immunoprecipitated PPEF1, further studies are required to determine whether PPEF1 localization and phosphatase activity are modulated during capacitation. Acknowledging that PPEF1 is an ortholog

of RdcC, a calcium-dependent phosphatase present in the retina of *Drosophila*, our study is the first to investigate the contribution of this newly identified sperm calmodulin binding protein in the context of reproductive biology and sperm function. Overall, this project will help to better understand the molecular mechanisms underlying sperm capacitation, a process controlling sperm fertilizing ability. Ultimately, our findings might open new avenues on the development of non-hormonal male contraceptives targeting sperm enzymes.

P 11.8

Characterization of human sperm intracellular metabolites to understand their association with functional ability and genetic integrity

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Introduction Male factor alone contributes to approximately 50% of the infertility cases. Although semen analysis is a primary diagnostic test in understanding the male reproductive potential, it does not address the fertilizing capacity and genetic integrity of spermatozoa. Since cellular metabolism is influenced by genetic factors and pathophysiology involved with it, characterization of the endogenous metabolites from spermatozoa may help us in better understanding the hidden cause of male infertility.

Patients (or Materials) and Methods In this study, using 1H Nuclear magnetic resonance (NMR) spectroscopy intracellular metabolites of sperm cells from 14 normozoospermic men and their association with functional and genetic parameters such as motility, viability and DNA damage and were studied.

Results Twenty-one metabolites from spermatozoa were identified, out of which the concentration of leucine, isoleucine, valine, glucose, lactate, putrescine and ethanolamine were negatively associated with the spermatozoa motility. On the other hand, arginine, betaine and glycerol were positively correlated with sperm motility. Further genetic integrity and viability were positively correlated with all metabolites tested except putrescine which was negatively correlated with the viability.

Conclusion Thus, spermatozoa metabolites can aid in understanding the sperm functional and genetic integrity. Further, metabolomics analysis of sperm may serve as a screening diagnostic tool in elucidating the pathology associated with male infertility.

P 11.9

Results of testicular biopsy evaluation and reproductive hormones analysis in infertile men with Sertoli-cell only syndrome

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Introduction The aim of the study was to evaluate testes with Sertoli cell only syndrome (SCOS) focusing on morphometric signs of testicular dysgenesis and markers of Leydig cell (LC) function in relation to hormonal status of studied men.

Materials and Methods Forty nine testicular biopsies of patients with SCOS and 15 controls with normal spermatogenesis (NOR) were assessed for the seminiferous tubule diameter (STD), thickness of tubular membrane (TM), areal fraction of intertubular space (AFIS) and LC number (LC-score). The results of histological examination were correlated with serum levels of FSH, LH, testosterone (T) and T/LH ratio.

Results In SCOS testicular volume (Median (M): 16.0 vs 29.5; $p < 0.001$) and STD (M: 141.7 vs 190.2; $p < 0.001$) were lower, while TM (M: 9.8 vs 6.4; $p < 0.001$) and AFIS (M: 47.6 vs 27.6; $p < 0.001$) were significantly higher in comparison to NOR. LC-score was higher in SCOS than in NOR group (M: 2.2 vs 1.1; $p < 0.001$). Abnormal AFIS and STD were present in 43% of SCOS biopsies and among them in 81% the increased LC-score was found. In SCOS group the subjects had significantly higher levels of both gonadotropins (FSH, M: 19.9 vs 3.4; $p < 0.001$; LH, M: 7.1 vs 4.2; $p < 0.001$). Total serum testosterone level did not differ between studied groups, however, T/LH ratio was significantly decreased in SCOS group (M: 2.3 vs 3.8; $p < 0.001$). Negative correlation between LC-score and STD was observed in SCOS group ($r = -0.48$; $p < 0.001$). AFIS correlated positively with serum FSH level in both groups (NOR, $r = 0.53$; $p < 0.05$; SCOS, $r = 0.41$; $p < 0.05$), while with LH, and negatively with T/LH ratio, only in SCOS (LH, $r = 0.37$; $p < 0.05$; T/LH, $r = -0.36$; $p < 0.05$).

Conclusions Presented data confirm that substantial number of subjects with SCOS presents signs of testicular dysgenesis and impaired function of LC. Increased serum levels of LH and FSH may reflect LC dysfunction, and additionally significant histological changes in testicular structure.

P 11.10

Mapping men's reproductive decision-making pathways

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Introduction While men are half of the reproductive equation, research examining men's reproductive decision-making has

been limited. This research uses reproductive life histories to construct a schematic map of men's decision-making pathways about pregnancy and contraceptive (non)use.

Methods Individual telephone interviews (n = 48) were conducted with cisgender, heterosexual men, ages 25–67, living in seven U.S. southern states from May–December 2019. Men were recruited using targeted Facebook advertisements. Interviews explored men's reproductive histories, including contraceptive use and contribution to pregnancies (average time 70 minutes). Interviews were audio-recorded, professionally transcribed, and analyzed using a constructivist approach to grounded theory.

Results Participants' narratives were used to develop a map of questions and outcomes related to pregnancy and contraceptive use. The schematic reveals complex reproductive pathways where men have few options to manage their fertility and need to negotiate contraceptive (non)use and potential parenthood with their partners. Feedback loops emphasize that men are repeatedly confronted with these questions and negotiations until they or their female partner(s) are infertile; it is not a one-time process but a continuous journey lasting over men's reproductive lives.

Conclusions Findings expand our understanding of men's reproductive life course and demonstrate how men negotiate aspects of this journey with their partners. The map provides a starting point for classifying the continuum of pregnancy intentions and contraceptive use for men, who are often excluded from such work. Future research may draw on these pathways as areas of inquiry, thereby expanding the small body of research on men's reproductive lives and concerns that need to be understood.

P 11.11

Ex vivo 7 T magnetic resonance imaging of testicular tissue: preliminary results

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Men with azoospermia who wish to have children undergo TESE which has a success rate of only about 60%. Our study aimed to assess ex-vivo MRI of testicular tissue, including diffusion-weighted imaging (DWI) as a potential tool in developing a prediction model for sperm retrieval as well as assess the differences between fresh tissue and frozen-thawed tissue. The study included 18 samples of testicular tissue obtained via TESE, 9 that underwent 7 T MRI as fresh tissue (group 1) and 9 that were frozen in liquid nitrogen, thawed and then imaged (group 2). Groups were divided into two subgroups, samples with sperm (A) and without sperm (B). T1, T2 and ADC values were measured in all samples and values were compared between

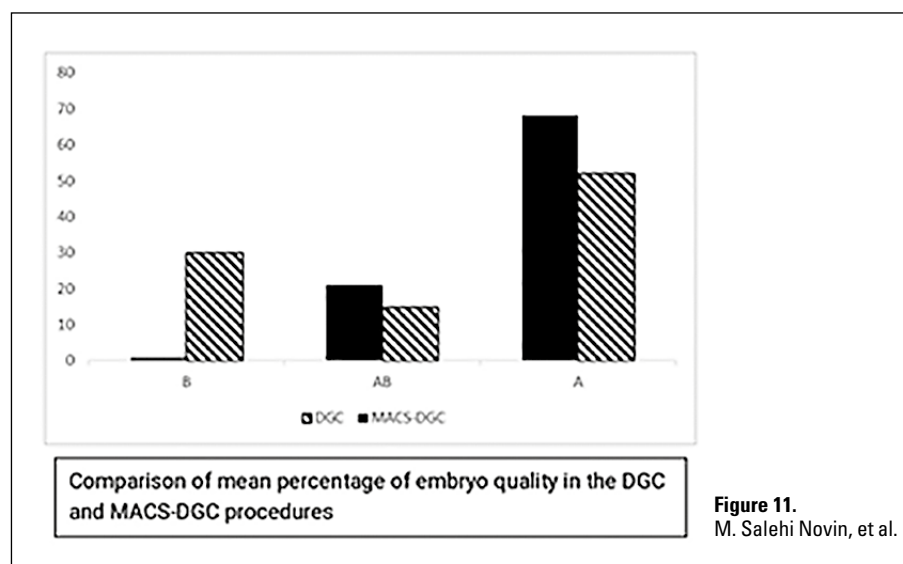


Figure 11.
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groups and subgroups. T2 was increased in group 2 compared to group 1 ($p = 0,00012$) while no statistically significant difference in ADC and T1 values was observed between groups. Within group 1, ADC was increased in subgroup B (n = 4) compared to subgroup A (n = 5) and the increase was statistically significant ($p = 0,019$). No statistically significant difference in ADC values was observed between subgroups 1A and 2A, 1B and 2B or 2A and 2B. In conclusion, T2 is significantly increased in frozen and thawed tissue compared to fresh tissue which should be considered when interpreting data and could reflect the effect of freezing and thawing on the consistency and increased water diffusion into the tissue. ADC could be a useful parameter in evaluating testicular tissue and predicting sperm retrieval but should be evaluated in a study with larger sample sizes.

Grants This study was supported by the Scientific Center of Excellence for Reproductive and Regenerative Medicine, Republic of Croatia, and by the European Union through the European Regional Development Fund, under grant agreement No. KK.01.1.1.01.0008, project "Reproductive and Regenerative Medicine – Exploring New Platforms and Potentials".

P 11.12

Annexin-V MACS sperm selection method could be effective on sperm parameter and embryo quality in male factor patients with high DNA fragment

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Introduction Sperm selection based on morphology and motility in ART techniques,

is not enough for choosing the best sperm especially in male factor patients. In Annexin-V magnetic activated cell sorting (MACS) technique, apoptotic sperm are separated from non-apoptotic one by negative selection. So, this method can help selecting good quality sperm for ICSI.

Method Semen samples from 30 male factor infertile couples (DFI > 30%) were selected and divided into two group in each patient. control was washed with DGC and experimental one was selected by MACS-DGC. Retrieved eggs in each patient, were divided in 2. Control and experimental group were injected by DGC and MACS respectively. Semen parameters and DFI (SCD test) were analyzed before and after processing. After ICSI, rate of fertilization and embryo development were evaluated. Comparison between results of control and experimental groups was assessed by SPSS analysis.

Results Results showed that, sperm motility and morphology after MACS method (45%, 1.7%) was significantly higher than DGC method (40%, 1.1%) and before washing (35%, 0.9%). Percent of DFI in MACS group (36%) was significantly decreased compared to DGC (45%) and primitive group (55%). The number of oocytes were injected in DGC group was 93 and in MACS group was 111. Fertilization rate in both groups was almost the same (72.07% in MACS vs 73.11 in DGC). Rate of day 3 embryo with good grade in MACS group (72. 5%) was significantly higher than DGC (51.47%, $p < 0.05$). The pregnancy rate, from MACS embryos was 35.4%.

Conclusion Results indicated, sperm selection by MACS-DGC method can improve sperm motility, morphology and reduce sperm DNA fragmentation. No significant difference was observed in fertilization rate, but the percent of high-quality embryo was significantly higher by this method. All pregnant had very high DNA fragmentation (> 45%), according to the mechanism of MACS method, it can be suggested as a good choice for patients with high DFI (Fig. 11).

P 11.13

Sperm Izumo1 immuno-fluorescence comparison of unexplained infertile men and men without fertilization problems

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Unexplained infertility means that the couples unable to achieve pregnancy with no reason after two years of unprotected intercourse. Semen analysis is first diagnostic test for evaluation of the male fertility and normal results are seen in the unexplained infertility. However most molecular impacts are not clear. For fertilization, Izumo1 redistribution is necessary after acrosome reaction. Animal experiments shows that the defects related to Izumo1 cause the fertility problems. From June 2017 to March 2018, experiments are conducted fresh semen specimen of 48 males. 18 control whose partners get spontaneously pregnant within a year and 30 males of unexplained infertility. Semen analysis and chromatin condensation assessment were done with fresh sperm samples. After density gradient centrifugation, acrosome reaction was assessed with progesterone. Antibodies against to Izumo1 and CD46, and DAPI used for triple immunofluorescence of sperm.

Age, semen analysis and chromatin condensation parameters haven't differ statistically significant among the groups. Sperms were evaluated in 4 groups: triple positive, one positive (DAPI+), Izumo1 negative (Izumo1-, CD46+, DAPI+), DAPI negative (Izumo1+, CD46-). Triple positive sperms were higher in the control group than the experiment group but statistically insignificant ($p = 0.075$). Izumo1 negative and DAPI negative sperms have observed in some of the samples. Some sperms with the anomalies like double nuclei also have Izumo1 immunopositivity. There is statistically significant negative correlation between the chromatin condensation and the triple positive sperms within the groups ($p < 0.05$). Even Izumo1 immunopositivity haven't differed among the groups, sperms have reacted different to the progesterone. The physiologic reactions of the sperms are regulated with the molecular level. Understanding the acrosome reaction in molecular level will be helpful for the diagnosis of the male infertility as well as the treatment options.

P 11.14

First Genome Wide Association Study (GWAS) in men with unexplained infertility – Identification of a genetic region determining follicle-stimulating hormone action

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Introduction 30–40% of infertile men remain unexplained. Follicle stimulating hormone (FSH) plays a key role in initiation and maintenance of spermatogenesis; action might be hampered in some of these patients. One well-studied single nucleotide polymorphism (SNP) (rs10835638) is associated with *FSHB* mRNA transcription and directly affects FSH serum levels, testicular volume and spermatogenesis. The aim was to identify further SNPs associated with FSH levels in unexplained infertile men.

Patients and Methods Retrospectively, around 1900 characterized men with unexplained infertility were identified from our clinical database Androbase®. In the discovery cohort of 742 men a genome wide association study (GWAS) was performed (Illumina PsychArray_v1.3®) and analyzed (Illumina®GenomeStudio, PLINK and R). As validation, the SNPs rs1103005 and rs10835638 were genotyped in an independent cohort of 1127 patients by TaqMan SNP-PCR and association to clinical parameters was evaluated.

Results Imputation analysis revealed 9 SNPs with genome-wide significance ($p < 3.994e-07$) at the *FSHB* locus on Chr. 11p.14.1. All SNPs, including rs10835638, were highly linked to one another (linkage disequilibrium). A validation study on 1127 patients for the newly identified rs1103005 and for rs10835638 revealed a significant association with FSH ($p < 1.93e-06$; $p < 4.04e-7$), FSH/LH ratio ($p < 4.84e-13$; $p < 4.94e-12$) and bi-testicular volume ($p < 7.36e-03$ and $p < .29e-03$).

Discussion Our study revealed that not one single SNP, but rather the *FSHB* gene region is the main genetic determinant affecting FSH action. This study is the first delineating this region as of crucial importance in the regulation of FSH serum levels in infertile men with unexplained infertility. We suggest to include one of these SNPs into routine diagnostics in infertility workup, to identify a subgroup of so far unexplained infertile men, who putatively benefit from FSH treatment.

Grants Supported by the DFG CRU326.

P 12.1

In vitro effects of aqueous extract of unfermented rooibos on human sperm function

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Introduction *Aspalathus linearis* (Rooibos) is a popular plant owing to its antioxidant properties. Animal studies have demonstrated the beneficial effects of the extract on sperm functions. This study aimed to investigate the effects of unfermented rooibos extract on sperm function *in vitro*.

Material and Methods Semen samples were collected after 3–5 day's sexual abstinence by masturbation from healthy donors ($n = 25$) at the University of the Western Cape, and infertile patients ($n = 25$) attending Tygerberg and Vincent Pallotti Hospitals, South Africa for fertility problems. Samples were liquefied, washed (300 x g; 10 min) with human tubular fluid in 1 % bovine serum albumin (HTF-BSA) and incubated with unfermented rooibos aqueous extract (0, 0.15, 1.5, 150 µg/ml) for 1 h at 37 °C, where HTF-BSA served as control.

Results Sperm motility, vitality and DNA fragmentation remained unchanged in both groups ($p > 0.05$). Intracellular reactive oxygen species (ROS) production (15 µg/ml; $p < 0.01$) and acrosome reaction (1.5 µg/ml; $p < 0.0001$) increased significantly, while the percentage of intact mitochondrial membrane potential (MMP; 150 µg/ml; $p < 0.01$) decreased in the donor group, but were unaffected in the patient group ($p > 0.05$).

Conclusion Lower concentrations of aqueous extract of unfermented rooibos induced ROS production and acrosome reaction in the donor group. However, higher concentrations of the extract appear to be harmful to MMP, which might impair sperm functions and fertility thereof.

P 12.2

Impact of metabolically healthy obesity in patients with andrological problems

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Introduction While the pathogenic role of metabolically complicated obesity (MCO) in erectile dysfunction (ED), major adverse cardiovascular events (MACE) and male infertility has been widely studied, that of metabolically healthy obesity (MHO) has been poorly investigated. We herein evaluated the role of MHO in ED pathogenesis, MACE prediction and male reproductive health.

Methods We studied a consecutive series of 4945 men (50.5 ± 13.5 years) with sexual dysfunction (SD) (cohort 1) and 231 (37.9 ± 9.1 years) males of infertile couples (cohort 2). A subset of SD patients ($n = 1687$) was longitudinally investigated to evaluate MACE. All patients underwent clinical, biochemical, erectile function (EF) and flaccid penile color-Doppler US (PCDU) assessment. Infertile men underwent also scrotal and transrectal US, semen analysis (including IL-8), prostatitis-like symptoms (PLS) assessment. MHO was defined as BMI > 30 kg/m² with HDL > 40 mg/dl and absence of diabetes or hypertension. The rest of the obesity sample was defined MCO. MHO or MCO were compared with the rest of the sample (normal weight (NW) men).

Results In cohort 1, 816 men (16.5%) were obese, 181 (3.7%) MHO, 635 (12.8%) MCO. In cohort 2, 68 men (28.4%) were obese, 19 (8.2%) MHO, 49 (21.2%) MCO. After adjusting for confounders, in both samples, either MHO or MCO showed lower testosterone and worse PCDU parameters, compared to NW. However, only MCO showed a worse EF compared to NW. In the longitudinal study, both MHO and MCO were independently associated with a higher incidence of MACE, compared to NW (both $p < 0.05$). In cohort 2, MHO and MCO showed a higher prostate volume, and MCO also higher US and biochemical (sIL-8) features of prostatic inflammation compared to NW, but no differences in PLS or seminal parameters.

Conclusions MHO is associated with sub-clinical ED, increased cardiovascular (CV) risk and prostate enlargement. MHO men should be considered at high CV risk, as MCO, and followed-up for EF and prostate abnormalities.

P 12.3

Does maternal smoking cessation before the fetal masculinization window mitigate adverse effects on offspring semen quality?

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Introduction The negative impact of maternal smoking during pregnancy on offspring semen quality is well established. We investi-

gated if smoking cessation before the fetal masculinization window, starting in gestational week seven, can mitigate the adverse effects on semen volume, sperm concentration, total sperm count, motility, morphology and testicular size.

Methods In 980 18-year old sons of women from the Danish National Birth Cohort, we estimated the differences in semen characteristics and testicular volume among early quitters (prior to week seven), continuous smokers and non-smokers. Maternal smoking was reported around gestational week 16. Negative binomial regression analyses were adjusted for parents' ages at birth, maternal pre-pregnancy BMI, maternal alcohol intake, family socioeconomic status, partner's smoking, and son's abstinence time, place of ejaculation (home or clinic) and recent fever. Analysis of motility was further adjusted for interval from ejaculation to analysis.

Results Of 230 (24%) women who smoked during first trimester, 62 (27%) quit before week seven. Sons of early quitters had a 19% lower sperm concentration (95%-CI: -36%, 2%) and 23% lower total sperm count (95%-CI: -41%, 0%) than sons of non-smoking mothers. Similarly, sons of continuous smokers had 18% lower sperm concentration (95%-CI: -31%, -3%) and 37% lower total sperm count (95%-CI: -39%, -12%). Maternal smoking was not associated with other semen characteristics or testicular size.

Conclusion Smoking cessation before gestational week seven did not appear to mitigate the negative impact of smoking on offspring semen quality. While results are based on relatively small numbers, they suggest that adverse effects are not only caused by acute disruption of the development of fetal reproductive organs during week seven to fifteen, but that other mechanisms, such as delayed effects of smoking compounds, epigenetic changes or pre-conceptional effects, may be at play.

P 12.4

Sperm quality and features of the antioxidant protection system in men living in different regions of Siberia

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Currently, one of the risk factors for disorders of the male reproductive system is the formation of reactive oxygen species (ROS) in cells and tissues. The formation of ROS is balanced by the action of various antioxidants. The complexity and intensity of the process of spermatogenesis sensitive to violations of the balance of vitamins, coenzymes, trace elements, necessitates the determination of the content of these substances in the ejaculate. The study of antioxidant system, lipid peroxidation and the quality of semen in men of reproductive age living in Ulan-Ude, Republic of Buryatia, in Irkutsk

and Novosibirsk. All men were somatically healthy. The collection and analysis of semen was performed according to who recommendations. It is found that in the group of somatically healthy men living in the city of Irkutsk spermogram characterized by the presence of higher sperm count (million per ml), 34.4% more than in men of Novosibirsk and 23.6% more than men Ulan-Ude. Thus, in the group of men of Irkutsk revealed a significant increase in concentrations of α -tocopherol by 44% compared with men of Ulan-Ude and by 38% compared with men of Novosibirsk. In the study of semen of men of Ulan-Ude is installed active high content of spermatozoa in relation to this indicator in men of Novosibirsk, Irkutsk and 16% and 11% respectively. For men Ulan-Ude is also characterized by a higher level of total antioxidant activity, 34% higher than that in men of Novosibirsk and 13%, than men of Irkutsk. The studies of the quality of ejaculate and lipid peroxidation processes in men of various Siberian cities show that the place of residence and ecological and geographic position are the main causes that determine fertility problems. Regional peculiarities and ecological and geographical environment can be important factors affecting the functioning of the reproductive system and determine the heterogeneity of male infertility under conditions of anthropogenic press.

P 12.5

Inhibition of autophagy impairs sperm quality and promotes cell death in human spermatozoa exposed to oxidative stress

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Autophagy is a regulated pathway of lysosomal degradation which helps eukaryotic cells to maintain or restore homeostasis, being a stress response mechanism. Oxidative stress (OS) is a main cause of impaired sperm function linked to male infertility and previously we demonstrated that human sperm subjected to OS activate an autophagic response; however, the impact of blocking autophagy in human sperm under OS conditions is unknown. The objective here was to evaluate the impact of autophagy inhibition in human sperm exposed to OS. For this, spermatozoa from normozoospermic donors were used. Chloroquine was used as the autophagy blocker, since it has been reported as an autophagy inhibitor in different mammalian cells, including human sperm. Ionomycin and hydrogen

peroxide (H_2O_2) were used separately to induce OS. Sperm cells were co-incubated with ionomycin plus chloroquine and separately with H_2O_2 plus chloroquine. An untreated control and a control incubated only with the OS inducer were included. Viability, mitochondrial membrane potential ($\Delta\Psi_m$), phosphatidylserine (PtdSer) externalization and caspase activation were evaluated by flow cytometry. Metabolic parameters including glycolysis, measured as extracellular acidification rate (ECAR) and oxidative phosphorylation based on the oxygen consumption rate (OCR) were analyzed with a Seahorse XFp extracellular flux analyzer. The results showed that OS decreases viability and $\Delta\Psi_m$, while increases PtdSer externalization and caspase activation. The OS-mediated impairment of sperm parameters is accentuated when autophagy is blocked. Also, autophagy inhibition directly impairs ECAR and OCR and negatively affect metabolic response under OS conditions.

In conclusion, blocking of autophagy results in increased oxidative damage, which negatively impacts the functionality and lifespan of sperm cells, suggesting a crucial role of autophagy as a stress response mechanism by male gametes.

Grants Supported by FONDECYT 11170758

P 12.6

Impact of Electronic Cigarette Smoke Exposure on Testicular and Spermatozoa Performances in Albino Rats

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Introduction This study was done to investigate the toxic impact of Electronic Cigarettes smoke exposure on the testicular functions, semen parameters and the antioxidant status in Albino rats.

Materials and Methods A total of 24 adult fertile Male Wistar albino rats were included in the study, randomly distributed into three groups as follows: control group (n = 8), E-cig exposed group without nicotine (n = 8) and E-cig 6 mg/ml of nicotine exposed group (n = 8). Rats in group 2 and 3 were exposed to 2 cycles/hour. Each cycle included 15 min exposure and another 15 min for recovery, 6 hours/day for 8 weeks.

At the end of the study, the testes were isolated for sperm examination and histological analysis, as well as blood sampling and biochemical analysis for testosterone level and antioxidant activity.

Results Result showed that e-cig exposure led to alteration of semen parameters with a significant drop of sperm concentration in nicotine group, a significant increase of morphologically abnormal in both e-cig groups

and a significant increase of Sperm motility in no- nicotine group. Testosterone show significant increase in e-cig groups, more evident in the nicotine group. Catalase enzyme activity is SS decrease 31.10%, $P < 0.0001$ in nicotine group.

Conclusion The present study showed that E-cig exposure is associated with decreased sperm concentration, increase abnormal forms, increase testosterone level more evident in nicotine group. E-cig exposure affects ROS neutralization by decrease CAT enzyme activity and induce histological testicular degeneration of spermatogonia. The decrease in catalase activity and sperm concentration can be attributed to nicotine rather than the E-liquid. On the other hand, as regards increased abnormalities, E-liquid exerts this effect, but the effect is enhanced further by nicotine.

P 12.7

Determination of promoter methylation of MLH1 and MSH2 genes and their effect on sperm DNA fragmentation and sperm chromatin condensation in men with varicocele

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Introduction MLH1 and MSH2 are DNA mismatch repair (MMR) enzymes which have essential roles in DNA repair and meiosis. Studies have shown that polymorphisms and mutations of MMR genes might have effect on male fertility. Varicocele is the most frequent curable cause of male infertility and may lead to sperm DNA fragmentation (SDF). The aim of the study is to analyze the promoter methylation of *MLH1* and *MSH2* genes, sperm DNA fragmentation index (DFI) and high DNA stainability (HDS) and to investigate probable associations among methylation alterations, SDF and sperm chromatin condensation in men with varicocele.

Patients and Methods Semen samples from 27 infertile men with varicocele and 10 normozoospermic men as a control group were included in this study. Pyrosequencing was used to analyze methylation of *MLH1* and *MSH2* promoters quantitatively. DFI and HDS were assessed by sperm chromatin structure assay (SCSA).

Results The means of total sperm counts in infertile men with varicocele and control group were 42.14 million vs 88.31 million, respectively. *MLH1* promoter methylation was significantly higher in infertile group than in controls (means 3.4886% vs 2.7750%, $p = 0.017$). Methylation of *MLH1* promoter was positively correlated with methylation of *MSH2* promoter ($p = 0.398$, $p = 0.024$). HDS was higher in infertile men with varicocele than normozoospermic men (means 5.3355% vs 2.4559%, $p = 0.018$). DFI did not differ between varicocele and

control groups ($p = 0.453$), however DFI was inversely correlated with total sperm count ($\rho = -0.384$, $p = 0.025$). No association was found between DFI, HDS and either *MLH1* or *MSH2* promoter methylation.

Conclusion These data suggest a potential association between *MLH1* promoter methylation and varicocele-related infertility, but larger cohort studies are needed to verify the results.

P 12.8

Paternal preconception methotrexate exposure and perinatal characteristics of the children – a nationwide register study in Sweden 2006–2014

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Introduction Methotrexate (MTX) is a folate antagonist used at low doses in the treatment of inflammatory diseases. Due to a lack of evidence and the known teratogenic effect in women, men have been recommended to cease MTX treatment 3 months prior to conception. In recent years, a number of studies investigating paternal preconception MTX exposure have failed to demonstrate any significantly increased risk for adverse pregnancy outcomes. However, the evidence is still scarce and current national guidelines differ. The aim of this study was to further investigate the effect of male preconception MTX exposure on offspring health.

Patients and Methods A nationwide register study was carried out, analyzing singleton live births recorded in the Swedish Medical Birth Register (MBR) 2006–2014. By linking MBR to the Swedish Prescribed Drug Register, 224 offspring to fathers with preconception MTX exposure were identified, along with 810 178 offspring to unexposed fathers. Preconception MTX exposure was defined as fulfilling both 1) at least one MTX withdrawal from pharmacies 0–3 months prior to conception and 2) at least one more withdrawal 0–12 months prior to conception. The effects of paternal MTX exposure on the risk of major congenital anomalies, preterm birth and small-for-gestational age were analyzed using logistic regression, adjusted for medical and socioeconomic factors.

Results Given paternal preconception MTX exposure, the odds ratio (95% CI) for congenital anomalies was 0.99 (0.41–2.41), for preterm birth 0.98 (0.54–1.81) and for small-for-gestational age 1.04 (0.43–2.53).

Conclusion No increased risk for adverse pregnancy outcomes was observed after paternal preconception MTX exposure. This is the largest study to date investigating the safety of paternal MTX use prior to conception. Along with a growing body of evidence, this study suggests MTX can safely be continued among men trying to conceive.

P 12.9

Epididymis-on-a-chip approach to elucidate the impact of environmental plastic pollution on male fertilityE. Stoimenou¹, K. Pondman¹, B. Venzac¹, T. Burgers¹, S. Sharma², S. Schlatt^{1,2}, S. Le Gac¹¹University of Twente, Enschede, Netherlands;²CeRA, Münster, Germany

The blood-testis and blood-epididymis barrier (BTB and BEB), are amongst the strongest barriers in the body, protecting the post-mitotic germ cells from the immune system. Studying these barriers is crucial not only in the field of reproductive medicine, but also to identify chemicals and pollutants such as endocrine disruptors possibly disturbing these barriers, as exposure to certain environmental cues is considered to contribute to the significantly increased prevalence of male infertility [1].

Due to the absence of valid test systems the adverse effects of environmental chemicals and pharmaceuticals on adult human male reproductive function could not be systematically investigated. Therefore, establishing a robust and physiologically relevant *in vitro* screening platform using human male gonadal tissue is essential. Here we present an epididymis-on-a-chip microfluidic system, for studying the BEB barrier, using samples obtained from gender dysphoria patients undergoing sex reassignment surgery. The epididymal tissue is clamped between pillars in a custom-designed 3D printed cartridge, which is inserted in a housing chip. In our system we aim to study the possible impact of, e.g., environmental pollutants and chemicals on the integrity of the BEB.

Presently, the system and culture conditions are being optimized for prolonged *ex vivo* culture of epididymal tissues [2]. This model will be thereafter employed as a screening platform to study the impact of environmental chemicals, e.g. degraded by-products of environmental plastics, microplastics and endocrine disruptors on the BEB integrity. Later, this platform can be adapted for similar exploratory studies on the BTB using seminiferous tubules.

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P 12.10

Widely used pesticides and biocides interfere with Ca²⁺-signaling through effects on the CatSper channel in human sperm cellsA. Rehfeld, A. M. Andersson, N. E. Skakkebaek
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Introduction Pesticides and biocides are widely used in modern agriculture and humans are broadly exposed to these chemicals. Our previous study [Schiffer et al. 2014]

identified selected pesticides and biocides that induced Ca²⁺-signals in human sperm. We hypothesized that multiple pesticides and biocides may interact with the human sperm-specific CatSper Ca²⁺-channel.

Materials and Methods We screened 53 pesticides and biocides for effects on Ca²⁺-signaling in human sperm cells using a Ca²⁺-fluorimetric assay. Using the specific CatSper inhibitor RU1968 we examined whether an induced Ca²⁺-influx involved CatSper. Dose response relations were assessed for the Ca²⁺-influx inducing pesticides and biocides. These chemicals were examined for effects on sperm penetration into viscous media and acrosomal exocytosis.

Results We found that 28 of the 53 pesticides and biocides induced a mean Ca²⁺-influx > mean Ca²⁺-influx of the negative controls $\pm 3SD$ (10 μM , n = 7). The most efficacious chemicals Milbemectin A4 and A3, as well as Chlorpyrifos induced mean Ca²⁺-influxes at 10 μM reaching 87%, 85% and 76% respectively of the maximal Ca²⁺-influx induced by progesterone. The majority of the chemicals were found to induce an Ca²⁺-influx by activating CatSper (n = 4). Dose response relations were assessed for the 28 pesticides and biocides with mean EC50 values in the μM -range (n > 6). Several of the pesticides and biocides inhibited subsequent CatSper-responses induced by progesterone and prostaglandin E1 (n = 3). Several pesticides and biocides affected sperm penetration into viscous media and acrosomal exocytosis (n = 3).

Conclusions 28 of 53 examined pesticides and biocides induced Ca²⁺-influxes in human sperms, the majority by activation of CatSper. Some of the pesticides and biocides were found to interfere with sperm functions *in vitro*. Clinical studies are needed to investigate if exposure pesticides and biocides have effects on human fertility.

P 12.11

Associations between semen quality and age, meteorological variables, clinical conditions and lifestylesG. L. Verón¹, A. D. Tissera², G. M. Estofan³, R. Bello⁴, F. Beltramone³, R. I. Molina², M. H. Vazquez-Levin¹¹Instituto de Biología y Medicina Experimental (IBYME), Ciudad Autónoma de Buenos Aires, Argentina; ²Laboratorio de Andrología y Reproducción (LAR), Córdoba, Argentina; ³Centro Integral de Ginecología, Obstetricia y Reproducción (CIGORI), Córdoba, Argentina; ⁴Universidad de Tres de Febrero (UNTREF), Ciudad Autónoma de Buenos Aires, Argentina

Semen analysis is an indispensable tool for assessing male fertility potential since it provides relevant information on male genital tract functionality. The evaluation of associations between risk factors and semen quality is fundamental for clinical management. This blind cross-sectional study assessed the impact of age, clinical (obesity) and lifestyle (cigarette smoking, alcohol consumption) as well as meteorological variables during

spermatogenesis (temperature, humidity, pressure, sunshine duration, and related variables) upon human semen quality. A large cohort of men (n = 11657) was subjected to a thorough semen evaluation (semen volume, sperm concentration, motility, vitality, morphology, round cells, HOS test, nuclear maturity, and sperm kinematics: VSL, VCL, VAP, LIN, STR, WOB, BCF, ALH, and MAD) following WHO 2010 guidelines and internal/external quality assurance standards at LAR andrology laboratory (Argentina). As a result, age ≥ 40 years, obesity (body mass index [BMI] ≥ 30), and spermatogenesis during summer were individually found deleterious to semen quality, as reflected by lower sperm routine and kinematic parameters in samples from men ≥ 40 years, BMI ≥ 30 or obtained in summer, when compared to those of men < 40 years, normal BMI, or obtained in winter. Moreover, several confounding factors (obesity, cigarette-smoking, alcohol-consumption, and spermatogenesis in low humidity/long sunshine duration periods) contributed to worsen the already decreased sperm quality in men ≥ 40 years. Interestingly, a predictor selection algorithm followed by a multiple linear regression analysis of meteorological variables identified humidity and sunshine duration as the most frequent semen quality predictors. Altogether, findings from this wide study highlight the relevance of risk factors studies for predicting reproductive health alterations associated to diseases, lifestyle, and environment and for professional counseling.

P 12.12

Are ZnO-NPs harmless to spermatogenesis? Focusing cytoskeleton and nucleoskeleton dynamics in spermatogonia cellsA. R. Pinho^{1,2}, F. Martins^{1,3}, M. E. V. Costa^{4,5}, A. M. R. Senos^{4,5}, O. A. B. da Cruz e Silva^{1,3}, M. D. L. Pereira^{1,4}, S. Rebelo^{1,3}¹Aveiro University, Department of Medical Sciences, Aveiro, Portugal; ²CICECO-Aveiro Institute of Materials, University of Aveiro, Aveiro, Germany; ³Neuroscience and Signaling Laboratory, Institute of Biomedicine—IBIMED, University of Aveiro, Aveiro, Portugal; ⁴CICECO-Aveiro Institute of Materials, University of Aveiro, Aveiro, Portugal; ⁵Aveiro University, Department of Materials and Ceramic Engineering, Aveiro, Portugal

New era of nanomedicine has brought excellent alternatives that benefit diagnosis and medical treatment, due to the exceptional set of characteristics of nanoparticles. Zinc Oxide Nanoparticles (ZnO-NPs) are used in a variety of biomedical applications. However, its small size and its capacity to increase ROS levels leading to apoptosis are very useful in cancer therapy and microbial treatment, raising also concerns on its biosafety. ZnO-NPs, in addition to being cytotoxic to the male reproductive system, can also interfere with the cytoskeleton and nuclear structure in different cell types. Since the dynamics of the cytoskeleton and nucleoskeleton is extremely important for the progression of

meiosis in spermatogenesis, it is urgent to clarify the consequences of ZnO-NPs in the cytoskeleton and nucleoskeleton dynamics using cells in pre-meiotic stage, namely spermatogonia cells. Therefore, our proposal was to evaluate *in vitro* the cytotoxicity of ZnO-NPs using spermatogonia cells focusing on cytoskeleton and nucleoskeleton alteration. Pre-meiotic cell model of mouse spermatogonia stage GC-1 spg cells were used to evaluate the effect of ZnO-NPs on key proteins of the cytoskeleton (tubulin and actin) and of the nucleoskeleton (SUN1, nesprin-1, lamin A/C, and LAP1). Our results clearly indicate that higher concentrations of ZnO-NPs have a cytotoxic effect on GC-1 cells, leading to a decrease of viability with an increase of cell death, a consequence of changes in cytoskeleton and nucleoskeleton dynamics. As a conclusion, ZnO-NPs, in a time and dose dependent manner, impact the dynamics and structure of the GC-1 cell line in mouse sperm cells, and its nuclear movement, positioning and stability, which can compromise spermatogenesis and, thus male fertility.

Grants Project CICECO-Aveiro Institute of Materials, UIDB/50011/2020 & UIDP/50011/2020, national funds through FCT/MCTES.

P 12.13

Lyophilized acai berry (*Euterpe oleracea* Martius) prevents senescence-related decay in male reproductive outcome

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The use of functional foods with high anthocyanin index, such as acai berry (*Euterpe oleracea* Mart.), has been an ally in the treatment of many diseases related to Metabolic Syndrome and aging. The experimental model of induction of senescence, using D-galactose-treated rodents, displays indicative characteristics of cognitive, motor and reproductive impairment similarly to those observed in natural aging mice. Thus, this project seeks to determine the level of spermatogenic alterations and the implications for paternal contribution to embryonic and fetal development conceived by the animals induced to senescence; we also propose to verify the possibility of avoiding such damages with the concomitant consumption of acai berry. For this, 18 male Wistar rats were used and distributed into 3 groups (n = 6 each): one group of animals (n = 12) was submitted to accelerated aging undergoing treatment with D-galactose for a period of 4 weeks, in daily doses of 200 mg / kg of body weight, at mid-day, via gavage; half of these animals (n = 6) received, at the same day, supplementation with 200 mg / kg of lyophilized acai berry diluted in water, 5 times a week at 5 P.M., since the first week of aging induction; a group of animals (n = 6) received, also via gavage, 0.6 ml of distilled water for 4 weeks,

once or twice a day to mimic the treatments applied in the experimental groups (control). Once completed the treatment period, all animals were exposed to cohabitation with females (total n = 60) in estrus stage. Vaginal smears were collected in the following morning and sperm-positive females were submitted to laparotomy at gestational day 20. Three days after mating, males were anesthetized by intraperitoneal injection with Dopolen® / Anasedan® solution and underwent laparotomy to collect the testes, epididymides, ventral prostate and seminal vesicles. Sperm samples were frozen for sperm DNA integrity analysis. Male and female rats were submitted to euthanasia following the current guidelines established by the local council for animal experimentation control (CEUA, protocol number 3829240419). The results we have so far suggest that animals aged with D-galactose showed detrimental effects to male fertility, evidenced by a significant raise in embryonic resorption and pre-implantation losses, while animals co-treated with acai berry generated a significant increase of live fetuses, in addition to a lower number of pre- and post-implantation losses.

Our results suggest a strong positive nutraceutical effect of acai berry on sperm DNA quality, for improving paternal contribution to embryo development and pregnancy outcome in a model known for causing a high oxidative stress status.

P 13.1

Inhibin B and FSH – The best male fertility report

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Introduction Sperm cells are produced in the testes. Inhibin B is produced by the Sertoli cells in tubules seminiferous in the testes of males. These cells are stimulated by FSH. Can Inhibin B be a good marker of spermatogenesis? Should we use other marker (FSH?, testosterone? testis volume?)

Patients We examined 100 patients with infertility.

Methods Semen analysis was performed according to World Health Organization guidelines (WHO 2010). Hormone analyses include: FSH (follicle stimulating hormone), LH (luteinizing hormone), testosterone, estradiol, prolactin, TSH, and inhibin B. We analysed the dependencies between semen parameters, testicular volume and hormones, especially inhibin B.

Results The sperm count was significantly and positively correlated with Inhibin B ($r = 0.75$, $p < 0.001$). The Inhibin B was negatively correlated with FSH ($r = 0.66$, $p < 0.001$).

The lower was the concentration of inhibin B, the lower was the number of sperm in the semen. There was also a relationship between seminogram and FSH – the higher was the FSH, the lower was the number of sperm.

There was no relationship between the number of sperm and the concentration of

LH, testosterone, Estradiol, TSH, prolactin, DHEAS.

Conclusions It seems that we can use the value of inhibin B and FSH to assess the intensity of spermatogenesis. The decreased concentration of inhibin B correlates with the number of sperm (the lower the concentration of inhibin B the lower the efficiency of spermatogenesis) and with FSH (the higher FSH, the lower the sperm count). **High levels of FSH and reduced levels of inhibin B clearly indicate impairment of spermatogenic function in addition to the testes.**

The concentration of testosterone is not good predictor of spermatogenesis. (Inhibin B and testosterone are produced from different types of cells in the testis).

P 13.2

Differential tissue-specific damage caused by bacterial epididymo-orchitis in the mouse

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Ascending bacterial urinary tract infections can cause epididymo-orchitis. In the cauda epididymidis, this frequently leads to persistent tissue damage. Less coherent data is available concerning the functional consequences of epididymo-orchitis on testis and caput epididymidis. In an *in vivo* study, the functional and spatial differences in responsiveness of murine epididymis and testis to infection with uropathogenic *Escherichia coli* (UPEC) were addressed. Whole transcriptome analysis (WTA) was performed on testis, and on caput, corpus and cauda epididymidis of adult C57BL/6 J wildtype mice. Following UPEC-induced epididymo-orchitis in these mice, epididymal and testicular tissue damage was evaluated histologically and semi-quantitatively at 10 days and 31 days post-inoculation. Expression of inflammatory markers and candidate antimicrobial genes were analysed by RT-qPCR. WTA revealed distinct differences in gene signatures between caput and cauda epididymidis, particularly amongst immunity-related genes. Cellular and molecular signs of testicular inflammation and disruption of spermatogenesis were evident at day 10, but recovery was observed by day 31. In contrast to the cauda, the caput epididymidis did not reveal any signs of gross morphological damage or presence of pro-inflammatory processes, despite confirmed infection. Known UPEC-associated antimicrobial peptides (AMPs), like *Lcn2*, *Camp* and *Lyph8*, were inherently highly expressed and upregulated in the caput following infection, poten-

tially allowing an early luminal protection from UPEC. At both time points, the caput epididymidis exhibited no obvious infection/inflammation-derived tissue damage. Studies addressing earlier time-points will examine whether or not a pro-inflammatory response in the caput epididymidis is essential for effective protection from UPEC.

P 13.3

Medice, cura te ipsum: case report of a first-person experience with male infertility

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Case report The male partner accounts for almost 50% of cases of couple infertility, as either the only or a contributing factor. Genetic causes are considered responsible for 15% of cases, and are most frequently associated with severe oligozoospermia or azoospermia. In the present case report, the Author – an andrologist mostly working on male infertility and sexual dysfunctions – describes his own experience with the diagnosis of male infertility, and the relevant psychological consequences, as well as the consequent changes in patient management learnt from this experience. The Author/patient had a three years history of failing to achieve a clinical pregnancy with a healthy partner two years younger than him, despite normal semen analysis (sperm count: $98 \times 10^6/\text{ml}$, 3.2 ml, total 313.6×10^6 ; total motility 60%; normal sperm morphology: 13%) and normal hormone levels (FSH 2.71 mIU/mL, LH 5.48 mIU/mL, inhibin B 188 pg/mL, total testosterone 27.65 nmol/L). The Author/patient and his partner sought fertility treatment: during the course of pre-FIVET evaluation, both underwent karyotype analysis, and a paracentric inversion of chromosome 15 [46,XY,inv(15)(q15q24)] was found, explaining the cause of male infertility. While pregnancy is largely feasible via preimplantation genetic diagnosis, receiving the diagnosis had severe psychological consequences for the Author/patient, with the onset of moderate anxiety (General Anxiety Disorder-7 scores increased from 4 to 13) and depression (Patient Health Questionnaire-9 increased from 1 to 7) which lasted for several months. Diagnosis also changed the Author's approach to infertile patients, with an increased tendency to investigate genetic causes of couple infertility, and to suggest assisted reproduction techniques earlier than before in cases of unexplained infertility.

P 13.4

Prolactin in patients of a specialized andrology clinic

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Introduction The role of prolactin in male reproductive function is less known compared to its role in female reproduction, however the measurement of prolactin in the investigation of male infertility is considered necessary. Our aim was to assess the prevalence of hyperprolactinemia – as a possible cause of male infertility – in the laboratory work-up required in patients attending a specialized andrology clinic for infertility or erectile dysfunction.

Materials and Methods We studied 656 men, with mean age (SD): 34 (11) years, who presented at the andrology clinic either due to dyspermia ($n = 249$) or due to erectile dysfunction ($n = 396$); plasma prolactin was measured in 292 men. Assessment of normal/elevated values ($> 30 \text{ ng/mL}$) of prolactin (as the dependent variable), and the patients' group (individuals with dyspermia/erectile dysfunction), plasma testosterone & gonadotrophins, as well as age (as covariates/factors), was done with the chi square test & logistic regression.

Results In men with dyspermia 121 had prolactin within normal limits and 6 had increased levels, while in men with erectile dysfunction 149 had prolactin within normal limits and 16 had increased levels ($p = 0.17$). No effect of age, testosterone or gonadotrophins on prolactin was noted ($p = 0.47$).

Conclusion The measurement of prolactin is often considered to be necessary in the investigation of male factor infertility, however, it is not often found to be high due to pathological causes (prolactinoma, secondary hypogonadism).

P 13.5

The (patho)physiology of CatSper Ca^{2+} -channels in human sperm

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Introduction Male infertility is often due to low sperm counts, impaired motility, and/or abnormal morphology. However, for about 30% of infertile men, semen parameters are normal, and infertility is rather due to an unexplained sperm dysfunction. The underlying mechanisms are enigmatic. We surmised that human sperm dysfunction involves the Ca^{2+} -channel CatSper that translates changes in the chemical microenvironment into changes in swimming behavior.

Patients and Methods We developed a rapid test to assess CatSper activity in sperm from patients undergoing a fertility workup. We characterized seven patients and their sperm identified as CatSper-deficient using genetic tests, audiometry, semen analysis, electrophysiology, Ca^{2+} -fluorimetry, and motility analysis.

Results We show that all seven patients feature a similar genetic aberration, i.e. a

homozygous deletion of *CATSPER2*. The patients' sperm lacked functional CatSper channels, but according to standard semen analysis, the patients were normozoospermic. Thus, a loss of CatSper function escapes current methods used to assess male fertility. The *CATSPER2*-deficient patients failed to conceive naturally, upon IUI, and/or upon IVF; conception was achieved only through ICSI. We show that this is due to the failure of CatSper-deficient sperm to hyperactivate, wherefore the sperm fail to penetrate the egg coat. Remarkably, four of the seven patients also suffer from a deletion of the *STRC* gene and, thus, from the deafness-infertility syndrome.

Conclusion We conclude that human sperm dysfunction may involve loss of CatSper function, that CatSper-deficient sperm fail to penetrate the egg coat, and that the homozygous deletion of *CATSPER2* represents the most common genetic cause underlying loss of CatSper function. Our results also demonstrate that a deletion of *CATSPER2* is often, but not always, associated with a deletion of *STRC*, causing the deafness-infertility syndrome.

P 13.6

The role of activin A and chemokines in the development of inflammatory responses during chronic testicular inflammation in mice

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Experimental autoimmune orchitis (EAO) serves as a model of autoimmune-based chronic testicular inflammation and shares many features in common with male subfertility and infertility. EAO is characterized by severe immune cell infiltration into the interstitium, testicular fibrosis and an increase in the production of pro-inflammatory cytokines and chemokines, including TNF, IL-6, activin A and CCL2. An increase in $\text{CD4}^+\text{CD8}^+$ double positive T cells also has been observed in the EAO testis. However, the influence of CCL2 and activin A on the testicular immune response remains unclear.

Preliminary results using azan and Giemsa staining established that *CCR2*^{-/-} mice, defective in recruitment of monocytes during inflammation, are protected from EAO damage. Since activin A is increased in orchitis, its effect on bone marrow derived macrophages (BMDM) as an in vitro surrogate for testicular macrophages was investigated. Results from five independent experiments show that activin A (50 ng/ml) decreased mRNA expression of CCL7 and CCL8 ten-fold and increased CXCL16 and CXCL14 3-fold and 60-fold, respectively.

Our preliminary analysis by flow cytometry revealed that the treatment of murine single-cell suspensions of splenocytes with activin A for 4 days led to a proportional increase in CD3+ T cell numbers. Within the population of T cells, we observed a decrease in CD4+ T cells and an increase in CD8+, CD8+CD25+ and CD4+CD25+ T cells. Further analysis by flow cytometry showed that activin A added for 2 days to phorbol-12-myristate-13-acetate (PMA) and ionomycin-activated splenocytes increased the expression of IL-17A in CD8+ T cells, TNF, IFN- γ and IL-17A in CD4+ T cells and IL-17A, TNF, IFN- γ and IL-10 in CD4+CD8+ double positive T cells.

Our initial observations identify a role of activin A and CCL2 in altering the immune cell phenotype, where activin A shows potential to regulate the chemokine network, and CCL2 plays a crucial role in exacerbating testicular inflammation.

P 13.7

Development of a multivariable prediction model for congenital unilateral absence of the Vas Deferens (CUAVD)

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Introduction CUAVD is a rare condition that is associated with a high risk of CFTR mutations and/or unilateral renal agenesis. No seminal profile is specific of this condition and the gold standard for its diagnosis is transrectal ultrasonography (TRUS). The aim of this study was to develop a multivariable prediction model for CUAVD in a cohort of male partners of infertile couples.

Patients and Methods We conducted a retrospective review of medical records of 147 patients that had TRUS in a single tertiary-centre. Amongst them, 69 had CUAVD and 78 were controls. Thirty predictors were explored through uni- and bivariate analysis. Selected predictors were used in a complete multivariable logistic regression model.

Results Sperm concentration, total sperm number, sperm progressive motility and vitality were not significantly different between CUAVD patients and controls. CUAVD patients presented a lower semen volume than controls (1.5 vs 2.4 mL respectively, $p < 0.000$). However, no difference of histories of fertility was recorded (23 vs 32% of previous successful pregnancies respectively, $p = 0.23$).

After variables selection, we obtained a final multivariate model based on the three following predictors: history of cryptorchidism, total seminal fructose below 13 mmol/ejaculate, total seminal alpha-1,4 glucosidase (mUI/ejaculate). The ROC curve of the model presented an AUC value (95%-CI) of 0.866 (0.808–0.924). This was in favour of a good selection between cases and controls. The model was then internally validated by bootstrapping.

Conclusion Despite several limits (retrospective design, no external validation), this multivariable prediction model presented a good discrimination and open the way to a screening test for CUAVD.

P 13.8

Are tests for sperm antibodies useful in clinical routine to identify men with immunologic cause for infertility?

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Background The testing for ASAB (anti-sperm antibodies) has become part of routine workup of an infertile patient since an article in 1952 described a test for “antisperm agglutinins”. However, in the last years there has been a decline in published research articles indicating diminishing interest in this topic. Moreover, the production of some commercial kits ceased, resulting in issues with inhouse preparation of reagents, validation and external quality control.

Objective and Rationale A literature review of original research articles and review articles published in the last 20 years that use tests for ASAB as a method to identify men with immunologic cause for infertility.

Search Methods The MEDLINE database was searched from inception until August 2020 with following search terms: “antisperm”, “antibody”, “mar” and “asa”, combined with “sperm”. A deselection of the articles was performed by both investigators separately, the differences were discussed to prevent any bias. Deselection criteria were: published before the year 2000, fulltext not available in English, other than human spermatozoa, publications not answering the objective of this study.

Outcomes Available data does not support, that ASAB testing is an essential part of the routine workup for infertility in an individual patient:

1. Until present day, no causative treatment for ASAB has been found.
2. Presence of ASAB does not appear to influence the success of ICSI treatment of the infertile couple.
3. Screening tests rely on the presence of motile spermatozoa, meaning that cytotoxic antibodies that cause death of the spermatozoa cannot be detected with these methods.

Wider implications Screening tests for ASAB should no longer be used in the routine workup of an infertile patient.

P 13.9

Impact of genetic risk scores on association between childlessness and the risk of cardiovascular mortality

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In a population-based cohort of 22,000 men gathered in the 1970s, we previously showed that childlessness can be regarded as a predictor of cardiovascular disease (CVD) associated mortality independently of other well-known risk factors. Before the era of assisted reproduction sub-fertile men remained childless, why the latter condition can be considered as proxy of poor male fertility. We wished to explore if the higher risk for CVD mortality among childless, and presumably infertile men is due to a more common inheritance of genetic risk for CVD or, if not, to assess if the two factors work synergistically as predictors of CVD mortality.

We used data from a population-based cohort of 2130 middle-aged men from Southern Sweden, specifically designed to study the epidemiology of CVD. Among them 311 (14%) were childless at the age of 45 years. Two sets of genetic risk scores (GRS) based on either 27 or 50 single nucleotide polymorphisms (SNPs) – GRS27 vs. GRS50 – were tested. In previous studies each of the included SNPs has been shown to be associated with CVD at genome-wide significance level. The GRS of each individual in the study was calculated based on the previously reported risk estimate of each SNP. Six different study categories according to fatherhood status (+/-) and 3 groups of GRS – high, intermediate or low genetic risk – were defined.

GRS distribution did not differ between fathers and childless men ($p = 0.29$ for GRS 27 and 0.49, for GRS 50). However, high GRS was a stronger predictor for CVD mortality among childless men (HR: 3.12 [95%-CI: 1.39–7.04 for GRS50] and HR: 3.73 [95%-CI: 1.75–7.99 for GRS27]), than it was case among fathers (HR: 1.92 [95%-CI: 1.1–3.36 for GRS50] and HR: 1.54 [95%-CI: 0.87–2.75 for GRS 27]).

Thus, combining GRS with fertility status may improve identification of high-risk group for CVD mortality and, thereby, help defining individuals who might benefit from early preventive measures aiming to improve the lifespan and quality of life.

P 13.10

Does the FSHB c.-211G > T polymorphism impact the spermatogenic potential in infertile male patients?

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Introduction Follicle stimulating hormone (FSH) is a key driver of spermatogenesis in the male. Via the FSH receptor it binds at Sertoli cells (SC), which play a decisive nursing role for maturing germ cells. A single nucleotide polymorphism (SNP) (rs 10835638 *FSHB* c.-211 G > T) reduces the promoter activity, thereby leading to reduced FSH values and lowered sperm counts. The histo-pathological mechanisms behind this have not been studied so far. This project aimed to determine the impact of the *FSHB* c.-211 G > T SNP on human spermatogenic efficiency. Testicular tissue of azoospermic patients homozygous for the T- allele was analysed and results compared to patients homozygous for the G- allele with respect to their spermatogenic potential.

Patients and Methods 62 patients homozygous for T or G, from our inhouse database were selected, with testicular biopsies available. Patients were subgrouped into obstructive azoospermia (OA) vs. non-obstructive (NOA) and adjusted according to their genotype: NOA: n = 26 with GG, n = 26 with TT; OA (serving as controls): n = 5 with GG, n = 5 with TT). Immunohistochemistry was performed applying the following antibodies: SOX9 for SC, MAGE A4 for spermatogonia and CREM for round spermatids (RS). Quantification of distinct germ cell types were carried out using the Vectra® microscope by Perkin Elmer, allowing subsequent calculation of the Sertoli cell Workload (SCWL), defined as the number of germ cells served for by one SC, was calculated.

Results There were no significant differences in SC-, spermatogonia- and RS- numbers between patients carrying the wildtype *FSHB* c.-211 G > T (GG) and those with two T-alleles (TT) within the NOA cohort. Neither was the SCWL significantly different between the genotypes.

Conclusion The polymorphism *FSHB* c.-211 G > T does not limit the spermatogenic potential by lowering Sertoli cell numbers or the Sertoli cell workload.

Grants Funded by DFG CRU 326.

P 13.11

Association of seminal plasma infections with oxidative stress

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Introduction Oxidative stress in seminal plasma can negatively affect sperm function, hence it can reduce male fertility. Infections are able to increase local concentrations of reactive oxygen species (ROS). Recent studies describe the assessment of the oxidation-reduction potential (ORP) as feasible method to measure simultaneously both oxidants and antioxidants. However, the association of seminal plasma infections with ORP in se-

men samples and the putative impact of subsequent antibiotic therapy is unknown.

Aim To study the association of seminal plasma infections and antibiotic therapy with ORP in semen samples of infertile men.

Methods We prospectively measured the ORP in semen samples of 25 idiopathic infertile men who visited our Center of Reproductive Medicine and Andrology for control visits after having been diagnosed as idiopathic infertile. In addition, to standard semen analysis according to WHO guidelines, we performed seminal bacterial analysis and additional sperm function tests, e.g. DNA fragmentation index (DFI), CASA motility assessment and used the MiOXSYS® System (Aytu Bioscience, Engelwood, CO, USA) for ORP analysis. Results of ORP were then compared with the results of all semen parameters, specifically in relation to a coexistence of seminal plasma infections.

Results Preliminary data exhibited a significant association of ORP with acute infections in seminal plasma in 2 of 25 patients (mean ORP: 16.35 vs. 2.83 mv/10⁶ sperm, p < 0.001) and a relevant increase in ORP after antibacterial treatment in another 3 patients, while no association was seen for standard semen parameters and DNA fragmentation in this study population.

Conclusion We want to study the association of ORP with seminal tract infections in a larger cohort and follow up the ORP after antibacterial therapy. ORP might prove as a useful marker indicating recovery of sperm function after treatment of seminal infections.

P 13.12

Effect of bariatric surgery on reproductive hormones and semen quality

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Introduction Excess of adipose tissue would produce greater conversion of testosterone into estradiol leading to secondary hyperestrogenic hypogonadism hypogonadotropic with possible effects in the production of reproductive hormones (RH) and spermatogenesis. Our aim was to evaluate the RH and seminal quality (SQ) of young obese patients that have undergone bariatric surgery.

Material and Methods This prospective matched study was performed in 9 young (40). Patients were evaluated before and 6 to 10 months after bariatric surgery. The SQ was analyzed according to WHO (2010) using a computerized motion analysis system. Normal forms and deformity index was assessed

by Strict Criteria. DNA fragmentation index (FI) was assessed by the Sperm Chromatin Dispersion tests and nuclear immaturity (IN) by Aniline blue. The RH (FSH, LH, albumin, Total, free and bioavailable Testosterone, sex hormone-binding globulin (SHBG) and Estradiol) were determined by ECLIA. All patients signed a written consent. Data were showed as median and interquartile range and analyzed by Wilcoxon signed rank test. A p < 0.05 was considered significant.

Results A decreased in post-surgery values was observed in: BMI 45.4 (8.1) vs 29.0 (5.4) kg/m², p = 0.003; semen volume 3.5 (0.5) vs 3.0 (1.5) mL, p = 0.04; total sperm count 218 (186) vs 74 (170) × 10⁶ sperm, p = 0.02; curvilinear velocity 58.7 (6.8) vs 55.0 (16.40) um/s, p = 0.02 and amplitude of lateral head displacement 3.1 (0.3) vs 3.0 (0.8) um, p = 0.01. No significant differences on sperm progressive motility, viability, normal forms, deformity index, FI and IN were found. RH showed an improvement in FSH 2.7 (1.3) vs 3.3 (1.6) (mIU/mL), p = 0.02 and SHBG 27.4 (20.0) vs 69.4 (58.7) (nmol/l) p = 0.007. Variation in BMI correlates positively with semen volume (r: 0.6, p = 0.05) and with SHBG (r: 0.6, p = 0.01).

Conclusion Semen quality may not be improved after bariatric surgery despite successful weight loss and the improvement of reproductive hormones. Further studies are required to discard possible nutritional deficiencies and/or very near post surgery evaluation as causing the effects observed on spermatogenesis (Fig. 12).

P 14.1

Sperm retrieval and cryopreservation in testicular torsion patients: Benefits and limitations

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Purpose To assess the value of sperm retrieval and cryopreservation in postpubertal testicular torsion (TT) patients.

Patients and Methods This was a prospective study conducted on postpubertal males with TT. Surgical exploration was urgently performed and detorsion with fixation or excision was done according to the intra-operative assessment of the viability of testicular tissue. Also, the contralateral testis was fixed to prevent future torsion. Conventional or microsurgical sperm retrieval was tried in all cases with the intention of cryopreservation. Patients came back to clinic 1 week after surgery and monthly thereafter for 3 months with new hormonal assay and sperm analysis. Sperm retrieval rate, changes in hormonal and sperm parameters were reported to assess the potential benefit of sperm retrieval in these patients at the time of scrotal exploration.

Results Out of the 99 patients initially assessed in this study, a total of 62 were eligible to be enrolled. The mean age was 19.5 ys. A total of 20 testicular units could be salvaged

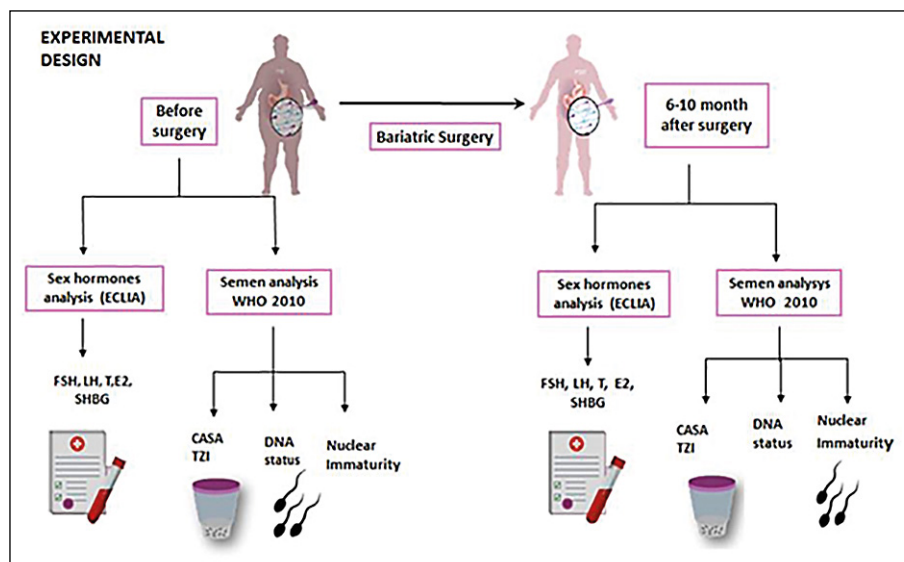


Figure 12. M. C. Craia, et al.

(32.3%). Successful sperm retrieval was achieved in 93.5% of patients. On follow up, 33.8% of patients developed azoospermia and a similar proportion showed OAT.

Conclusions Pubertal TT patients may develop azoospermia after surgical intervention and should benefit from sperm retrieval and cryopreservation.

P 14.2

Does Anti-Müllerian hormone have a place in the management of azoospermia? A single-center study of 134 patients

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Background The aim of the present study is to evaluate the contribution of serum Anti-Müllerian hormone (AMH) in the management of infertile men with azoospermia as well as the prediction of the outcome of surgical sperm extraction (TESE).

Materials and Methods Clinical data and hormonal profiles (FSH, LH, inhibin B, AMH, and total testosterone (T) plasma levels) from 134 men with azoospermia submitted to TESE, between 2017 and 2019 in Lille University Hospital, were retrospectively analysed. Patients were classified according to the aetiology of azoospermia and the result of sperm retrieval (SR) at TESE.

Results AMH plasma levels were significantly lower in unexplained, cryptorchidism and genetic nonobstructive azoospermia (NOA) compared to obstructive azoospermia (OA) (medians = 28.3; 21.6; 7.2 and $p = 0.04$; $p = 0.002$; $p < 0.0001$, respectively). Lowest values were observed in cases of genetic NOA ($p = 0.005$ compared to unexplained NOA). A significant positive correlation was observed between AMH, T and inhibin B in all subgroups of NOA. The best sperm retrieval rate (SRR) was obtained in OA (100%). Among NOA subgroups, cryptorchidism had the best SRR (13/22; 59.1%). The lowest SRR were

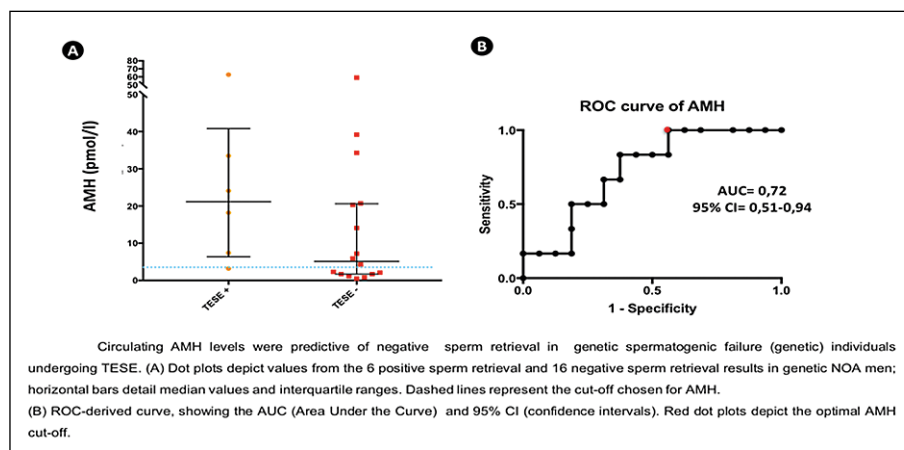


Figure 13. H. Benderradi, et al.

observed in cytotoxic NOA (1/8; 12.5%) and in genetic NOA (6/22; 27.3%).

Only AMH serum levels was predictive of a negative TESE results in genetic NOA (figure 1-A). Using the Receiver Operating Characteristic (ROC), we calculated a cut-off for serum AMH (below 2.6 pmol/l) which allowed us to predict the negative SR with 100 % sensitivity (Fig. 13).

Conclusions: Our results suggest that serum AMH could help us to predict the negative results of sperm retrievals at TESE in genetic NOA, and on the other hand, plasma AMH is a good marker of Sertoli cell alteration's function and it decrease is related to the origin of NOA and specially in genetic NOA which was associated with negative results of sperm retrievals at TESE.

P 14.3

Centriolar defects, centrin 1 alterations and FISH studies in human spermatozoa of a man of a couple that produces aneuploid embryos in natural and artificial fertilization

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The large maternal contribution to embryo aneuploidies is known and overshadows the clinical relevance of the spermatozoon resulting in a fair consideration of paternal influence to this phenomenon.

We studied sperm from a man of a couple that obtained aneuploid embryo with natural and assisted fertilization. After conventional sperm evaluation, repeated three times, immunofluorescence analysis (IF) for localization of tubulin and centrin 1 and ultrastructural study by transmission electron microscopy (TEM) were carried out. The sperm chromosomal aneuploidies were assessed by fluorescence in situ hybridization (FISH) analysis for chromosomes 18 and 9. Sperm of a proven fertility man were used as control. Sperm concentration ranged from 20 to 35.5×10^6 sperm/ml, progressive motility from 18% to 25%, normal morphology from 3% to 4%, viability was 70%–75%. In a discrete percentage of cells, an irregular head-tail attachment was observed together with the presence of isolated and coiled tails.

Centrin 1 was rare in sperm of the patient, whereas in sperm from control it was visible as two distinct spots in the head-tail junction. The labeling with anti-tubulin antibody indicated that the 80% of patient's sperm showed two spots in the connecting piece area, whereas control sperm displayed a single spot, indicating the proximal centriole. By TEM analysis we demonstrated that the second spot detected in patient's sperm was the centriolar adjunct, that appeared very long, distinctive tract of human sperm immaturity. FISH analysis revealed high frequencies of

sperm aneuploidies: 18 disomy 1.5%; 9 disomy: 2.1%, diploidy: 1%.

In conclusion, the sperm of this naturally fertile patient showed alteration in the connecting piece, immature centriolar adjunct and scarce presence of centrin 1, concomitant with high frequency of aneuploidies, for this reason a paternal contribute to sperm aneuploidies cannot be ruled out.

P 14.4

The functionality of the sperm cell in the presence of antisperm antibodies

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Introduction The presence of antisperm antibodies (ASA) has been described as a significant contributor to male infertility. Yet, the literature in this topic is scarce and controversial, being the clarification of the exact impact of ASA on sperm and male reproductive function imperative. Our aim is thus to characterize the sperm function in ASA patients, using a comprehensive and integrated approach.

Materials and Methods Human semen samples were divided in 4 groups based on the presence of antisperm antibodies (IgG and IgA; MAR test): (1) ASA > 50%, pathological group; (2) ASA 10%–50%; (3) ASA < 10%; (4) without ASA. Several sperm functional parameters were assessed microscopically: motility, morphology, viability (eosinY), chromatin status (Diff-Quik), acrosomal integrity (PSA-FITC) and capacitation status (phosphorylated tyrosines); and by flow cytometry: mitochondrial membrane potential (JC-1) and superoxide production (MitoSOX-red).

Results The data collected so far has showed a 3% prevalence of ASA pathological patients in our center. Even with a reduced number of samples in the pathological group, it was clear that besides some conventional parameters such as viability and motility, also sperm capacitation was impaired in the presence of ASA. Additionally, the sperm mitochondrial function of these patients seem to have some dysfunctions. In fact, our preliminary data has showed a tendency towards a higher mitochondrial superoxide production in ASA patients and more data is being collected to allow for more definitive conclusions.

Conclusions Overall, we have obtained relevant and new information regarding the effects of ASA on sperm function, identifying new compromised aspects in these patients.

Variables over the entire population (n=70)		SCI n=30	Neuro n=12	PS n=9	Blad. n=3	MN n=5	Iatrog n=3	Psy n=8	Total n=70
Before treatment									
Ejac., n (%)	AE	6 (21%)	2 (17%)	5 (56%)	1 (33%)	4 (80%)	0	3 (38%)	21 (30%)
	RE	2 (7%)	7 (58%)	1 (11%)	2 (67%)	0	0	3 (38%)	15 (21%)
Medical treatment									
PVS only, n (%)		9 (30%)	0	0	1 (33%)	3 (60%)	1 (33%)	0	14 (20%)
PVS + midodrine, n (%)		25 (83%)	2 (17%)	2 (22%)	0	2 (49%)	0	0	31 (44%)
Midodrine only, n (%)		1 (3%)	9 (82%)	7 (78%)	2 (67%)	1 (20%)	2 (67%)	8 (100%)	30 (43%)
Results of medical treatment									
Attempt, median [IQR]		2 [2-3]	1 [1-2]	2 [1-3]	2 [1.5-2.5]	2 [2-2.5]	2 [1.5-3]	1.5 [1-2.2]	2 [2-3]
Ejac, n (%)	AE	18 (60%)	7 (58%)	3 (33%)	2 (67%)	1 (20%)	1 (33%)	3 (38%)	35 (50%)
	RE	8 (27%)	4 (36%)	4 (44%)	1 (33%)	0	1 (33%)	5 (62%)	23 (33%)
	total	26 (87%)	11 (92%)	7 (78%)	3 (100%)	1 (20%)	2 (67%)	8 (100%)	58 (83%)
Ejaculated sperm cryopreservation, n (%)		19 (63%)	9 (75%)	6 (67%)	3 (100%)	0	1 (33%)	7 (88%)	45 (64%)
Surgical treatment									
TESE, n (%)		6 (20%)	1 (8%)	1 (11%)	0	2 (40%)	1 (33%)	1 (12%)	12 (17%)
Results of surgical treatment									
Surgical sperm cryopreservation, n (%)		4 (13%)	1 (8%)	1 (11%)	0	1 (20%)	0	0	7 (10%)
Results of medical and surgical treatments									
Cryopreservation rate, n (%)		23 (77%)	10 (83%)	7 (78%)	3 (100%)	1 (20%)	1 (33%)	7 (87%)	52 (74%)
Samples, median [IQR]		7 [2-11]	9 [5-16]	7 [5-14]	8 [5-13]	0 [0-0]	0 [0-4]	11 [10-16]	8 [0-13]

SCI: spinal cord injury, Neuro: neurologic disease, PS: pelvic surgery, Blad.: bladder neck incompetence, MN: myelomeningocele, Iatrog: iatrogenic, Psy: psychogenic, Ejac: ejaculation, AE: antegrade ejaculation, RE: retrograde ejaculation

Figure 14. M. Deknuydt, et al.

Nevertheless, the exact impact of ASA in the sperm function warrants further investigations and we now focusing on different approaches to obtain new insights on the impact of these antibodies on male fertility.

P 14.5

Non-invasive management of ejaculatory dysfunction in patients referred for sperm cryopreservation: a retrospective study

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Background The non-invasive management of ejaculatory dysfunction (ED) that impact fertility is poorly documented in literature. A sperm retrieval procedure using penile vibratory stimulation (PVS) and/or midodrine was studied in patients with ED causing infertility whatever the cause.

Results Data about sperm retrieval were retrospectively collected for 70 patients with ED referred to Andrology Department of Lille University Hospital for fertility preservation or before assisted reproductive

technique (ART) between January 2015 and October 2018. Semen was collected by PVS if a neurological lesion impaired the glans sensitivity. If PVS failed, it was combined with midodrine. Midodrine only was used in all other causes of ED. A testicular sperm extraction (TESE) was performed as a last resort. Ejaculation occurred in 58 of 70 patients (83%) after midodrine and/or PVS with a median of 2 attempts (Interquartile Range [IQR] 1–2). The most represented cause was spinal cord injury (SCI) with an ejaculatory rate (ER) of 87%. The ER and the cryopreservation rate were significantly increased when the level of lesion was above T10 segment. The ER was of 92% in neuro-pathic diseases. It reached 100% in case of bladder neck incompetence or in psychogenic AN, explaining the high level of ejaculated sperm cryopreserved. After a pelvic surgery (PS), the ER rate was of 78%. The use of a psychotropic treatment was associated with 67% of ejaculation despite midodrine. Poorer results of ER (20%) were obtained from patients with MN who could not cryopreserve sperm. Specimens from 45 of 58 patients who ejaculated (78%) were frozen. TESE allowed semen cryopreservation in 7/12 (58%) more patients. 52 patients have frozen sperm with a median of 8 samples (IQR 0–13). Ten pregnancies occurred. The pregnancy rate (PR) per cycle and per embryo transfer were of 28% and 23% respectively.

Conclusion The non-invasive management of ED using PVS and/or midodrine was associated with an ER of 83% (Fig. 14).

P 14.6

Effect of vas ligation on TESE results in non obstructive azoospermic rats

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Introduction To this date, there is neither a method to predict the results of sperm retrieval in patients with non-obstructive azoospermia, nor methods to enhance its results, with the exception of cases with hypogonadotropic hypogonadism and large grade varicoceles. This work evaluates a proposed method for enhancing sperm accumulation (not sperm production), by ligation of the vas deferens, in an effort to enhance TESE results in NOA rats by sperm accumulation.

Materials and Methods Sixty mature male rats with equally sized testes were included in this study. 50 were in the study group and 10 were in the control group. Bilateral testicular biopsies were performed for the 60 rats to confirm equal spermatogenesis in all testes. Non-obstructive azoospermia was induced using DNG (40 mg/kg body weight [bw]) + TU (25 mg/kg bw) every 30 days for three months. Monthly FNA's confirmed NOA from all rats. All rats became azoospermic after three months. Rats of the study group were divided into 2 subgroups. In the first group, ligation of the right vas was performed (24 rats). In the second group, ligation of the left vas was performed (24 rats). Two rats died afterwards, one from each group. On the other hand control group rats were not ligated. After a period of 90 days (2 spermatogenic cycles), TESE was performed for the 56 rats in the study group (46 rats) and rats in the control group (10 rats). The hormonal injection proceeded till the final TESE and scarification of all rats in all groups.

Results On day 90 TESE was performed for all rats. Sperm retrieval was compared between different groups. Number of sperms and round spermatid in biopsies were measured. These numbers were significantly higher in the ligated side when compared to the other side or to the control group.

Conclusion Ligation of the vas deferens in rats with NOA could enhance the results of sperm retrieval.

P 14.7

Oxytocin as a new treatment option for ejaculatory disorders – S19 of the rat epididymis as a testing model

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The adult rat epididymis can be segmented into 19 segments with S19 being the most distal part where the sperm is stored. During ejaculation the sperm is driven forward through the vas deferens by strong contractions of the cauda epididymis. Patients with benign prostatic hyperplasia (BPH) medicated with alpha-1 receptor adrenergic blockers often suffer from ejaculatory disorders.

We investigated the contractile response to oxytocin (OT) (500 nM) and norepinephrine (NA) (10 µM) in multiple segments of the rat epididymis by using live-imaging with special focus on the last two segments (S18 and S19). The response of S19 to three different doses of OT (1 nM, 10 nM, 100 nM) was tested. The effects of two OT-antagonists (atosiban and cligosiban), an arginine vasopressin antagonist (SR 49059), an alpha-1 blocker (tamsulosin) and a PDE5 inhibitor (tadalafil) on S19 were tested separately.

Both OT and NA increased contractions throughout the rat epididymis. In S19 both OT (dose-dependently) and NA were able to induce a very strong and complex series of contractions which could not be found in any other segment investigated. Atosiban or cligosiban were able to completely block the strong reaction of S19 to OT (500 nM) while SR 49059 was only able to block it partially. Tamsulosin could completely block the strong reaction of S19 to NA (10 µM) while tadalafil could not. OT was then still able to induce that strong reaction in the adrenergically blocked S19.

In S19 of the rat epididymis OT had a similar strong effect to NA. OT was still able to contract tissue important for the ejaculatory process which had been unresponsive to NA. This could indicate that OT-based medication might be a great treatment option for ejaculatory disorders, especially in cases of pretreatment with alpha-1 blockers such as tamsulosin which is common in patients with BPH. S19 might be a great model to test new medication for the treatment of ejaculatory disorders and BPH.

P 14.8

Sperm morphology and motility in mice and men: Investigations of defective male germ cell differentiation

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Male infertility can be caused by disturbances of sperm count, morphology and motility amongst other reasons. About 30–40% of

male infertility cases are caused by genetic abnormalities such as chromosomal defects or gene mutations, leading to decreased germ cell production or function. Somehow, only a few clinically relevant gene mutations/polymorphisms or novel genes have been identified so far. This study aims to identify novel genes related to sperm morphology and motility by next generation sequencing.

Testicular biopsies from azoospermic men were classified as normal spermatogenesis (NSP, n=3), spermatid arrest (SDA, n = 4) and Sertoli cell only syndrome (SCO, n = 3) by histology. RNA was extracted from adjacent cryopreserved biopsies followed by RNA-Seq (Illumina NextSeq 500 sequencer).

Differentially expressed genes (DEGs, filter for significance: base mean ≥ 5 , $-\log_{10} p \leq 0.05$) were NSP Vs SCO–10,253, NSP Vs SDA–1,873, SDA Vs SCO–4,017 and filtered based on Gene Ontology and KEGG pathway terms related to “spermatogenesis”, “calcium pathway”, “cAMP pathway”, “flagella proteins” and highly down-regulated DEGs between NSP Vs SDA. From which, 11 genes potentially involved in sperm morphology and motility have been identified: CFAP47, PDE4A, ZP1, SLC9C1, SLC8B1, SPATA31E1, ORAI1, CACNB2, TNC, TEK3, and TMEM37. Genes were validated using RT-PCR and initial immunohistochemistry (IHC) for ORAI1 and SPATA31E1 were performed. Further experiments for IHC and quantitative RT-PCR using different pathologies will narrow down the list of potential genes involved in defective male germ cell differentiation and to reveal its biological significance. Additional experiments utilizing high-resolution quantitative mass spectrometry (Orbitrap Eclipse) will be performed using ejaculates showing either normal or disturbed motility/morphology.

Grants Supported by DFG GRK 1871/1

P 14.9

Genetics and spermatology in CBAVD patients

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Materials and Methods A group of 73 unrelated Russian men of reproductive age with CBAVD syndrome. The average age was 31.1 ± 7.1 years. The examined men did not have any karyotype abnormalities or clinically significant microdeletions of the long arm of the Y chromosome.

Results The sample of men was divided into 2 groups: with mutations in the *CFTR* gene and without disorders in this gene. A comparative analysis of hematological parameters between these two groups was performed.

The volume of ejaculate samples studied in groups I and II varied from 0.2 to 2.0 ml

and 0.3 to 1.5 ml, with an average of 0.73 ± 0.42 ml and 0.70 ± 0.31 ml, respectively. Oligospermia was observed in group I in 56 (94.9%) patients, in group II – in 12 (92.3%) patients.

The viscosity of the ejaculate samples significantly varied from normal (1–20 mm) to elevated values (up to 100 mm), and averaged 12.3 mm for the group of patients with CFTR gene mutations and 25.6 mm for the group of patients without CFTR gene mutations, respectively.

The acidity of the ejaculate varied from acidic to alkaline (pH from 5.5–7.7). In the first group, only one male (who had the *mutCFTR/5T* genotype) had a normal pH value. In the second group of patients, the pH value was lower than normal and did not exceed 6.9.

The concentration of white blood cells in the ejaculate was within the reference values (not exceeding 1 million / ml), exceeding this indicator (leukospermia) was observed in 5 patients (6.76%).

Conclusion In the studied sample of men, spermatological signs of VAS deferens obstruction and aplasia/hypoplasia of seminal vesicles were found, which are characteristic of patients with CBAVD syndrome and more than 90% of men with CF: azoospermia, oligospermia, and a reduced pH. There were no significant differences in spermogram parameters between groups of patients with mutations and/or the 5T allele of the CFTR gene and without them.

P 14.10

Sperm recovery and ICSI outcomes in men with non-obstructive azoospermia: a systematic review and meta-analysis

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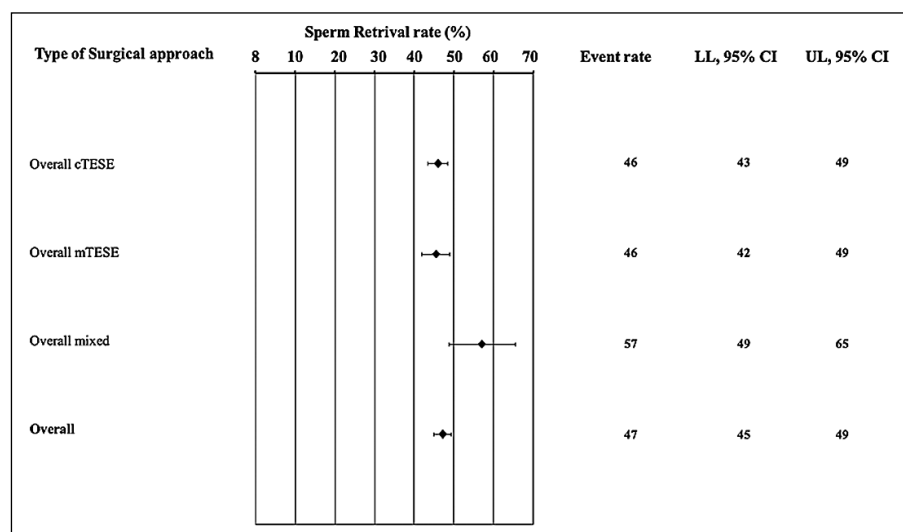


Figure 15. W. Vena, et al.

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Introduction Factors affecting sperm retrieval rate (SRR) or pregnancy rates (PR) after testicular sperm extraction (TESE) in patients with non-obstructive azoospermia (NOA) have not been systematically evaluated. In addition, although micro-TESE (mTESE) has been advocated as the gold standard for sperm retrieval in men with NOA, its superiority over conventional TESE (cTESE) remains conflicting.

Materials and Methods An extensive Medline, Embase and Cochrane search was performed. All trials reporting SRR derived from cTESE or mTESE in patients with NOA and their specific determinants were included.

Results 117 studies met the inclusion criteria for this study, enrolling 21,404 patients with a mean age ($\pm$ SD) of 35.0 ± 2.7 years. cTESE and mTESE were used in 56 and 43 studies, respectively whereas 10 studies used a mixed approach and 8 compared cTESE with mTESE. Overall, a SRR per procedure of 47 % was found. No differences were observed when mTESE was compared to cTESE 46% for cTESE versus 46% for mTESE. Meta-regression demonstrated that SRR per cycle was independent of age and hormonal parameters at enrolment. However, the SRR increased as a function of testis volume (TV). In particular, by applying ROC curve analysis, a mean TV higher than 12.5 ml predicted SRR > 60% with an accuracy of 86.2%. A total of 1096 biochemical pregnancies were reported (cumulative PR=29% per ICSI cycle. No influence of male and female age, mean TV or hormonal parameters on both PR and LBR per ICSI cycle was observed.

Conclusions This analysis shows that cTESE/mTESE in subjects with NOA results in SRRs of up to 50%, with no differences when cTESE was compared to mTESE. Retrieved sperms resulted in a LR of up to 28% ICSI cycle. Although no difference between techniques was found, to conclusively clarify if one technique is superior to the other, there is a need for a sufficiently powered and well-designed randomized controlled trial to compare mTESE to cTESE in men with NOA (Fig. 15).

P 14.11

Factors associated with abnormal epididymo-testicular unit in a cohort of French boys operated for unilateral cryptorchidism

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Introduction Abnormalities of the epididymo-testicular unit (ETU) are frequently reported in cryptorchidism. The goal of this study was to identify the main factors associated with these abnormalities in order to gain more insight into their pathogenesis.

Patients and Methods A retrospective review of medical files of 396 boys undergoing orchiopexy for unilateral nonsyndromic cryptorchidism was conducted. ETU was classified as normal or abnormal (for both complete and incomplete dissociation). Adjusted odds ratio (aOR) with 95% confident intervals (95%CI) were calculated using multivariate logistic regression.

Results Abnormal ETU was reported in 121 boys (30.6%). They were younger than those with normal ETU (2.7 vs 4.8 year, $p < 0.001$). Age was associated with a lower probability of abnormal ETU (aOR 0.81; 95%-CI 0.73–0.90). Conversely, small testis and short spermatic cord length were associated with a high probability of abnormal ETU (aOR 5.40, 95%-CI 3.19–9.25 and aOR

4.69, 95%-CI 1.63–15.48, respectively). Open peritoneo-vaginal duct was associated with a lower probability of abnormal ETU (aOR 2.62, 95%-CI 1.44–4.93). However, testis position (external inguinal ring, inguinal canal or abdominal) was not associated with a probability of abnormal ETU.

Conclusion Our study pointed out some factors associated with abnormal ETU in unilateral cryptorchidism and shed light on involved mechanisms. Further research is needed to confirm these results.

P 14.12

Asymptomatic urogenital infections and their impact on male and female fertility

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This research focuses on asymptomatic urogenital infections (*Ureaplasma urealyticum*, *Mycoplasma hominis*, *Mycoplasma genitalium*, *Chlamydia trachomatis*) as a potential cause of infertility. These infections might have a negative impact on fertility by activating pro-inflammatory immune responses in both males and females. It is assumed that antibiotic treatment (ATB) suppresses the immune reaction, and thus increases the chance of conception. On the other hand, ATB might have a negative impact on the sperm quality.

Aim of the study To assess how urogenital infections and its treatment with ATB affect the quality of sperm. Five hundred males and their female partners from infertile couples were examined. Pathogens were diagnosed in urine samples using cultivation and PCR. In 243 couples (48.6%) at least one of the four infections was detected. All infected couples were treated with Doxycycline 100 mg bid for 10 days.

The couples were divided into 4 groups according to the presence/absence of any infection in the male/female partner. Interestingly, no significant difference in the quality of sperm was found among the groups. In 235 men (133 without infection and 102 after ATB treatment), a follow-up sperm analysis was performed 29–149 days later (median 78 days). Lower sperm concentration was found in the group after ATB treatment (at the border of significance, $p = 0.08$). No other significant difference was found.

Conclusion At least one asymptomatic urogenital infection is present in almost half of infertile couples. In women, it was previously found that ATB improves the chance of conception. In this study, we have not found any

benefit of ATB treatment on the sperm quality. However, we suppose that proportion of apoptotic sperm, as well as leucocyte count and antisperm-antibodies might decrease – that will be studied in the next phase of our study.

P 15.1

Sperm motility subpopulation structure in stallions: breed differences

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The introduction of computer-assisted semen analysis (CASA-Mot) systems allowed for an unprecedented degree of sophistication in the study of sperm kinematics patterns. The aim of this study was to analyze kinematic characteristics of two horse breeds and to calculate the subpopulation structure based on these parameters. A total of 42 fresh semen ejaculates (26 of Spanish breed and 16 Arabian breed) were collected by artificial vagina and subsequently analyzed for kinematic parameters using the ISAS[®]v1.2 CASA-Mot system and a Spermtrack[®] 10 µm depth reusable counting chamber. Eight kinematic parameters were evaluated automatically at 250 frames per second. All kinematic parameters showed significant differences among breeds. Spanish horse had higher VAP, VCL and BCF compare to Arabian horse. Spanish horse sperm was faster but Arab horse was more linear. Principal component (CP) analysis was done as the first step of subpopulation analysis, rendering three CP named velocity, linearity and oscillation. Subpopulation analysis showed three, being the dominant subpopulation for stallion that composed by fast, straight and lineal motility with a high beat spermatozoa (41.7%). Slight differences in the distribution of these three subpopulations were observed between Arabian and Spanish horses. In conclusion, higher frame rate of video capturing permitted to have new interpretation of the sperm kinematic subpopulation structure in stallion.

P 15.2

Equine sperm morphology analysis: alive vs dead stained sperms techniques

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The evaluation of the male fertility potential is based on the analysis of the basic spermatic characteristics (concentration, motility and morphology), as well as the seminal fluid.

Thus, the study of sperm morphology is a fundamental element in the seminal analysis, but its real meaning has been biased by the techniques used for its evaluation. These classical techniques involve dehydration and subsequent staining, which involves the production of artifacts. The objective of the present work was to compare two methods for equid semen morphology evaluation. The first one is the new technique Trumorph[®] analyzing the spermatozoa suspended in their seminal fluid and the second is eosine-negrosin staining technique. For that purpose, a total of 49 ejaculates from 20 stallions and 29 donkeys were used. Semen samples were obtained with artificial vagina. After semen collection and dilution, an aliquot was placed on the slide and covered with cover-slide that was placed in the Trumorph[®] device. The observation was made with a 40× negative phase contrast objective. Another aliquot was stained using eosine-negrosine stain, and analysed using 100× bright field objective. The general morphology was very similar in both species showing no significant differences in the total amount of abnormalities comparing both techniques. Nevertheless, the use of Trumorph[®] technique rendered a perfect differentiation of head (both acrosome and post-acrosome areas), midpiece and principal piece of the spermatozoa allowing to a better determination of morpho-abnormalities. So, we can conclude that Trumorph[®] could be a good and simple alternative for morphology analysis that use alive sperms avoiding artifact production.

P 15.3

Topological distribution of functional-biochemical features of spermatozoa by Raman Microspectroscopy

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Introduction Understanding the complex biochemistry of spermatozoa is critical to explore their pathophysiology. Since this complexity is ignored by conventional sperm analysis, alternative approaches have to be considered and implemented. In this regard, Raman Microspectroscopy represents an innovative tool for the assessment of biochemical features otherwise undetectable. Previously, using this technique, different sperm regions head, midpiece and tail, were easily disentangled based on their biochemical components and several organelle-related bands were identified. These novel readouts are of value for clinical settings especially if their specificity could be confirmed within the sperm structures. Thus, this study was carried-out to disclose the topological distributions of Raman organelle-specific features of sperm.

Materials and Methods Sperm suspensions from normozoospermic donors were smeared onto Suprasil slides. Raman multi-spectral mappings with signals of 14 spectral traits were recorded in all spermatozoal compartments.

Results The average spectrum of the sperm head is characterized by bands ranging from 600 to 1150 cm⁻¹, mainly associated with nucleic acids. Five bands at 1094, 1180, 1373, 1483, and 1509 cm⁻¹ showed to be specific to the post-acrosomal area of the nucleus. In the midpiece, the spectra are dominated by bands from 1150 to 1700 cm⁻¹, which are related to mitochondrial components. One band associated with cytochrome-C at 1546 cm⁻¹ showed high specificity for this area. The signals regarding the sperm tail, due to the structural aspects, tend to be too weak to be deeply evaluated.

Conclusions Raman bands associated with different functional elements were consistently distributed along specific sperm regions and represent potential markers of functionality related to DNA integrity and mitochondrial activity. Raman analysis has the potential to be considered as a new strategy for biochemical sperm assessment in infertile patients.

P 15.4

Development of a “Rapid-Mixing-CASA” technique to quantify transient ligand-induced motility responses in sperm populations

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Introduction For more than 30 years, computer-aided sperm analysis (CASA) has been used to quantify kinematics of sperm in both research and clinical environments. Replacing the subjective human eye with objective computation, CASA has provided unique insights into sperm physiology and the basics of reproduction. However, in vivo, the motility of sperm is modulated by chemical cues provided by the female to assist sperm in navigating across the oviduct and fertilizing the egg. In the past decade, a picture has emerged that these chemosensory motility responses are both rapid and transient. Due to their low time resolution, classical CASA approaches are ill-suited to study the ligand-control of sperm motility. We developed a kinetic, “Rapid-Mixing CASA” technique to study, in a quantitative fashion, ligand-evoked motility responses in human sperm.

Materials and Methods We combined the well-established CASA approach with an advanced microfluidics setup. After contacting sperm with a chemical stimulus by rapid mixing, a virtually instantaneous onset of microscopic data acquisition for motility analysis is enabled.

Results We used this “Rapid-Mixing CASA” setup to characterize motility responses evoked by chemical stimuli in human sperm.

Conclusion Embedded into an easy-to-operate automatic setup configuration, we envisage the common use of “Rapid-Mixing-CASA” to elucidate how human and other mammalian sperm translate changes in their local chemical microenvironment into changes in their swimming behavior.

P 15.5

Assessment of DNA fragmentation in live spermatozoa: a novel and rapid application of flow cytometry for sperm analysis

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Introduction Sperm DNA integrity has become one of the most discussed and promising biomarkers for the assessment of male fertility. However, an easy-to-apply method capable of evaluating DNA fragmentation in the live fraction of spermatozoa, which is the one used and wanted for assisted reproductive technologies (ART), has remained elusive, preventing this parameter from being fully applied in clinical settings. Herein we propose a novel, rapid and easy to perform co-staining for the analysis of DNA fragmentation in membrane-intact spermatozoa.

Materials and Methods Normozoospermic semen samples from donors (n = 10), were used to validate a co-staining consisting of Acridine Orange (AO) and LIVE/DEAD Stain (LD), against established methods for the evaluation of cell viability, propidium iodide stain (PI), and DNA fragmentation, the Sperm Chromatin Structure Assay (SCSA), to rule out cross-interference. Furthermore, the accuracy of the method was tested by the evaluation of samples prepared with different amounts of membrane and DNA damage.

Results No significant differences were observed between the co-staining and the established staining procedures (membrane integrity, P = 0.755; DNA fragmentation P = 0.976). Moreover, high R square values were obtained from the analysis of samples of known membrane (R² = 0.9959) and DNA damage (R² = 0.9843). Combining simultaneous assaying for sperm membrane integrity and nuclear DNA fragmentation allowed the analysis of four relevant sperm categories and assess the proportion of membrane-intact spermatozoa with compromised DNA integrity.

Conclusions This novel co-staining showed no cross-interference between its elements, providing sensitive, accurate, and reliable results. This new protocol has the potential to provide clinically relevant information about the DNA fragmentation in membrane-intact spermatozoa, improving the diagnostic of male infertility and enabling a better understanding of sperm dysfunction.

P 15.6

Effect of incubation and analysis temperature on kinematic and morphometric human semen values

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Introduction Human semen analysis should begin a simple inspection soon after liquefaction, preferably at 30 minutes, but no longer than 60 minutes after ejaculation. During liquefaction, the sample can conserve either in room temperature (RT 23 °C) or at 37 °C. The incubation temperatures and its impact on sperm motility are crucial but never considered. This study aims to examine the effect of different incubation temperatures on sperm parameters in both qualitative and quantitative analysis. Likewise kinematics and morphometrics which refers to the quantitative analysis of form.

Material and Methods Seminal samples from thirteen donors were incubated semen samples of were obtained by masturbation after 2–4 ejaculatory abstinence days and incubated for 30 min at 23 °C (RT) and at 37 °C and prepared following WHO 2010 criteria. Morphometric and kinematic parameters evaluated using an ISAS[®]v1 CASA-Morph and CASA-Mot systems, respectively.

Results The obtained data show that there were no significant differences (P > 0.05) on the subjective sperm quality parameters. On the other hand, the sperm head morphometric parameters were significantly higher after room temperature incubation; additionally, with lower ellipticity (P < 0.05). Furthermore, kinematic parameters were evaluated at two different temperatures (23 °C and 37 °C) during the incubation time and analysis process. Mainly, the four temperature combinations showed that the results of kinematic parameters followed this order according to the incubation and analysis temperatures 23°-23 °C < 23°-37 °C < 37°-37 °C < 37°-23 °C.

Conclusion Our data demonstrate the optimal correlation in kinematics parameters between the incubation and analysis temperature is 37 °C and 23 °C, respectively. The temperature control during both incubation and analysis is needed for accurate semen analysis.

P 15.7

Atlas of human sperm morpho-anomalies using Trumorph[®]

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Introduction The morphology of the spermatozoa is defined along the process of sperm formation in the testicle (spermatogenesis and spermiogenesis), epididymal transit maturation and mixing with accessory glands secretions during ejaculation. The semen analysis is used to assess the fertile potential of the male, based on the concentration, motility, and morphology of the spermatozoa. Most of them, including that recommended by WHO 2010, implies different fixation and staining processes that involve the production of morphology artifacts. The Trumorph® is based on the examination of in vivo preparations of a fresh semen drop of 3 µl between slide and a conventional coverslip, after increasing the temperature to 45 °C in just 10 seconds and subjected to a pressure of 5 kP. To obtain a homogeneous distribution of the drop is achieved in the whole area of the cover, with a height of approximately 6µm, which “forces” the cells to arrange themselves according to their maximum projection.

Material and Methods Samples from 30 voluntary donors were obtained by masturbation after 2–4 ejaculatory abstinence days. After liquefaction, the Trumorph® device was used to prepare the sample. The observations were made with a 40x objective PHN and images were captured and recorded using an ISAS®v1 CASA system.

Results The most representative images of each type of anomaly were classified according to head, midpiece or neck, and tail defects. Since each sperm has these three components, in each image can be occur combination of various morphoanomalies. For this reason, the images are accompanied by the corresponding explanatory tables, where the morphoanomalies to be indicated. In the other hand, other non-spermatic cell types were evaluated.

Conclusion Elaboration of the atlas of morpho-abnormalities sperm using the new technique Trumorph® following, classification WHO 2010.

P 15.8

Optimal capture frame rate for kinematic human sperm analysis using CASA-Mot systems

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Introduction Conventional semen analysis suffers for lack of precision and repetitiveness, reducing its impact in predicting male fertility. The introduction of computer assisted semen analysis (CASA-Mot) systems for the evaluation of sperm motility and kinematics has overpassed these problems. Nevertheless, former technological limitations have restricted the generalization of the use of CASA-Mot both for research and clinical purposes. One of these limitations is the frame rate (FR) at which images are captured.

The aim of the present work was to define the optimal FR, considering the curvilinear velocity (VCL) as the most sensible parameter.

Material and Methods Semen samples of 12 adult volunteers were obtained by masturbation after 2–4 ejaculatory abstinence days. For the study, a reusable counting chamber Spermatrack® of 10 µm depth was used in combination with an ISAS®v1 CASA-Mot system. Image sequences were recorded at 500 frames per second using a digital camera able to capture at 500 fps. These original videos were fragmented at different FRs (25, 50, 100, 150, 200, and 250) to define the asymptotic value of the exponential curve that defines the behavior of VCL relative to the FR.

Results The a value of the exponential curve was 150,23 fps, corresponding to a VCL of 130.58 m/s, long from the value of 98.89 m/s corresponding to 50 fps (the highest FR used by most of the present CASA-Mot systems).

Conclusion As a conclusion, almost 150 fps must be used for capture and analysis of human sperm kinematic studies to have confidence on the results.

P 15.9

Synthesis of caged prostaglandin E₁ derivatives to study the ligand-control of CatSper Ca²⁺-channels

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Introduction The sperm-specific CatSper channel controls the intracellular Ca²⁺-concentration and, thereby, the motility of human sperm. Human CatSper is activated by rise in intracellular pH, depolarization of the membrane, and by a range of small molecules including steroids and prostaglandins. To elucidate the mechanism underlying prostaglandin activation of CatSper and to study the ensuing motility responses, we planned to synthesize a caged PGE₁ derivative. Using caged-PGE₁ as a tool, we want to quantify the kinetics of CatSper activation and the swimming path of sperm while navigating in a light-sculptured prostaglandin gradient. PGE₁ harbors several O-containing functional groups, i.e. a carboxylic acid, an allyl alcohol, and a β-hydroxy ketone for chemical modification.

Methods Both alcohols are chemically unstable and, thus, difficult to functionalize. Therefore, we identified and employed a general procedure for esterification of the carboxylic acid using aliphatic and benzylic halides and base. The residual activity of the caged-PGE₁ derivatives was characterized by kinetic (Ca²⁺) fluorimetry, using sperm loaded with the fluorescent Ca²⁺-indicator Fluo-4 and a fluorescence plate reader. The photocleavability of photolabile groups was investigated by exposing the caged-PGE₁ to UV irradiation.

Results We showed that esterification of the carboxylic acid dramatically reduces the potency of PGE₁ to activate CatSper. As a proof of concept, two photolabile caged-PGE₁ derivatives were synthesized and characterized. Next, other photolabile groups will be attached to PGE₁ to optimize the solubility, quantum yield, uncaging kinetics and resistance to hydrolysis.

Conclusion Our results show the feasibility of the approach to synthesize an effective caged PGE₁ to investigate prostaglandin activation of CatSper and ensuing motility responses.

P 15.10

Novel perspective in human spermatozoa RNA content assessment and morphology evaluation with in vivo acridine orange staining

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Introduction Transgenerational effect carried by spermatozoa that fertilizes egg can be attributed not only to DNA sequence transmitted to offspring but also to epigenetic characteristics of the sperm. Even though spermatozoa are considered transcriptionally silenced, various types of RNA are actively coordinated in different compartments inside the cell. Aim of our study was to evaluate morphology, sperm DNA damage and localize RNA in the sperm cell with In vivo acridine orange (AO) staining that detects sperm DNA damage and DNA/RNA content.

Methods Ten men suffering from oligoasthenoteratozoospermia (OAT) syndrome and 10 normozoospermic fertile men were analyzed using in vivo AO staining. After liquefaction, 10 µl of sperm at concentration 5 million/ml was dropped on slide pretreated with AO stain. Sample was gently covered with cover slip and within 2 hours analyzed. Microscopic analysis was performed by fluorescent and confocal fluorescent microscopy.

Results Fluorescent microscopy imaging showed classic sperm morphology and red, orange or green sperm staining with red indicating damaged and green intact genome. Confocal imaging showed clear contrast between red and green staining on the level of single sperm. With red coloring, we could precisely localize RNA in residual cytoplasm in damaged and intact sperm. Furthermore, detailed morphological characteristics of the spermatozoa could be determined.

Conclusion In vivo AO staining of spermatozoa and imaging with confocal microscopy provided precise insight into level of DNA

damage, localization of RNA in the sperm and detailed morphology within single sperm.

Grants This study was supported by the Scientific Center of Excellence for Reproductive and Regenerative Medicine, Republic of Croatia, and by the European Union through the European Regional Development Fund, under grant agreement No. KK.01.1.1.01.0008, project “Reproductive and Regenerative Medicine – Exploring New Platforms and Potentials”.

P 15.11

Unique features of human sperm circular RNAs (circRNAs)

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Introduction CircRNAs are a new type of non-coding RNAs which are more stable than the linear RNAs. We aimed to characterize human sperm circRNAs profile.

Patients and Methods KNIFE algorithm was applied to predict circRNAs from human sperm RNA-seq datasets. Additionally, we have tested the resistance of these predicted circRNAs to the RNase R treatment in sperm and testis samples.

Results More than 700 circRNAs derived from 545 different genes were predicted in all sperm samples assessed. Three distinct types of sperm circRNAs were defined according to their pattern of resistance to the RNase R treatment: 1) Resistance to the RNase R treatment only for divergent primers amplification indicating circRNAs and their linear cognates co-exist; 2) Resistance to the RNase R treatment for both convergent and divergent primers amplification, indicating that the RNAs were mainly circRNAs; 3) High sensitivity to the RNase R treatment for both convergent and divergent primers amplification indicating the decircularization of the circRNA. Additionally, we demonstrated that this decircularization is not a stochastic event.

Conclusion As a differential feature between sperm and somatic cells, in which linear and circular forms derived from the same gene co-exist, we have observed that many RNAs from the sperm are exclusively present in their circular form. In addition, since, the linear cognates of these sperm circRNAs were detected in the human testis, it is suggested that while linear forms are gradually degraded during spermatogenesis, the circular forms are selectively retained in the mature sperm. However, some sperm circRNAs showed sensitivity to the RNase R treatment. Interestingly, a non-random decircularization of those non-intact sperm circRNAs was ob-

served, which led us to hypothesize that those circRNAs could co-exist with their circRNA turnover machinery.

Grants Supported by the Ministry of Economy and Competitiveness PI16/00346 to RO.

P 15.12

The Ca²⁺-channel CatSper is not activated by cAMP/PKA signaling but directly affected by chemicals used to probe the action of cAMP and PKA

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Introduction The sperm-specific CatSper channel controls the influx of Ca²⁺ into the flagellum and, thereby, the swimming behavior of sperm. A hallmark of human CatSper is its polymodal activation by membrane voltage, intracellular pH, and oviductal hormones. Whether CatSper is also activated by signaling pathways involving an increase of cAMP and ensuing activation of protein kinase A (PKA) is, however, a matter of controversy.

Methods To shed light on this question, we used kinetic ion-sensitive fluorimetry, patch-clamp recordings, and optochemistry to study transmembrane Ca²⁺ flux and membrane currents in human sperm from healthy donors and from patients that lack functional CatSper channels.

Results We find that human CatSper is neither activated by intracellular cAMP directly nor indirectly by the cAMP/PKA-signaling pathway. Instead, we show that non-physiological concentrations of cAMP and membrane-permeable cAMP analogs used to mimic the action of intracellular cAMP activate human CatSper from the outside via a hitherto unknown extracellular binding site. Finally, we demonstrate that the effects of common PKA inhibitors on human CatSper rest predominantly, if not exclusively, on off-target drug actions on CatSper itself rather than on inhibition of PKA.

Conclusion We conclude that the concept of an intracellular cAMP/PKA-activation of CatSper is primarily based on unspecific effects of chemical probes used to interfere with cAMP signaling. Altogether, our findings solve several controversial issues and reveal a novel ligand-binding site controlling the activity of CatSper, which has important bearings on future studies of cAMP and Ca²⁺ signaling in sperm.

P 15.13

RAC1 controls progressive movement and competitiveness of mammalian spermatozoa

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Motility is essential for mammalian sperm to reach their goal. Although the main mechanisms generating sperm motility are known, the complete molecular pathways controlling progressive movement towards the eggs remain elusive.

Mice with the *t*-haplotype were used. Pharmacologically tools to inhibit RAC1 were employed. Comprehensive sperm kinematics analyses were performed by computer assisted sperm motility analysis (CASA). Single sperm with distinct progressiveness were genotyped by PCR.

Our data shows that RAC1 plays an important role in controlling progressive movement. Upon Rac inhibition of wild type sperm, progressive movement is impaired. Moreover, sperm from mice homozygous for the genetically variant *t*-haplotype (*t/t*), which are sterile, show strongly enhanced RAC1 activity in comparison to wild type (+/+) controls, and quickly become immotile *in vitro*. Sperm from heterozygous (*t/+*) males, on the other hand, display intermediate RAC1 activity, impaired progressiveness and transmission ratio distortion (TRD) in favor of *t*-sperm. We show that *t/+*-derived sperm consist of two subpopulations, highly progressive and less progressive. The majority of highly progressive sperm carry the *t*-haplotype, while most less progressive sperm contain the wild type (+) chromosome. Dosage-controlled RAC1 inhibition in *t/+* sperm rescues progressive movement in (+)-sperm *in vitro*, directly demonstrating that impairment of progressive movement in the latter is caused by enhanced RAC1 activity.

The combined data show that RAC1 plays a pivotal role in controlling progressive motility in sperm, and that enhanced or reduced RAC1 activity interferes with progressive movement. Differential RAC1 activity within a sperm population impairs the competitiveness of sperm expressing suboptimal RAC1 activity and thus their fertilization success, as demonstrated by *t/+*-derived sperm.

P 15.14

Tumor necrosis factor-alpha receptor-1 (TNFR1) is present in human spermatozoa but is not functional in triggering apoptosis pathways

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Although TNF- α is detectable within the male genital tract, especially under pro-inflammatory conditions, its effects on sperm functions are controversial, and no direct evidence has been produced to date for the presence of its receptor, TNFR1, in human spermatozoa.

Mitochondrial membrane potential (MMP) and activation of caspase-8 (receptor caspase) and caspase-3 (executioner caspase) were assessed by flow cytometry in motile sperm suspensions exposed for 2 h to scalar concentrations of TNF- α (0.5–100 μ M). Sperm motility was analysed by CASA. The sperm expression of TNFR1 as well as adaptor proteins of its signalling pathway, TNFR1-asso-

ciated death domain (TRADD) and Fas-associated protein with death domain (FADD), was investigated by Western blot analysis where Jurkat cells were used as the positive control. TNFR1 was immunolocalized in human spermatozoa by confocal microscope.

Sperm motility and MMP were not significantly affected by the exposure to increasing concentrations of TNF- α , which did not induce caspase-8 or caspase-3 activation. The Western blot analysis of sperm extracts from different ejaculates demonstrated, in all samples, the presence of a single immunoreactive band of the expected molecular size of TNFR1 (~55 kDa). At the confocal microscope, TNFR1 appeared to be colocalized

with Caveolin-1 on large areas of the principal piece. When the components of TNFR1 signaling cascade were explored by Western blot analysis, similar to Jurkat cells, spermatozoa displayed TRADD; nevertheless, band density in the FADD molecular size was generally faint and poorly detectable, suggesting a potential defective signaling downstream of TNFR1/TRADD interaction.

Although human spermatozoa display the TNFR1, it appears to be not functional in triggering apoptosis pathways in these cells, likely due to a defective FADD signaling. The claimed detrimental effects on male fertility of TNF- α should be reconsidered in the light of the present findings.

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