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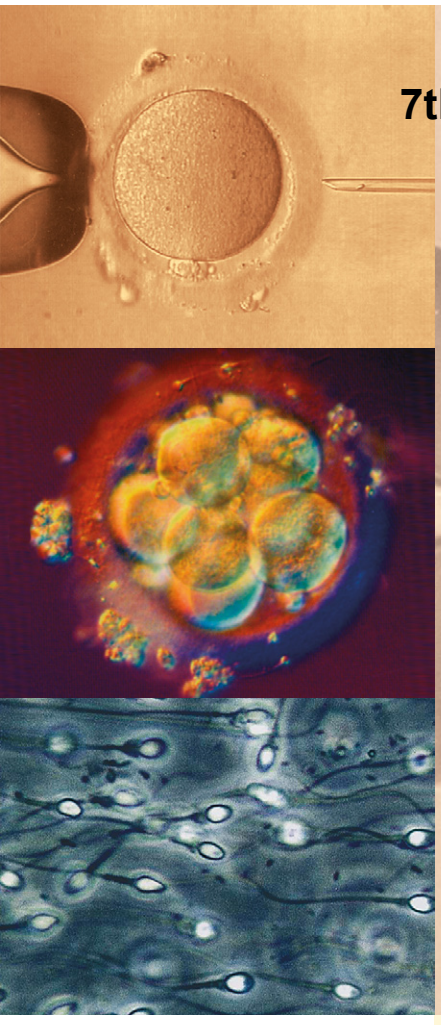

7th European Congress of Andrology (ECA) November 28-December 1, 2012, Berlin Abstracts

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FÜR KLINIK & PRAXIS

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& Anmeldung unter

7th European Congress of Andrology (ECA)
November 28 – December 1, 2012, Berlin
Abstracts*

Oral Presentations

Opening Lecture

01
What Makes a Normal Man?

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Sex development is regulated by hormones. Normally 46,XY fetuses have testes that produce large amounts of testosterone during pregnancy. If testosterone is normally reduced to dihydrotestosterone and androgen receptors are acting properly, the fetus will masculinise and look like a boy as newborn. Dysgenesis of the testes leading to defective androgen production, defects in steroid biosynthesis and androgen insensitivity lead to impaired masculinisation, 46,XY Disorder of Sex Development (DSD). Exogenous anti-androgens disrupting the hormone biosynthesis or action, or causing gonadal dysgenesis can also cause 46,XY DSD. Mild forms of this are hypospadias and cryptorchidism, both of which are very common birth defects. Developmental disorders are linked to an increased risk of testicular germ cell cancer and impaired semen quality in adult men. Testicular Dysgenesis Syndrome (TDS) is a term to describe the possible background of all these problems. Rodent studies have demonstrated that there is a specific masculinisation programming window in fetal development that is decisive for later development. Disruption of androgen production or action at this window has permanent effects on penile and testicular development. It is apparent that a similar critical window can be found also in human development. The time and width of the window varies for different endpoints, and e.g. sperm production capacity is determined over a wide window spanning from fetal life to puberty, whereas penile size may have much shorter programming window. The challenge for andrologists is to find reasons for abnormal development of a man. We have only a handful of genetic defects that are known to explain TDS, and it is likely that environmental factors play a major role. It is our task to identify those factors to be able to prevent male reproductive health problems.

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Short Oral Presentation 1: Free Communications, New Horizons in Andrology

02
Compartmentalization and Regulation of Iron Metabolism Proteins Protect Male Germ Cells from Peripheral Iron Fluctuation

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The high mitotic rate and avid mitochondriogenesis of developing male germ-cells imply high iron requirements. Yet, access to germ cells is tightly regulated by the blood-testis-barrier. To elucidate how iron supply to developing sperm works and affects mature sperm function we localized iron deposition by Perls stain and analyzed iron related proteins in testes of wild-type, Irp2–/– and Hfe–/– mice by in situ hybridization, immunohistochemistry and immunoblots. In addition, ferritin secretion and sperm function were analyzed. Iron deficiency as well as the absence of IRP2 affected mature sperm function in a complex way. In the testes, iron accumulated mainly around seminiferous tubules (SFT) and only small amounts localized within the SFT. The colocalization of transferrin receptor (TfR1) with transferrin (Tf) and the divalent metal transporter-1 (DMT1) suggested an active role for TfR1 in Tf dependent iron import to primary spermatocytes, within the SFT. The expression of IRP2 in later primary spermatocytes and spermatids may explain a detachment of TfR1 regulation from iron levels that we observed in the SFT. DMT1 accumulation in the luminal compartment of the SFT supported our hypothesis that its main role may be in iron transport during the final steps of germ cell maturation. Ferritin within the SFT was mainly synthesized in Sertoli cells that are capable of secreting ferritin, which may be taken up by primary spermatocytes. We suggest that during spermatogenesis, iron moves from primary spermatocytes to spermatids, which deliver during elongation most iron to the apical compartment of Sertoli cells. From there iron is routed back to a new generation of spermatocytes. Losses are replenished by the peripheral circulation. Such an internal iron cycle detaches iron homeostasis within the SFT from the periphery. This newly developed model explains how compartmentalization can protect male germ-cells from peripheral nutrient fluctuations.

20
Epigenetic Biomarkers of Testis and Epididymis Existing in Extracellular Nucleic Acids from Human Semen

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Introduction Developing epigenetic biomarkers for male infertility is desired. Small RNAs and DNA methylation in testis/epididymis are essential epigenetic regulations for male fertility. Recently, we and others have found extracellular mRNAs, miRNAs, and DNA in human seminal plasma. We reasoned some testis/epididymis specific or enriched small RNAs and DNA methylations should be detected in these extracellular nucleic acids, and thus hold promise as non-invasive epigenetic biomarkers for infertility.

Materials & Methods The vasectomy on fertile men makes it possible to identify seminal small RNAs and DNA methylations predominately secreted from human testis and epididymis, because the ejaculate of a successfully vasectomized man does not contain the secretion from testis and epididymis. By comparing between normozoospermic donors and vasectomized men, solexa miRNA sequencing and promoter array were used to screen testis/epididymis specific or enriched seminal miRNAs, piRNAs and promoter methylation, respectively. Candidates were further validated by quantitative PCR in individuals with normozoospermia and vasectomy.

Results Solexa miRNA sequencing and subsequent validation in individuals identified 61 miRNAs reliably predominately secreted from testis/epididymis. Interestingly, 28 miRNAs, which contain 5 miRNA clusters, reside on the X-chromosome. At least 995 seminal piRNAs were identified in nor-

mozoospermic donors while were absent in vasectomized men. The promoter array identified promoters of 1834 testis and epididymis-specific hypomethylated genes and 1017 testis and epididymis-specific hypermethylated genes. Subsequent validation confirmed the result of promoter microarray.

Conclusion The present study identified epigenetic informations that predominately derived from testis and epididymis from human semen. These epigenetic informations may be useful noninvasive molecular markers for human male infertility on revealing the epigenetic etiology and physiopathological status of impaired sperm production and maturation.

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04

Worsening Inhibin B, but not Testosterone, Secretion after Kidney Transplantation in Male Patients < 45 years

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Introduction It is well established that male patients with end-stage chronic kidney disease often exhibit biological and/or clinical hallmarks of an abnormal hypothalamo-pituitary-gonadal axis. Several studies reported that haemodialysis (HD) does not reverse this impaired endocrine status while others reported controversial results about the recovery of LH, FSH and testosterone secretions after successful kidney transplantation (KT). Beyond the endocrine status, infertility is an important issue for young male patients. Since inhibin B has been recently recognized as a valuable marker of spermatogenesis, it would be of interest to investigate it together with serum gonadotrophins, testosterone levels in men on hemodialysis and after successful KT.

Material & Methods Fifty-three male patients under 45 years (mean: 37 years) were studied longitudinally while undergoing HD (median length: 36 months) and six months after KT. Pre- and post-operative serum specimens were collected to assess LH, FSH, testosterone and inhibin B levels by immunoassays. Hormonal status was also compared to that of 46 fertile semen donors (mean age: 37 years).

Results For patients under HD, LH and FSH levels were higher than that of control men, those of testosterone and inhibin B were not significantly different. After KT, LH levels returned to normal range whereas those of FSH significantly rosed. Testosterone levels remained constant within the normal range but, unexpectedly, those of inhibin B significantly dropped. A detailed analysis of individual secretion profiles revealed that 60% of the grafted patients remained eugonadic but only a minority of them (40%) retained normal inhibin B levels. These finding and the fact that pre-operative levels of testosterone and inhibin B were not correlated suggest that Leydig and Sertoli cells were differently and independently impacted by the transplantation. We then investigated whether clinical, biological or therapeutic (immunosuppressive regimen) parameters could help to expect post-graft inhibin B levels. We could identify a cut-off for pre-graft inhibin B levels that is able to predict a post-graft level < 80 pg/mL with a sensibility of 77% and a specificity of 92%.

Conclusion Our study suggests that endocrine testicular secretions of uremic male patients under 45 years are differently impacted by kidney transplantation. These preliminary and unexpected findings raise new issues in the management of transplanted patients' fertility.

06

Detection of Polysialylated NCAM on Mammalian Sperms

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Introduction Fertilisation in mammals is a complex sequence of biochemical events leading to embryogenesis. In this process, initial recognition of negatively charged carbohydrates motifs by complementary receptors seems to play a critical role in the sperm binding to zona pellucida glycoproteins surrounding the plasma membrane of an oocyte. Studies by Kitajima and co-workers demonstrated the presence of α2,9-linked polysialic acid (polySia) on sea urchin sperm. Intriguingly, binding of an anti-α2,9-polySia antibody leads to an inhibition of sperm motility as well as fertilisation due to a decrease of the intracellular Ca2+ level. Based on these studies, we became interested in the potential involvement of sialic acid polymers in mammalian fertilisation.

Material & Methods Therefore, we isolated human sperms for Western blotting and immunohistochemistry. To identify potential polySia-carriers a glyco-proteomic approach was employed. For detailed analysis of the degree of polymerization a DMB-HPLC approach was used.

Results Our experiments demonstrated that α2,8-linked polySia was present in protein lysates of purified sperms. The polySia chains were bound to N-glycans of the neuronal cell adhesion molecule (NCAM) displaying chains with more than 40 sialic acid residues. Interestingly, polySia-NCAM was present in the postacrosomal region of sperms, which is known to play an essential role during sperm-egg binding.

Conclusion The localization of polySia-NCAM together with the already described presence of un-polysialylated NCAM on the egg surface let assume that polySia together with NCAM is involved in distinct fertilisation processes.

07

Insulin-like Factor 3 (INSL3): a New Clinical Parameter for Andrology

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Introduction Insulin-like factor 3 (INSL3) is a member of the relaxin family of peptide hormones, and is produced by both fetal and adult-type Leydig cells of the testes in amounts which are dependent only upon the number of Leydig cells and their differentiation status. Fetal and adult-type Leydig cells are discrete population of cells, developing in the embryo and at puberty, respectively. INSL3 expression is constitutive and independent of acute regulation by the hormones of the HPG axis. As a result, INSL3 measurements in peripheral blood are highly consistent within individuals, varying little over periods of several weeks. INSL3 produced by fetal Leydig cells can be measured in amniotic fluid collected at routine amniocentesis at 12-16 weeks. This fetal INSL3 is responsible for the first phase of testicular descent and represents a uniquely fetal-gender specific hormone potentially able to influence placental physiology, as well as acting as a biomarker for fetal health, particularly in the context of environmental endocrine disruption.

Materials & Methods We have developed a series of highly sensitive time-resolved fluorescence immunoassays (TRFIA) able to measure INSL3 from humans, rodents and domestic species, in serum and amniotic fluid down to limits of detection of around 5 pg/ml. We have recently completed several cohort studies, including 1200 men from the Australian general population, as well as men from infertility clinics, and also amniotic fluid samples from 250 pregnant women carrying male fetuses.

Results and Conclusion INSL3 has an average concentration in healthy human males of 1.0 + 0.5 ng/ml. This declines from a maximum in young men (aged 35–40) of 1.3 + 0.5 ng/ml to a low value of 0.8 + 0.4 in elderly men (aged 75–80), correlating also with circulating testosterone values. Whereas

total testosterone declined ca. 6% per decade, INSL3 declined ca. 12% per decade, reflecting again that there is no compensatory acute regulation by the HPG axis, and that INSL3 is a direct estimate of Leydig cell functional capacity. In fact INSL3 was more strongly correlated with total testosterone than any other factor, including LH, suggesting that INSL3 might be a more robust predictor than testosterone of testis status in the context of metabolic syndrome or late-onset hypoandrogenemia. Current studies are aiming to confirm this, and also to broaden the application of INSL3 measurement to a more precise assessment of pubertal development in boys. In regard to INSL3 detection in amniotic fluid, we have shown a significant response at 12–14 weeks gestation to later symptoms of preeclampsia and IUGR, strongly suggesting that INSL3 concentration reflects male fetal health in early pregnancy. It remains to be evaluated whether this is also relevant for postnatal health outcomes.

08

Membrane Transporters for Sulfated Steroids and Steroid Sulfatase Expression in the Human Testis – A Functional System?

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Introduction Sulfated steroid hormones can be transported into the cells by various membrane localized uptake carriers. In the human testis, sodium dependent organic anion transporter (SOAT), organic anion transporting polypeptides (OATPs) and the organic solute carrier protein 1 (OSCP1) are predominantly expressed. By the enzyme steroid sulfatase (StS), intracellular sulfated steroids can be converted into the biologically active, non conjugated steroid hormone.

Methods The expression of membrane transporters and StS was analyzed on mRNA level using qRT-PCR (testis homogenate), RT-PCR (single cell populations, assessed via laser assisted microdissection) and in situ hybridisation (ISH). The cellular localization of SOAT and StS was examined applying immunohistochemistry and Western blotting. Additionally, the functional analysis of human SOAT protein was conducted using stably transfected HEK293 cells and liquid chromatography tandem mass spectrometry (LC/MS-MS).

Results RT-PCR and ISH using biopsies showing normal spermatogenesis revealed the expression of SOAT mRNA in primary pachytene spermatocytes. By using qRT-PCR we were able to show, that SOAT expression is severely diminished or even absent in biopsies showing an arrest of spermatogenesis or a complete loss of germ cells. The same, to a minor degree, was shown for other membrane transporters as OATP6A1 and OSCP1. In contrast to that, StS mRNA was expressed in specimens showing normal or impaired spermatogenesis, respectively.

SOAT protein was shown to be present in testis biopsies using WB of tissue homogenate and in pachytene spermatocytes using IHC. StS protein was detected in Sertoli and interstitial Leydig cells, confirming the data from qRT-PCR analysis.

HEK293 cells stably expressing the SOAT carrier protein showed significant transport activity for DHEAS and E1S. This was demonstrated by using radiolabeled [^{3}H]-DHEAS and [^{3}H]-E1S compounds as well as by direct analysis of the cell associated uptake fraction by LC/MS-MS. Furthermore, β-estradiol-3-sulfate and androstendiol sulfate were identified as novel substrates of SOAT. In contrast to SOAT, OATP6A1 and OSCP1 had no transport activity in stably transfected HEK293 cells for sulfated steroids.

Conclusions SOAT and other membrane transporters are expressed in germ cells, whereas StS is expressed in various cell populations. Co-expression within the seminiferous tubule hints to an involvement of sulfated steroids and their biology in hormonal regulation of spermatogenesis. SOAT seems to be the most relevant uptake carrier for the local supply of the testis with sulfated steroid hormones, since transport ability of other membrane transporters was significantly low.

09

Combined Effects of the Variants FSHB-211G/T and FSHR 2039A > G on Male Reproductive Parameters

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Introduction Recently, a polymorphism in the FSHB promoter (-211G > T, rs10835638) was found to be associated with lower serum FSH levels in cohorts of Baltic young men. Concurrently, an increased frequency of the T-allele in patients with oligozoospermia was shown, a finding that was confirmed in a later Italian study. In contrast, a polymorphism in the FSH-receptor gene (FSHR, 2039A > G, rs6166) was previously shown to be associated with FSH levels in women only. Since no study addressing joint effects of both FSHB-211G > T and FSHR 2039A > G has been conducted so far, we analyzed effects of both SNPs on male reproductive parameters.

Subjects & Methods 1,213 male partners in infertile couples without known causes for male infertility attending the Department of Clinical Andrology, Centre of Reproductive Medicine and Andrology, University Clinic Muenster, a tertiary-referral centre for infertility were genotyped by TaqMan assay. Associations between single and combined SNP genotypes and clinical parameters were evaluated.

Results The FSHB-211G > T T-allele showed significant dosage effects for FSH (–0.51 U/l per T-allele), LH (0.28 U/l) and bi-testicular volume (–3.2 ml). Statistical significance was enhanced several fold following meta-analysis including the previously published Baltic studies totalling 3,017 men. TT-carriers were found more than twice as frequently among men with sperm counts below 39 Mill. (3.2%) than in those with high sperm counts (1.4 %, $p = 1.7\times10^{-3}$). In contrast, The FSHR 2039A > G G-allele exhibited non-significant trends for associations with higher FSH and reduced testicular volumes. However, in the combined model, FSHR 2039A > G significantly modulated the more dominant effect of FSHB -211G>T on serum FSH and testicular volume among the T-allele carriers.

Conclusions By analysing both SNPs for the first time, we convincingly show that indeed FSHR 2039A>G has an effect also in males. In the proposed model of the combined effects, FSHB-211G > T acts strongly on male reproductive parameters while the FSHR 2039A > G effects were approximately 2 to 3 times smaller. Since oligozoospermic patients carrying unfavourable variants affecting FSH action may benefit from FSH treatment, both SNPs are promising candidates to make their way into the routine clinical workup of the male with oligozoospermia. However, first, controlled treatment studies are urgently warranted.

The study was supported by a Research Group Linkage grant (to J.G. and M.L.) from the Alexander-von-Humboldt foundation and by the Deutsche Forschungsgemeinschaft (grant TU 298/1-1 to F.T.).

10

Accumulation of Chymase-Positive Testicular Mast Cells in Infertility Patients and Evidence for Local Angiotensin II Effects

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Question Testicular mast cells (MCs) are significantly increased in number in the testes of men with impaired spermatogenesis. This insight is based on gene expression studies and immunohistochemical analysis of tryptase, the major MC product [Meineke et al., 2000]. The phenotype of testicular MCs may change, depending on their location and the underlying pathology [Welter et al., 2011] but whether the enzyme chymase (CHY) is present in testicular MCs is not fully known. CHY promotes differentiation and growth of interstitial connective tissue [Hirata et al., 2007]. It can cleave angiotensin I (Ang I) to Ang II, which may be involved in contraction and growth of peritubular myoid cells [Rossi et al., 2002]. However, Ang II can also fuel inflammatory responses in vascular smooth muscle cells [Li et al., 2009 and supports tissue fibrosis [Fan et al., 2009; Rüster and Wolf, 2011].

Hence, the present study was performed to further characterize testicular MCs and to explore the effects of Ang II on cultured human testicular peritubular cells (HTPCs).

Methods Testicular biopsies of patients with germ cell arrest (GA), mixed atrophy (MA), Sertoli cell only (SCO) syndrome as well as patients with normal spermatogenesis were stained immunohistochemically with an anti-CHY antibody. Cultured HTPCs were used to study Ang II effects on contractile abilities. The inflammatory potential of Ang II was explored by RT-PCR.

Results CHY-positive MCs were hardly seen (0.03–0.49 per tubule) in samples with normal spermatogenesis. If present, they were mainly found in the interstitial spaces. CHY-positive MCs were increased in patients with MA (0.37–1.04/tubule) and GA (0.37–0.84 per tubule) and were most abundant in SCO (1.29–3.88 per tubule). In MA, the peritubular wall was identified as the principal site of CHY-positive MCs implying interactions between MCs and peritubular cells.

AT1R mRNA was detected in HTPCs, which contracted upon Ang II stimulation (n = 2 patients). On-going cell culture studies indicate that Ang II rapidly induces IL-6 and TLR-4 mRNA, while co-incubation of Ang II with the blocker losartan attenuates this Ang II effect.

Conclusions These preliminary data show that CHY-positive MCs increase in infertility patients. Thus MCs, via CHY and via Ang II may induce contraction of peritubular cells. Furthermore it is possible that the observed proinflammatory action of Ang II may contribute to male infertility.

(DFG MA 1080/21-1).

Short Oral Presentation 2: Hypogonadism, Metabolic Syndrome and Reproductive Function

11

Sustained Improvement of Features of the Metabolic Syndrome upon Normalization of Serum Testosterone in Hypogonadal Men – Follow-up up to 5 years

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Objectives Hypogonadal men tend to increase body weight, fat mass and develop features of the metabolic syndrome. Long-term effects of normalization of testosterone in hypogonadal men on weight, waist circumference and lipid metabolism, liver functions upon treatment with parenteral testosterone undecanoate were studied.

Methods A cumulative registry study of 255 men (mean age: 60.6 ± 8.0 years), with testosterone levels between 0.14–3.51 ng/mL with late onset hypogonadism (LOH).

Results A remarkable progressive and sustained decline of body weight and waist circumference over 5 years was observed. Fasting glucose decreased from 103.38 ± 14.44 mg/dL to 97.54 ± 2.34 ($p < 0.0001$). The proportion of patients who had glucose levels ≥ 100 mg/dL decreased from 45% at baseline to 16% at 60 months. HbA_{1c} was measured in 125 patients and decreased from 6.94 ± 1.55% to 6.01 ± 1.41%. Total cholesterol decreased from 281.58 ± 39.8 mg/dL to 188.12 ± 11.31 ($p < 0.0001$), LDL from 163.79 ± 41.44 mg/dL to 109.84 ± 35.41 ($p < 0.0001$), triglycerides from 276.16 ± 51.32 mg/dL to 189.78 ± 11.33 ($p < 0.0001$). HDL was stable over the first 2 years (62 mg/dL at baseline and 63.26 at 24 months) and then declined to 52.45 at 60 months ($p < 0.0001$ vs baseline). Mean systolic blood pressure declined from 153.55 ± 17.6 mmHg to 137.74 ± 10.92 ($p < 0.0001$) and diastolic blood pressure from 93.49 ± 11.21 mmHg to 79.61 ± 7.35 at 60 months ($p < 0.0001$). At baseline, 91% of men had a systolic blood pressure of ≥ 130 mmHg which declined to 80% after 60 months. At baseline, 75% of men had a diastolic blood pressure of ≥ 85 mmHg declining to 22% at 5 years.

Conclusions Normalization of serum testosterone leads to a sustained improvement of all components of the metabolic syndrome.

12

Hypogonadism as a Risk Factor for Cardiovascular Mortality in Men: a Meta-Analytic Study

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Question To verify whether hypogonadism represents a risk factor for cardiovascular (CV) morbidity and mortality and to verify whether testosterone replacement therapy (TRT) improves CV parameters in subjects with known CV diseases (CVDs).

Methods An extensive Medline search was performed using the following words "testosterone", "CVD", and "males". The search was restricted to data from January 1, 1969, up to January 1, 2011.

Results Of the 1178 retrieved articles, 70 were included in the study. Among cross-sectional studies, patients with CVD have significantly lower testosterone and higher 17-β estradiol (E_2) levels. Conversely, no difference was observed for DHEAS. The association between low testosterone and high E_2 levels with CVD was confirmed in a logistic regression model, after adjusting for age and body mass index (hazard ratio [HR] = 0.763 [0.744–0.783] and HR = 1.015 [1.014–1.017], respectively, for each increment of total testosterone and E_2 levels; both $p < 0.0001$). Longitudinal studies showed that baseline testosterone level was significantly lower among patients with incident overall- and CV-related mortality, in comparison with controls. Conversely, we did not observe any difference in the baseline testosterone and E_2 levels between case and controls for incident CVD. Finally, TRT was positively associated with a significant increase in treadmill test duration and time to 1 mm ST segment depression.

Conclusions Lower testosterone and higher E_2 levels correlate with increased risk of CVD and CV mortality. TRT in hypogonadism moderates metabolic components associated with CV risk. Whether low testosterone is just an association with CV risk, or an actual cause-effect relationship, awaits further studies.

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Body Mass Index Regulates Hypogonadism-Associated CV risk: Results from a Cohort of Subjects with Erectile Dysfunction

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Introduction Obesity is an independent cardiovascular (CV) risk factor. Testosterone (T) is inversely related to body mass index (BMI) in males. There is substantial evidence suggesting that low T could play a role as a moderator of CV mortality in men. This study is designed to assess the possible interaction between T and obesity in predicting major cardiovascular events (MACE) in a sample of subjects with erectile dysfunction.

Methods A consecutive series of 1687 patients was studied. Different clinical, biochemical and instrumental parameters were evaluated. According to BMI, subjects were divided into normal weight (BMI = 18.5–24.9 kg/m^2), overweight (BMI = 25.0–29.9 kg/m^2) and obese (BMI ≥ 30.0 kg/m^2). Hypogonadism was defined as total T below 10.4 nmol/L. Information on MACE was obtained through the City of Florence Registry Office. Information on MACE was obtained through the City of Florence Registry Office.

Results Among the patients studied, 39.8% had normal weight, whereas 44.1% and 16.1% were overweight or obese, respectively. Unadjusted analysis in the whole sample showed that, while hypogonadism and obesity were significantly associated with an increased risk of MACE, their interaction term was associated with a protective effect. In a Cox regression model, adjusting

for confounders, hypogonadism showed a significant increased risk of MACE in normal weight subjects, whereas it was associated with a reduced risk in obese patients.

Conclusions Hypogonadism-associated CV risk depends on the characteristics of subjects, being more evident in normal weight than in obese patients. Further studies are advisable to clarify if low T in obese patients is a (positive) consequence of a comorbid condition (i.e. to save energy) or if it represents a pathogenetic issue of the same illness. Hence, possible misuse/abuse of testosterone treatment in obese subjects must be avoided.

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Obesity, Insulin Resistance and their Correlation with Testosterone level in Caucasian Male Patients

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Introduction Obesity is widely recognized as an important public health problem; its prevalence has increased substantially in the recent decades. The relationship between obesity and testosterone levels is one of the longest running controversies in endocrinology. The data of several studies prove, that there is a consistent correlation among the low testosterone level, insulin resistance and the risk of type 2 diabetes mellitus.

Aim The objective of the study is to show correlation with obesity, insulin resistance and testosterone level in male patients. We also study the influence of testosterone replacement therapy on obesity and insulin resistance in men.

Materials & Methods 97 subjects with 30–65 years and BMI 27,0–48,0 kg/m^2 were enrolled in the study. The following analyses were done: anthropometric study, biochemical measurements, ultrasonography of the abdomen and prostate. According to the laboratory and clinical condition we divided patients into three groups. The appropriate treatment was prescribed to all patients.

1. First group with obesity and androgen deficiency were we used diet and physical activity.

2. Second group with androgen deficiency, obesity and insulin resistance, we used diet, physical activity and metformin.

3. Third same group as second group with androgen deficiency, obesity and insulin resistance, we used testosterone, metformin, diet and physical activity.

Results In all investigated patients abnormal lipid profile and increased level of leptin was observed, all patients had decreased level of free testosterone and had inversely correlated with the degree of obesity and insulin resistance. After three months of treatment: We had some positive results cholesterol, triglyceride and LDL levels decreased, and HDL increased. Free testosterone level increased in all groups but the best results was in III group which was treated by diet, physical activity, metformin and testosterone. HOMA-IR decreased in all group but I and III group had alike result. BMI decreased in all groups but bets results was in III group. leptin level after treatment was approximately same in all groups, but compared best results was achieved in III group.

Conclusion As our small study had shown testosterone therapy reduces insulin resistance and obesity in male patients and also decrease total cholesterol level. These observations suggest that an inverse relationship exists between serum androgens, obesity and insulin sensitivity.

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Insulin Resistance is associated with Increased Seminal Plasma Insulin Concentrations and Reduced Semen Parameters in Obese Patients

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Male obesity is associated with both infertility and insulin resistance (IR). Moreover, insulin is a central regulator of gonadal and sperm functions. As insulin concentrations in seminal fluid of obese males have not been previously investigated, this case-controlled study assayed serum and seminal insulin and glucose concentrations in obese and non-obese participants. A total of 37 males were divided into an obese (BMI ≤ 29.9; n = 20) and non-obese (BMI ≥ 30; n = 17) group. Blood samples were assayed for glucose (FBG) and insulin (FBI). IR was determined by the QUICKI. Semen samples were analyzed for sperm concentration, progressive motility, total motility, vitality, leukocytes, glucose (SG) and insulin (SI) concentrations. Subjects with leukocytospermia (> 10^6/ml), on hormonal therapy or any reproductive disorder were excluded. However, diabetics not on insulin were included (n = 3) in the study. Ages between the groups were matched. BMI, FBI and QUICKI significantly differed between the groups (p = 2 = 0.41), and negatively with total (r^2 = –0.4) and progressive motility (r^2 = 0.44) and vitality (r^2 = –0.37). BMI correlated positively with SI (r^2 = 0.54) and negatively with progressive motility (r^2 = –0.33) and vitality (r^2 = –0.37). FBG correlated negatively with total (r^2 = -0.33) and progressive motility (r^2 = –0.33) and vitality (r^2 = –0.34). SG correlated negatively with total motility (r^2 = –0.41) and vitality (r^2 = –0.33). QUICKI correlated negatively with BMI (r^2 = –0.74) and positively with sperm concentration (r^2 = 0.42) and progressive motility (r^2 = 0.03). FBI strongly correlated positively with SI (r^2 = 0.71) and negatively with sperm concentration (r^2 = –0.33). SI:FBI ratio correlated positively with SI (r^2 = 0.46).

Results demonstrate that obesity and IR are associated with reduced sperm parameters. Increased FBI and SI may directly or indirectly affect spermatogenesis or hormonal function as it regulates Leydig, Sertoli and sperm cell function. Insulin is highly concentrated in seminal fluid, and this requires further physiological explanations. Increased FBI and SI may provide novel avenues for investigation of the mechanisms of obesity associated infertility, and clinical evaluation of IR may be beneficial in the investigation of subfertile male partners.

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Impact of Metabolic Syndrome on Male Fertility Parameters

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Introduction & Objective Overweight and obesity are frequently associated with interrelated disorders including visceral obesity, hyperglycaemia, dyslipidaemia, and hypertension, defined as metabolic syndrome (MetS). MetS is well-known to be associated with a chronic systemic inflammatory reaction. However, the impact of MetS on the urogenital tract in terms of inflammation and its association with semen parameters is largely unknown.

Methods In a prospective study* we enrolled 18 men with MetS and 13 age-matched male controls from the general population. In all patients the complete medical history was recorded and the body mass index (BMI) calculated. Fasting glucose and insulin were determined to calculate the homeostasis model assessment (HOMA index). Blood analysis for systemic inflammatory markers included sensitive C-reactive protein (sCRP) and adiponectin. The sex hormones luteinizing hormone (LH), follicle-stimulating hormone (FSH), testosterone and estradiol were measured. Semen analysis was performed according to WHO 2010 recommendations including volume, pH, sperm concentration, progressive motility, normal morphology, peroxidase-positive leukocytes, and granulocyte elastase. Statistical analysis was performed using the Wilcoxon test.

Results Compared to controls, in patients with MetS BMI and HOMA index were significantly increased (for both p < 0.001) as well as sCRP (p < 0.05). In addition, testosterone was significantly reduced (p < 0.001) and inversely estradiol elevated (p < 0.05). No significant changes were noted for FSH, LH and all semen parameters investigated (**Tab. 1**).

Conclusions We provide evidence for a systemic inflammation associated with impaired sex hormones in patients with MetS. Further enrolment of patients and controls is

Table 1. A. Pilatz et al. Metabolic, Hormonal and Semen Parameters in Patients with MetS (n = 18) and Controls (n = 13).

Parameter	MetS median (range)	Controls median (range)	p
Age (years)	44 (30–57)	42 (32–62)	0.373
BMI (kg/m²)	38.4 (27.2–63.2)	26.5 (21.5–29.5)	< 0.001
HOMA index	4.6 (2.1–12.8)	1.0 (0.7–1.4)	< 0.001
sCRP (mg/l)	4.3 (1.3–12.6)	0.8 (0.1–8.2)	< 0.050
Adiponectin (µg/ml)	6.1 (2.6–10.8)	9.6 (4.0–11.5)	0.094
FSH (mU/ml)	3.8 (1.8–12.1)	4.3 (2.3–14.9)	0.299
LH (mU/ml)	3.4 (1.7–9.6)	2.9 (1.8–4.3)	0.131
Testosterone (ng/dl)	280 (134–494)	441 (233–726)	< 0.001
Estradiol (pg/ml)	38 (6–63)	26 (13–38)	< 0.050
Semen volume (ml)	2.6 (0.2–8.5)	3.1 (1.0–4.9)	0.499
Semen pH	8.2 (7.6–8.8)	7.8 (7.3–8.7)	0.107
Sperm concentration (Mio/ml)	52 (0.2–379)	63 (14–404)	0.679
Progressive motility (%)	49 (16–64)	43 (9–72)	0.478
Normal morphology (%)*	4 (0–14)	5 (2–14)	0.223
Peroxidase + Leukocytes (Mio/ml)	0.1 (0.0–0.9)	0.2 (0.0–0.7)	0.811
Elastase (ng/ml)	145 (10–885)	56 (10–526)	0.280

* according to strict criteria

necessary to evaluate possible negative influences of MetS on semen parameters.

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Testosterone Protects from Metabolic Syndrome-Associated Prostate Inflammation: an Experimental Study in Rabbits

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Background Metabolic syndrome (MetS) and BPH/LUTS are often associated. One of their common denominators is hypogonadism. However, testosterone (T) supplementation is limited by concerns for potential prostatic side effects.

Objective To determine whether MetS-associated prostate alterations are prevented by T supplementation.

Methods We used a previously described animal model of MetS, obtained by feeding male rabbits a high fat diet (HFD) for 12-weeks. Subsets of HFD rabbits were treated with T or with the farnesoid-X receptor agonist INT-747. Rabbits fed a standard diet were used as controls.

Results HFD-animals develop hypogonadism and all the MetS-features: hyperglycaemia, glucose intolerance, dyslipidemia, hypertension, visceral obesity. In addition, HFD-animals show a prostate inflammation. Immunohistochemical analysis demonstrated that HFD induced prostate fibrosis, hypoxia, and inflammation. The mRNA expression of several proinflammatory (IL-8, IL-6, IL-1β, TNFα), T-lymphocyte (CD4, CD8, Tbet, Gata3, ROR γt), macrophage (TLR2, TLR4, STAMP2), neutrophil (lactoferrin), inflammation (COX2, RAGE), and fibrosis/myofibroblast activation (TGFβ, SM22-α, α-SMA, RhoA, ROCK1/ROCK2) markers was significantly increased in HFD-prostate. T, as well as INT-747, treatment prevented some MetS-features, although only T normalized all the HFD-induced prostate alterations. Interestingly, the ratio between testosterone and estradiol plasma level retains a significant, negative, association with all the fibrosis and the majority of inflammatory markers analyzed.

Conclusion These data highlight that T protects rabbit prostate from MetS-induced prostatic hypoxia, fibrosis and inflammation, which can play a role toward the development/progression of BPH/LUTS.

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Negative Association between Levels of Testosterone and Inflammatory Markers in Young Men

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Introduction Low grade systemic inflammation (LGSI) as well as androgen deficiency has in older men been associated with several pathologies including cardiovascular disease (CVD). However, the direction of causality connecting these conditions is not clarified. We hypothesized that low testosterone levels can cause LGSI. To test this hypothesis, we investigated the association between testosterone levels and expression of markers of inflammatory response in young men without any manifestations of CVD.

Material & Methods In a nested cross-sectional study, forty subfertile hypogonadal (n = 20) or eugonadal (n = 20) subjects (mean age 37 years, SD = 4.3) and 20 age-matched controls were randomly selected. Blood sampling, interviews and anthropometric measures were undertaken. Serum levels of testosterone, LH, estradiol, SHBG and 20 LGSI-markers were assessed. Linear regression models, with adjustment for age and fertility status were used.

Results Among 20 inflammatory markers, MIP-1α (β = –0.026; p = 0.029), MIP-1β (β = –0.015; p = 0.049) and TNF-α (β = –0.015; p = 0.040) showed negative association to total testosterone (TT) levels. MIP-1α (β = –1.985; p = 0.001) and TNF-α (β = –0.947; p = 0.014) showed negative association to calculated free testosterone (cFT) levels. In comparison to men with normal TT and cFT levels, TNF-α levels were higher in men with subnormal levels of TT (ratio 0.62; p = 0.006) and cFT (ratio 0.63; p = 0.007). Also, MIP-1α levels were higher in men with subnormal levels of TT (mean ratio 0.54; p = 0.033).

Conclusions The hypogonadal men had significantly higher levels of inflammatory markers that are related to the risk of CVD than eugonadal counterparts. This supports the hypothesis that subnormal testosterone already in young age evokes LGSI, which might be contributing to the risk of CVD and other long term complications of male hypogonadism.

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Alpha-haemolytic Strains of Uropathogenic *E. coli* Prematurely Activate the Acrosome Reaction and Induce DNA Damage in Sperm

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Introduction Infection and inflammation of the urinary tract are considered the aetiology in 10–15% of male infertility cases. In ap-

proximately 40% of patients suffering unilateral acute epididymitis, persistent impaired semen quality is observed even after successful antimicrobial treatment. Recent investigations have identified that 50% of bacterial related epididymitis is due to the presence of α-haemolysin (hlyA) producing Uropathogenic *E. coli* (UPEC). To date no extensive characterisation of these pathogenic strains, or their toxin has been conducted. Our aim was to elucidate the consequences of hlyA on sperm integrity using a UPEC induced murine experimental epididymitis model.

Methods & Materials To evaluate the consequences of hlyA on acrosome integrity in vitro, sperm collected from C57BL/6N mice were infected with either an α-haemolytic *E. coli* strain (UPEC CFT073 or FOS 22), a non-haemolytic *E. coli* strain (NPEC 470, UPEC HDM536 or Epi 300) or left untreated. Acrosome integrity was assessed by Spermac™ staining. The murine experimental epididymitis model was established by injection of a total of 40,000 UPEC CFT073 or NPEC 470 cells into both left and right vas deferens of C57BL/6N mice. PBS was used as sham control. Three days after infection, animals were sacrificed, blood taken, the epididymides removed and sperm collected. Testosterone was measured by radioimmunoassay. Intact epididymides were either placed in Bouin's solution and embedded in paraffin for H&E staining, or snap frozen. *E. coli* were detected using immunofluorescent staining. Spag11b gene expression was quantified by qPCR. Alterations in the chemical constituents of the sperm head were identified using Raman microspectroscopy and sperm nDNA integrity assessed by flow cytometry.

Results We found both types of *E. coli* detrimentally influenced epididymal function, as illustrated in vivo by significantly reduced levels of circulating testosterone and reduced expression of androgen-dependent Spag11b. Our findings revealed α-haemolytic UPEC CFT073 prematurely activates the acrosome following in vitro and in vivo infection in contrast to PBS and NPEC 470. In addition, UPEC CFT073 infected mice had higher numbers of sperm with nDNA damage, evidence of lipid peroxidation in the form of malondialdehyde and perturbations of the DNA backbone consistent with fragmentation.

Conclusion For the first time we provide evidence that directly implicates hlyA in the subversion of sperm integrity. We found hlyA to have multiple effects undermining not only the hormonal (testosterone, Spag11b) milieu but also sperm's functional (acrosome) and structural (nDNA) integrity. Furthermore, our findings provide insight into possible mechanisms by which hlyA causes damage. The existence of lipid peroxidation (malondialdehyde) is indicative of the consequences of oxidative stress and more particularly attack by ROS. Hence, our findings provide new insights into the consequences and possible processes underlying the clinical manifestations of acute epididymitis.

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The Value of Prostate Biopsy in Diagnosis of Urotuberculosis

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Introduction 77% men who died from TB, had prostate TB, mostly overlooked alive. Prostate TB is a sexually transmitted disease, leads to infertility, results in chronic pelvic pain, decreases a sexual function. The aim of study was to estimate a diagnostic value of a prostate biopsy for prostate TB.

Material & Methods 93 patients suspicious on prostate TB were enrolled in study. All underwent ultrasound guided core prostate biopsy with local anaesthesia. Straws were investigated by PCR, pathomorphology and culture.

Results Common complaints were pain (96.8%), dysuria (79.6%); laboratory findings: leucospermia 73.1%, haemospermia 51.6%. 37.6% had TB history, 34.4% had active TB of another localization, mostly pulmonary. Results of PCR: HPV 10.7%, Ureaplasma 2.2%. Mycobacteria culture was positive in 6.9%. Pathomorphologically in 94.6% inflammation was found, in 65.6% fibrosis, in 9.7% intraprostatic neoplasia, in 5.4% cancer, in 24.7% TB.

Conclusion The diagnosis of prostate TB is a very difficult task, because clinical features and laboratory signs are non-specific, alike chronic prostatitis. Absolutely pathognomonic symptom is a cavern on urethrogram, but caverns mean late-diagnosed complicated form, cavernous prostate TB cannot be cured neither chemotherapy nor by surgery. Prostate TB in early infiltrative non-cavernous stage may be diagnosed by PCR, culture or pathomorphology. Possibility of these methods alone is poor, it is necessary to use its in combination.

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Bacteria and Leukocytes as Inducers of Molecular Changes in Human Ejaculated Sperm Plasma Membranes During In Vitro Semen Infection

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Introduction An in vitro model of semen inflammation created in this study was intended to assess the influence of selected bacterial strains and/or leukocytes on sperm plasma membrane integrity. The complex examination proposed in the present study may allow us to achieve a more clear picture of subcellular changes in sperm membranes occurring in the course of semen bacterial infection.

Material & Methods Three bacterial isolates (*Escherichia coli*, *Staphylococcus haemolyticus* and *Bacteroides ureolyticus*), were chosen for the study. Leukocytes were isolated from the whole heparinized blood using a density gradient centrifugation technique (Histopaque-1.077). Sperm pellets obtained from normozoospermic volunteers were incubated with bacterial strains and/or leukocytes for 2h at 37°C. Sperm plasma membrane integrity was assessed by the LIVE/DEAD Sperm Viability Kit (SYBR14 and propidium iodide – PI), by the merocyanine-540 (M540) test, and by the hypoosmotic swelling (HOS) test. The level of lipid sperm membrane peroxidation was assessed determining the concentration of malondialdehyde (MDA) in sperm lysates using high-performance liquid chromatography (HPLC). The Annexin V-FITC Kit was used for PS externalization analysis.

Results The presence of B. ureolyticus was associated with a significant increase in the percentage of dead (PI-positive) spermatozoa as compared to untreated cells ($p < 0.01$). In general, the addition of leukocytes resulted in increase in the percentage of PI-positive sperm, regardless of bacterial strain applied. All the bacterial strains used alone or together with leukocytes affected sperm plasma membrane architecture measured by M540 test ($p < 0.01$). Out of the bacterial strain tested, *B. ureolyticus* caused significantly decrease in sperm swelling as compared to the control ($p < 0.05$). The presence of leukocytes in co-incubated mixture was associated with further decrease in sperm swelling primarily caused by anaerobes ($p < 0.01$). *Escherichia coli* and *B. ureolyticus* had the greatest influence on MDA concentration in spermatozoa membranes ($p < 0.001$). Again, the leukocytes, additionally increased the harmful effect of bacteria. As for Annexin V test, there was no statistical differences as compared to untreated spermatozoa (control).

Conclusions The range of sperm membrane alterations seems to depend on the type of pathogen and the presence/absence of leukocytes. The presence of the latter in semen may be the additional factor worsening the structural and functional sperm plasma membrane integrity during semen infection/inflammation. A microbiological examination of semen samples should be recommended not only for aerobic but also for anaerobic bacteria.

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The Global Online Sexuality Survey: Sexual Function and Dysfunction in USA

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Question The Global Online Sexuality Survey (GOSS) is a world-wide epidemiologic study of sexuality and sexual disorders, investigating cultural characteristics and uniqueness, and comparing sexuality across cultures and races, launched in the Middle East in 2010, and USA in 2011. The current study investigates the rates of erectile dysfunction (ED), premature ejaculation (PE), their predisposing risk factors and treatment trends, preferences for sexual positions, polygamy, the value of penile size, and much more.

Methods GOSS was randomly deployed to English-speaking male web surfers in USA via advertising on Facebook®, comprising 146 questions.

Results 2022 males participated in the survey from the United States of America, completing the survey to variable extents. 37.7% suffered ED as per IIEF-5. Adjusted to the World Standard Population by the World Health Organization the collective prevalence was 33.7%. 49.6% of the population surveyed were diagnosed as having PE as per PEDT. Age adjusted prevalence of PE was 51.3%.

Participants used phosphodiesterase inhibitors (PDEis) on more consistent basis in 23.7%, increasing progressively with age. Participants diagnosed with erectile dysfunction (ED) used PDEis in 37.5%, while those without ED used them in 15.6%; recreational use, the motivation for which was analyzed. PDEis were mostly utilized on prescription basis, and so was the choice for the brand of PDEi. PDEis were mostly purchased from pharmacies (72.7%), followed by online purchase (16.5%). However, 5.3% of pharmacy sales of PDEis were without prescription, and 9.6% of those utilizing PDEis without prescription happened to have coronary heart disease. Efficacy of PDEis, experienced and theoretical side effects were also reported, with unrealistic concerns over safety detected.

The three most preferred sexual positions were the missionary position, the female on top and the rear vaginal entry positions. The most commonly practiced sexual position was the missionary position, with the latter two practiced much less than preferred. Anal intercourse was the least preferred and practiced, though practiced by 20.6%. 52% reported having had more than one partner in parallel (informal polygamy). 39.3% reported never using condoms on casual sexual encounters. 28.8% reported the use of one or more contraceptive measure for birth control. The most frequently used was condom, though least preferred. Vasectomy and female contraceptive measures were the most favored.

Conclusion Sexuality in the United States of America as of 2011/2012 is extensively analyzed.

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Associations between Asymptomatic Prostatitis and Erectile Dysfunction in Ageing Male

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Question Asymptomatic inflammation as new category of prostatitis is often found during evaluation of other reproductive and sexual disorders. The aim of this study was to determine the associations between asymptomatic prostatitis and erectile dysfunction (ED) in ageing male.

Methods A total of 132 men (mean age 58.9 ± 6.7 years) undergoing health screening were investigated for prostate-specific symptoms and sexual function, white blood cells (WBC) in expressed prostatic secretion (EPS) and post-prostatic massage urine specimen, total prostate volume, urinary flow rate and for certain organ-specific, hormonal and biochemical markers. Men with clinical symptoms of prostatitis were excluded. Subjects were divided into 3 groups: men without leukocytes in EPS (< 0.2 × 10^6 WBC/mL, group 1), men with moderate (0.2–1 × 10^6 WBC/mL, group 2) and significant (>1 × 10^6 WBC/mL, group 3) counts of white blood cells in EPS.

Results We found statistical difference in International Index of Erectile Function-5 (IIEF-5) score for all investigated groups (p = 0.048). The prevalence of erectile dysfunction (IIEF-5 score < 22) was 72.7%, 73.8% and 85.4% for group 1, 2 and 3, respectively.

The IIEF-5 score showed a positive correlation with maximum flow rate (r = 0.331, p = < 0.001) and a negative correlation with WBC count in EPS (r = -0.24; p = 0.006) and PSA level in serum (r = -0.225; p = 0.01).

Conclusions Our preliminary results suggest that asymptomatic prostatitis may be one of the risk factors for ED (and other sexual disorders).

However, the future research should directly define the relationships between (asymptomatic) prostatitis and erectile dysfunction as well as examine the effect of treatment in ageing male with prostatitis and sexual problems **(Tab. 2)**.

Table 2. K. Ausmees et al. Selected characteristics of men undergoing screening of reproductive health.

	Group 1 < 0.2 × 10^6 WBC/ml	Group 2 0.2–1 × 10^6 WBC/ml	Group 3 ≥ 1 × 10^6 WBC/ml	p-Value
No. patients (%)	53 (40.2)	40 (30.3)	39 (29.5)	
Prevalence of ED (%)	40 (75.5)	31 (77.5)	35 (89.7)	
Mean age ± SD (yr)	57.8 ± 6.3	59.9 ± 6.8	59.1 ± 7.1	0.252
Abstinence time before EPS (days)				
– Median	5.0	6.0	5.0	0.135
– Mean ± SD	5.5 ± 3.3	7.6 ± 5.3	8.3 ± 6.1	
WBC in EPS (per ml)				
– Median	0.2	0.5	2.2	< 0.001
– Mean ± SD	0.05 ± 0.06	0.5 ± 0.2	5.9 ± 5.9	
IIEF-5 total score				
– Median	19.0	19.0	16.0	0.021
– Mean ± SD	18.3 ± 4.4	16.0 ± 6.6	14.7 ± 6.4	
Testosterone (nmol/l)				
– Median	16.4	17.6	16.4	0.422
– Mean ± SD	15.2 ± 6.0	17.7 ± 4.8	17.2 ± 6.3	
PSA (ng/ml)				
– Median	1.9	2.8	3.2	0.410
– Mean ± SD	2.2 ± 2.0	3.4 ± 2.6	5.1 ± 6.8	
hs-CRP (mg/l)				
– Median	1.4	1.3	1.2	0.089
– Mean ± SD	1.8 ± 1.7	2.1 ± 2.5	3.1 ± 5.7	
BMI (kg/m²)				
– Median	27.5	27.7	27.1	0.552
– Mean ± SD	28.1 ± 3.5	27.9 ± 4.3	27.2 ± 3.8	
Maximum flow rate (ml/s)				
– Median	18.2	14.9	16.2	0.495
– Mean ± SD	18.9 ± 9.5	17.0 ± 10.2	17.8 ± 8.2	
Total prostate volume (cm³)				
– Median	36.0	37.5	34.0	0.265
– Mean ± SD	38.5 ± 16.9	42.1 ± 16.4	36.5 ± 12.0	

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Body Height and Sexual Activity – Hungarian Data

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In the last decades there has been considerable progress in the assessment of sexual practices and evaluation of sexual dysfunction. However, there is limited data available concerning the relationship of sexual activity and basic anthropometric parameters in different age and ethnic groups.

The aim was to elucidate this relationship.

The study population consisted of 1,146 male patients aged 25–45 years. All patients were recruited from an outpatient andrology clinic in Budapest, Hungary. A medical survey was conducted on anthropometric parameters including age, height, body weight and body mass index (BMI). Patients were allotted into three age groups (25–29, 30–39 and 40–45 years). Self-reported sexual activity was also reported. Chi-square test, one-way ANOVA and stepwise linear regression were used to characterize relationships between anthropometric parameters and sexual activity.

The youngest age group showed the highest coital activity. Although 61% of the patients were either obese or overweight no correlation between BMI and sexual activity were apparent. There was a significant difference regarding sexual activity between height categories. Patients with height below 170 cm reported higher weekly coital frequency than men over 180 cm. Excepting height and age, most anthropometric measures did not correlate with sexual activity.

There is a great need for large-scale studies worldwide, using similar methods, and larger representative samples from various ethnic and age groups.

Reference:
Rurik I, Szigethy E, Fekete F, Langmár Z. Relations between anthropometric parameters and sexual activity of Hungarian men. Int J Impot Res 2012. doi:10.1038/ijir.2011.57. [Epub ahead of print]

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The SIAMS-ED Study: a Spontaneous, Multicentre, Observational Study on the Efficacy of Vardenafil in Men with Erectile Dysfunction and Cardiovascular Risk Factors

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Question Observational study designed and conducted by the Italian Society of Andrology and Sexual Medicine (SIAMS) to assess health outcomes in men with erectile dysfunction (ED) who took vardenafil for five months in a real-life setting.

Methods This was a spontaneous, multicentre (18 sites), open-label, prospective, non-comparative, interventional study performed in a large cohort of unselected men (n = 604, mean age 55.3 ± 12.1 yrs) with ED. Enrolled patients had a 4-week washout period from previous pro-erectile drugs and prior to ED investigations that included established questionnaires (SIEDY, EDOS, IIEF5, SSLC90) and penile colour Doppler ultrasonography. All patient underwent metabolic, cardiovascular and hormonal screening, including pulse pressure and testosterone evaluation; cardiovascular risk (CVR) was stratified into six classes predicting the likelihood of a major adverse cardiovascular event (MACE) within 10 yrs (Progetto Cuore Algorithm).

Results One third of patients (30.8%) meet the diagnosis of metabolic syndrome (ATPIII criteria). In particular, 33.2% had reduced HDL cholesterol, 36% increased waist circumference, 67.8% high blood pressure (of which 49.9% systo-diastolic and 8.1% isolated diastolic hypertension), 37.2% hypertriglyceridemia and 31.9% high blood glucose. Mean pulse pressure was 49.2 ± 10.4 mmHg. Stratification of cardiovascular risk was 28.6% and 30.2% for class I and II class (< 10% of MACE), 21.5% for the III class (10–15% of MACE), 12.3% for the IV class (15–20%), 5.4% and 1.9% for the V and VI class (> 20% of MACE).

A higher PP and a significant reduction in T levels were found in men with higher CVR classes(^{3}IV, $p < 0.0001$); PP was higher in men with moderate/severe ED vs mild ED ($p < 0.001$) and negatively correlated with androgens levels and waist ($p < 0.01$). Follow-up data on 185 men treated with Vardenafil documented a significant increase in baseline IIEF5 score(D = 6.1 ± 4.8; $p < 0.0001$) for all CVR classes. Interestingly, greaterD-IIEF5 increments were observed in men with higher PP and CVR ($p < 0.0001$), when adjusted for confounding factors. Mild adverse events were reported in < 5% of the population, with no differences between high vs. low CVR classes.

Conclusions In the real life settings, ED is becoming a frequent presenting symptom for patients with an elevated, often ignored, risk of future MACE. Simple screening procedures to select patients deserving preventive measures are needed. Androgens levels and PP could be used in these patients as biomarkers of cardiovascular health. Vardenafil turned out safe and powerful in improving erections in severe ED patients, proving unaltered efficacy in higher CVR classes.

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Clinical Correlates of Erectile Dysfunction and Premature Ejaculation in Men with Couple Infertility

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Introduction The frequency and the clinical characteristics of erectile dysfunction (ED) and premature ejaculation (PE) in infertile men have been poorly investigated. The aim of this study was to assess the prevalence of ED and PE and their clinical correlates in men seeking medical care for couple infertility.

Methods A consecutive series of 244 men (mean age 35.2 ± 7.8) with couple infertility was systematically evaluated. Erectile function was investigated with the International Index of Erectile Function-15 erectile function domain (IIEF-15-EFD), whereas ejaculatory status with the Premature Ejaculation Diagnostic Tool (PEDT). An IIEF-15-EFD < 26 indicated ED. A PEDT score < 8 indicates no-PE. All patients underwent psychological (Middlesex Hospital Questionnaire, MHQ), prostatitis symptoms (National Institutes of Health-Chronic Prostatitis Symptom Index, NIH-CPSI), hormonal, seminal and interleukin 8 (sIL-8; a surrogate marker of prostatitis) evaluation, along with scrotal and transrectal colour-Doppler ultrasound (CDU) assessment.

Results ED was found in 43 (17.8%) and PE in 38 (15.6%) subjects. After adjusting for age, IIEF-15-EFD score was negatively associated with depressive symptoms (MHQ-D score), somatization (MHQ-S score), NIH-CPSI total and Quality of Life (QoL) subdomain score. In a logistic multivariate model, among all these variables, only depression was significantly associated with ED (adjusted OR = 1.19 [1.02–1.39]; $p < 0.05$). PEDT score was positively associated with prostatitis symptoms and signs, such as sIL-8 and prostate CDU abnormalities (including arterial prostatic peak systolic velocity, APPSV), phobic anxiety (MHQ-P score) and calculated free testosterone (cFT). The association between PE and NIH-CPSI score or APPSV was confirmed even after adjustment for age, MHQ-P score and cFT (adjusted OR = 1.11 [1.05–1.17]; $p < 0.0001$ and 1.22 [1.03–1.44]; $p = 0.02$, for NIH-CPSI score and APPSV, respectively).

Conclusions ED and PE are reported by one out of six infertile patients. ED is mainly associated with depressive symptoms, while PEDT score is positively associated with prostatitis symptoms and signs, phobic anxiety and cFT.

Short Oral Presentations 4: Sperm Quality and Selection for ART, Determinants of Male Reproductive Health, Genetics and Epigenetics

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Long-term Contraception with Risug and its Functional Reversal by DMSO and $NaHCO_3$ in Rats

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The success of functional reversal following long-term vas occlusion with RISUG and its reversal by DMSO and $NaHCO_3$ was carried out in adult Wistar albino rats. The study was divided into seven groups containing ten animals in each, viz., sham operated control (group I), vas occlusion with RISUG for 90 days (group II), vas occlusion for 90 days and reversal by DMSO (group III), vas occlusion for 90 days and reversal by $NaHCO_3$ (group IV), vas occlusion for 360 days (group V), vas occlusion for 360 days and reversal by DMSO (group VI), and vas occlusion for 360 days and reversal by $NaHCO_3$ (group VII). Animals were subjected to bilateral vas occlusion using 5–7 µl of one human dose of RISUG in each vas. 90 and 360 days following vas occlusion, animals were subjected to vas occlusion reversal, respectively, with 250–500 µl of DMSO and 500–700 µl of 5 % $NaHCO_3$. Ejaculated spermatozoa of RISUG injected animals and initial intervals following reversal exhibited necrospermic status. In groups II, III, IV and VI 100% sterility was recorded at all post-injection mating intervals, whilst a gradual decrease in per cent fertility was detected in groups V and VII which was found zero per cent following 90 days of vas occlusion. Fertility test in DMSO reversed animals indicated 100% sterility in group III at 15th day mating which was gradually improved from 30–100% following 30th day to 90th day post-reversal. Group VI showed 70% fertility resumption at the first mating and eventually completely restored on the 45th day post-reversal. Reversal carried out by $NaHCO_3$ in group IV also exhibited a similar trend, indicated gradual fertility restoration from 80–100% following 30th day to 90th day post reversal. In group VII, however, fertility restored to 80% on the 15th day post-reversal, which was resumed to 100% in all other mating intervals. After 90 and 360 days of vas occlusion with RISUG, the lumen of the occlusion site showed eruption of epithelium at certain places which regained completely and patency of the vas was evident by day 90 post-reversal in all groups. In conclusion, a rapid restoration of fertility was evident in long-term reversal by both methods when compared with short-term and are feasible and effective leading to cent percent return of fertility and could be a viable alternative for reversible approach of contraception in human.

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Deficient Testosterone Production during the Masculinization Programming Window is associated with Sertoli-cell Only Tubules and Focal Dysgenesis in Adulthood

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Introduction The testicular dysgenesis syndrome (TDS) hypothesis proposes that maldevelopment of the testis, which could have numerous primary causes, leads secondarily to malfunction of the Leydig (LC) and/or Sertoli (SC) cells and consequent downstream disorders. Normal reproductive tract development and anogenital distance (AGD) are programmed within the 'masculinisation programming window' (MPW, e15.5–e18.5 in rats; ~8–14 weeks of gestation in humans), and TDS disorders can arise because of deficiencies in this programming. We have already shown that experimentally-induced dysgenesis of the testis in rats and testosterone production in the MPW are interrelated in fetal life, and both are associated with reduced AGD at birth. The purpose of this study was to evaluate if disorders of the adult testis are also linked to events specifically in the MPW.

Material & Methods Testes from adult male Wistar rats which were exposed in utero to either vehicle (control) or to dibutyl phthalate (DBP; 750 mg/kg/day) from embryonic day (e)15.5–e18.5 (MPW) were used to determine the appearance of SCO tubules and focal dysgenesis based on H&E staining and immunohistochemistry for various cell-specific markers. Testosterone production was measured by radioimmunoassay. Linear regression analysis was used to determine the relationship between AGD, dysgenesis, testosterone production and the appearance of SCO tubules.

Results We have analyzed 10 adult males which were exposed to DBP during the MPW. Of these 10 males, 1 male had severe hypospadias, 2 had very mild hypospadias while the other 7 males had a normal penis; average (scrotal) testis weight was reduced by ~25%. We performed H&E staining on 11 testes (8 scrotal, 1 inguinal, 2 cryptorchid), and in 6 out of 8 scrotal testes we observed SCO tubules and/or focal dysgenesis. These 6 testes were from animals that had normal penis development but reduced AGD (indicating reduced androgen exposure in the MPW). No abnormalities were found in control rats. More detailed, quantitative studies are ongoing and will be reported at the meeting.

Conclusion We conclude that DBP-induced suppression of intratesticular testosterone during the MPW, which results in life-long reduction in AGD, also induces a significant increase in focal dysgenesis and SCO tubules in normally descended testes in adulthood. This demonstrates the importance of testis development during the MPW in programming the structure and normality of the adult testis and TDS disorders.

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Impact of the Slenoprotein nGPx4 on Male Fertility and Sperm Epigenome

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Introduction We recently demonstrated that the nuclear form of Glutathione Peroxidase 4 (nGPx4) has a peculiar distribution in sperm head, being localized to nuclear matrix and acrosome, and is required for proper paternal chromatin decondensation at fertilisation. While protamines are the major component responsible for sperm chromatin packaging, a small amount of histones is also retained, the relevant role of which has been recently highlighted in influencing early embryo development. We reasoned that paternal histone modifications could be implicated in the process of sperm chromatin disassembly in the zygote. We used nGPx4 KO mice as experimental model to address this issue and to investigate the impact of nGPx4 on sperm fertilizing potential.

Material & Methods We analyzed levels of acetylated Histone H3 and H4 in cauda epididymal spermatozoa from nGPx4 KO and WT mice by western blotting and immunofluorescence staining. Sperm were decondensed by glutathione and heparin treatment to allow signal detection. The fertility of nGPx4 KO mice was determined by in vivo and in vitro assays.

Results We first assessed the consequences of the nGPx4 lack on hyperacetylated histone H4 and histone H3 acetylated at K9 and K14 levels. Interestingly, significant higher amounts of modified histones appeared in nGPx4 KO sperm compared to WT sperm. In light of the fact that nGPx4 KO sperm decondense faster than WT sperm, present data may link the acetylation of histones retained in sperm to paternal chromatin remodeling at fertilisation. To investigate the function of nGPx4 localized to sperm acrosome we asked whether it was associated with male fertility. Matings of nGPx4 KO male mice to wild-type females yielded both litter sizes and percentages of plugged and pregnant females significantly smaller than those of WT male mice. In vitro fertilisation assays re-

vealed 70% reduction in the percentage of embryos at pronuclear stage when metaphase II oocytes were inseminated by nGPx4 KO capacitated sperm compared to WT ones. The reduced fecundity of nGPx4 KO males was due to a defect of sperm ability to penetrate zona pellucida, being sperm binding and sperm-oolemma fusion similar between the two genotypes. These results demonstrate the subfertility of nGPx4 KO male mice.

Conclusions These findings reveal a previously unrecognized role for the nuclear isoform of GPx4 in male fertility. Hitherto it was demonstrated that the mitochondrial GPx4 (mGPx4) confers the vital role of selenium in mammalian male fertility, being mGPx4 KO sperm unable to fertilize oocytes because of tail severe abnormalities. We also propose that acetylated histones retained in sperm might contribute to the development of the male pronucleus.

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Correlation between Spermatozoal DNA Integrity (TUNEL Assay) and Chromatin Condensation (GRAM staining) with Basic Semen Analysis in Subfertile Men and Normal Controls

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Introduction Spermatozoal DNA integrity and chromatin condensation are crucial factors for male fertility. TUNEL assay and Gram staining were used for the assessment of nuclear quality in seminal smears from patients consulting for infertility. The aim of this study was to investigate correlation if any between the two indexes and normal and abnormal parameters of basic semen analysis.

Materials & Methods TUNEL and Gram staining were applied to semen samples from 105 infertile men. DNA Fragmentation Index (DFI%) and Gram Stain Index (GSI%) referring to the percentages of abnormal spermatozoa (TUNEL positive and Gram negative) were raised. From the total number of men examined, 78 provided basic semen analysis results and thus they were divided into two groups, those with normal and those with abnormal parameters, according to the WHO 2010 semen analysis criteria. Statistical evaluation was made with Kolmogorov-Smirnov, T-test and the correlation between GSI% and DFI% with Pearson's parametric method. Statistical significance was set at $p < 0.05$.

Results Spermatozoa with fragmented DNA (TUNEL positive) were measured and DFI % was 24.79 ± 19.397. Normal condensed spermatozoa were stained blue (Gram positive) whereas those with abnormal condensation appeared red (Gram negative) and the GSI% was 50.68 ± 18.50. All variables follow normal distribution and parametric statistic analysis was used. Additionally, with Pearson correlation coefficient no statistically significant correlation was revealed ($r = 0.147$, $p = 0.135$). The T-test for men with normal and abnormal parameters of basic semen analysis showed no differences between DFI% and GSI%, with p-value 0.470757 and 0.846763, respectively.

Conclusion Spermatozoal DNA integrity and chromatin condensation are not correlated and both indexes appear independently of basic semen analysis parameters. Although further investigation is needed, it is suggested that the two techniques must be used simultaneously for the best possible assessment of sperm quality.

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The Methylation analysis of Histone H4K12ac associated Promoters in Sperm of Healthy Donors and Subfertile Patients

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Introduction The proper maturation of sperm cells requires two important mechanisms: histone to protamine exchange and hyperacetylation of remained histones. The acetylation of histone H4 at lysine 12 (H4K12ac) has been observed prior to full decondensation of sperm chromatin after fertilisation suggesting its important role for regulation of gene expression in early embryogenesis. Similarly, DNA methylation may contribute to the gene silencing of several developmentally important genes. Following the identification of H4K12ac binding promotes in sperm of fertile and subfertile patients, we aimed to investigate whether the depletion of histone binding was associated with alteration of DNA methylation in subfertile patients.

Material & Methods Chromatin immunoprecipitation with antibody against H4K12ac was performed with spermatozoa of subfertile patients with impaired sperm chromatin condensation as assessed by aniline blue staining and fertile donors as controls. H4K12ac immunoprecipitates were analyzed by hybrydisation on HG18 promoter array (NimbleGen). DNA methylation analysis of 10 developmentally important, H4K12ac interacted promoters in spermatozoa of 7 healthy donors and 20 subfertile patients was performed using pyrosequencing (PyroMark Q24, Qiagen).

Results Over 500 target gene promoters for H4K12ac has been identified for H4K12ac in healthy donors. In contrast, less binding sites of 149 gene promoters and lower enrichment of each binding site could be identified in infertile patients. Depletion of H4K12ac of following developmentally important promoters: TRIP13, AFF4, AXIN1, EP300, LRP5, RUVBL1, USP9X, NCOA6, NSD1, POU2F1 was observed. The pyrosequencing analysis showed hypomethylation (5–10%) within CpG islands of H4K12ac binding promoters in fertile sperm which has not been changed in a group of subfertile men.

Conclusions Our result showed that the accessibility of histone binding is not limited by DNA methylation in normozoospermic men. The depletion of H4K12ac in selected developmentally important promoters in subfertile patients was not accompanied by changing in methylation status. We therefore suggest that aberrant histone acetylation within developmentally important gene promoters in infertile men, not DNA methylation, may reflect insufficient sperm chromatin compaction and transfer of epigenetic mark to the oocyte.

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Even Globozoospermic Spermatozoa have Cephalic Vacuoles

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Introduction We report the cases of 2 patients diagnosed with total globozoospermia after conventional semen analysis. We studied sperm morphology by high-magnification interference contrast microscopy and electron microscopy, and assessed acrosomal status by fluorescent labelling with peanut agglutinin (PNA) lectins and anti-CD46 antibodies. The total absence of acrosome in these patients provided a good opportunity to further investigate the origin of the cephalic vacuoles.

Material & Methods We carried out detailed morphometric analysis under high-magnification (×6 000) interference contrast microscopy and measured the number of sperm-head vacuoles and their percentage area using digital imaging system software (Leica Application Suite v 3.6). We also studied acrosomal status by fluorescent labelling with PNA lectins and anti-CD46 antibodies and electron microscopy. Analysis of DNA fragmentation was performed by terminal deoxynucleotidyl transferase-mediated dUTP nick end labelling assay (TUNEL) and chromatin status was explored using sperm chromatin structure assay (SCSA) and aniline blue staining.

Results Our 2 patients with total globozoospermia (absence of acrosome confirmed by electron microscopy) had vacuolar areas of 6.3% and 5% (reference population of fertile men 5.9%). However, although both these patients were diagnosed with total globozoospermia, their profiles were somewhat different: patient 2 had positive lectin labelling for 9% of spermatozoa and Golgi residues

were seen under electron microscopy. A live birth was obtained after ICSI for patient 2 only.

Conclusion In our 2 patients in whom 100% of spermatozoa are acrosomeless, high-magnification examination with Nomarsky contrast revealed a number of vacuoles and a relative vacuole area very similar to those of fertile controls. These findings strongly support the hypothesis that these vacuoles, in particular the largest vacuoles, are not of acrosomal origin.

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Epimutations in RHOX Genes are Associated with Male Infertility

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The X-linked RHOXgene cluster encodes a set of homeobox transcription factors that are selectively expressed in the reproductive tract. Members of this homeobox gene cluster regulate key target genes important for spermatogenesis and they promote male fertility in mice. Here, we report that the human RHOX gene cluster is selectively hypermethylated in sperm from men with abnormal semen parameters. Hypermethylation in this region of the X-chromosome is restricted specifically to the RHOX cluster as it does not spread to the immediately adjacent genes on the X-chromosome. To determine whether DNA methylation has a causal role in regulating RHOX transcription, we defined the promoter regions of the human RHOX genes and performed gain- and loss-of-function experiments. The results showed that DNA methylation is both necessary and sufficient to strongly repress RHOX gene transcription. Our results suggest that DNA methylation is responsible for the tissue-specific expression pattern of the RHOX gene cluster and lead us to propose that RHOX hypermethylation is a useful marker for male infertility.

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Reference Values of Sperm Chromatin Dispersion Test in Sperm Donors with Known Fecundity

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Introduction Reference ranges of sperm DNA integrity markers are usually drawn from individuals classified as fertile based on permissive criteria. With the objective of defining our own reference values for Sperm Chromatin Dispersion (SCD) test, we have retrospectively studied a series of sperm donors fully assessed in our hospital and with a detailed record of their reproductive fitness after assisted reproductive techniques.

Material & Methods We included 47 sperm donors younger than 35 y/o, without medical history of significant diseases, drug abuse and hereditary conditions. Blood analyses were normal, including haematology, biochemistry and karyotype. HBs antigens, antibodies against HCV, CMV and HIV, as well as RPR test, were all negative at baseline and after 6 months final quarantine. All included donors had normal semen analysis. Sperm count was > 50 millions/mL, progressive motility > 40% and normal morphological gametes > 6% (Tygerberg criteria). Sperm samples from each donor were used at least in 10 insemination cycles, on a minimum of 4 different female recipients. Sperm DNA integrity was analyzed with the method of SCD (Halosperm®) as described by the manufacturer, on semen aliquots previously frozen. Duplicate assessments were done on 500 sperm by two different observers. Fragmentation Index (FI) was calculated as the ratio between damaged sperm ("degraded" sperm, without halo, small halo) and total sperm counted.

Results The average pregnancy rate per insemination cycle of donors was 15.3%. Miscarriages occurred in 15.8% of pregnancies. The readings of FI by the two observers were equivalent. The FI was 13.2% (6–25) (median [95%-CI]) and showed some correlation with normal morphology (R = 0.293; p = 0,057) but not with other semen parameters, or pregnancy rates of individual donors. There were two donors with FI above the limit of 95%-CI showing good pregnancy rates and normal semen parameters.

Conclusions Our results confirm that the upper limit of reference for SCD test can be established around 25 %. Donors showing SCD above the median did not display any trend towards worsening of reproductive outcome, compared with those below the median.

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UVB-induced Mitochondrial Dysfunction does not Correlate with Sperm DNA Fragmentation

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Introduction New molecular methods have highlighted the importance of sperm nDNA integrity to embryo quality and implantation. The focus of many studies, it is now acknowledged that the fragmentation seen in sperm may be the consequence of oxidative stress. The cause of this stress however remains a contentious issue. The current hypothesis is that either internal and/or external events trigger a cascade causing mitochondrial dysfunction and the release of reactive oxygen species (ROS) which attack sperm nDNA. Unable to explain various inconsistencies, the plausibility of this proposition has been questioned. Our aim was to induce mitochondrial dysfunction by UVb irradiation, evaluate the effect on sperm nDNA, localize the damage and assess the influence of seminal plasma.

Materials & Methods Semen samples were collected from 45 patients attending the Andrology laboratory of CeRA. Samples were placed in one of 6 groups: 0%, 20%, 40%, 60%, 80%, 100% seminal plasma. Aliquots of each group were irradiated with UVb for 15s, 30s, 45s, 60s, 120s, 180s, 240s. Sperm motility and viability were assessed (WHO criteria), nDNA status determined by acridine orange test, lipid peroxidation with BODIPY (latter two tests by flow cytometry). The position of nDNA damage was localized using Raman microspectoscopic spectral mapping. Samples were left for 15 mins, then irradiated for an identical time and again assessed using the same tests.

Results Even short UVb exposure was found to significantly decrease sperm motility and viability, however nDNA integrity remained unaffected. Damage was seen to increase at high dosages but only after the death of the sperm. At levels where sperm were immotile but not dead (i.e. mitochondria damaged but the cell survived), no difference was seen in nDNA integrity. Increasing concentrations of seminal plasma ameliorated the actions of UVb with as little as 40% capable of providing maximum protection. Raman spectral mapping found nDNA damage primarily in the proximal region of the head.

Conclusion No evidence was found that damaged sperm mitochondria and their proposed ROS leakage caused any nDNA damage (neither the quantity nor location). In light of these findings and the accumulating evidence from other studies, the current hypothesis is unable to adequately explain the origin of sperm nDNA fragmentation.

ECA Plenary Lecture 1: Metabolic Syndrome and Reproductive Function

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Effects of MS on the Male Reproductive System

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The MetS clusters metabolic and cardiovascular risk factors, including hyperglycemia, atherogenic dyslipidemia, hypertension and abdominal visceral obesity. Such disorders are not clustered coincidently, and visceral obesity has been evidenced to play an essential role in the pathogenesis of MetS. The numerous deleterious effects of MetS are being investigated throughout the medical community, as MetS may affect many aspects of human physiology due to its systemic nature.

Male factor infertility may represent one such perturbation in male patients with MetS. Adverse effects of obesity, and in particular visceral obesity, on male infertility are postulated to occur through several mechanisms.

First, visceral obesity per se, is a major determinant healthcare issue leading to MetS-induced hypogonadism. Several studies have reported defects of the hypothalamic-pituitary-gonadal axis, characterized by a decline in T levels associated with normal or low follicle-stimulating hormone (FSH) and luteinizing hormone (LH) levels, in obese and in MetS men.

More recent epidemiological studies have clearly indicated a strong and independent relationship between male male reproductive system alterations and metabolic syndrome (MetS). This association implies common factors in the pathogenesis of these entities. Common denominators include sustained hyperglycaemia, insulin resistance, low-grade chronic inflammation and hypogonadism.

In order to evaluate the pathogenetic mechanism underlying these associations we develop an animal model of MetS, closely resembling the human phenotype. This animal model was obtained by feeding adult male rabbits a high fat diet (HFD; 0.5% cholesterol and 4% peanut oil) for twelve weeks. Along with the classical features of MetS, such as hyperglycemia, impaired glucose tolerance, dyslipidemia, hypertension, increased visceral obesity, HFD rabbits demonstrated also an overt hypogonadotropic hypogonadism characterized by a substantial reduction of testosterone levels, atrophy of seminal vesicles, prostate and testis, and an impairment of erectile function. Interestingly at hypothalamic level we found a reduction of GnRH immunopositivity that suggest a HFD-induced reduction of GnRH secreting neuron. At testicular level several enzymes involved in the steroidogenesis were down-regulated. However, more recently we investigated the effect of HFD-induced MetS at low urinary tract (LUT) level, including bladder and prostate.

Interestingly, MetS induced not only bladder dysfunction but also a prostatitis-like syndrome. A florid infiltration of inflammatory cells, including macrophages, neutrophils, $CD4^+$ and $CD8^+$ T lymphocytes was observed within the interductal stroma and epithelium of HFD prostate. Besides inflammation, marked tissue remodeling and hypoxia were observed in HFD-treated rabbit prostate. Prostate ischemia, along with inflammation and stromal reorganization, characterized by fibroblast trans-differentiation to myofibroblasts, has recently been recognized as a key pathogenetic factor in the development of BPH. Also HFD-treated rabbit bladder showed several histological alterations and dysfunctions. Histomorphological evaluation of bladder samples from HFD rabbits evidenced the presence of widespread hypoxic areas in both urothelial and fibromuscular areas, as well as fibrosis with marked reduction of muscle/fiber ratio compared to control bladders from rabbits fed a regular diet. The increased mRNA expression of several inflammatory markers, such as COX2, IL-6 and lactoferrin in HFD-treated rabbit bladder further suggests the presence of chronic inflammation.

The association between obesity/MetS and prostate diseases has been extensively investigated in benign prostatic hyperplasia (BPH), often with a positive correlation. Conversely the relationship between obesity and prostate inflammatory diseases has been poorly studied. We have recently demonstrated that seminal IL-8 is the most reliable predictive marker of prostate inflammatory diseases. In addition, we recently retrospectively studied 222 consecutive male patients attending our outpatient clinic for the first time from January 2008 to October 2010, seeking medical care for couple infertility to investigate possible correlations among body mass index (BMI) and signs/symptoms of prostatitis including seminal IL-8. And sperm parameters and color-doppler-ultrasound (CDU) features of the entire male genital tract. In this study we found an association of BMI with several CDU features suggestive of prostatitis and IL-8 level.

However, an association between MetS components and prostate inflammation has not yet been demonstrated in humans. We have therefore examined the histological characteristics of inflammatory infiltrates in prostatectomy specimens from a cohort of BPH patients and their correlation with preoperatory MetS features, including hypogonadism. Histopathological examination of BPH specimens demonstrated the presence of prostatic inflammation in all cases. The inflammatory score (IS) significantly increased as a function of MetS components. Patients with MetS had significantly higher IS as compared to the rest of the sample. Accordingly, each incremental unit of IS significantly increased the risk of having MetS, even after adjustment for age. Among MetS components, in a age-adjusted model, reduced HDL cholesterol and elevated triglycerides – but not high waist circumference, glycaemia, or blood pressure – were significantly associated with elevated IS . Considering triglycerides and HDL cholesterol as continuous variables, a significant correlation with IS was observed after adjusting for age.

In the subset of patients in whom testosterone evaluation was available (n = 92), the prevalence of hypogonadism (TT < 10.4 nM) was detected in 24.2% of subjects. In this sample, increased IS was significantly associated with hypogonadism. Furthermore, after adjustment for hypogonadism and age, higher triglycerides and lower HDL cholesterol levels were still associated with increased IS.

To investigate whether metabolic factors could directly trigger prostate inflammation, we performed preliminary studies in hBPH. Among the different factors, oxidized low-density lipoprotein (oxLDL) showed the highest secretion of IL-8 (> 10-folds)-a surrogate marker of prostate inflammation-as well as IL-6, and bFGF. Co-treatment with DHT significantly inhibited oxLDL-induced secretion of IL-8, whilst an AR-antagonist, bicalutamide, reversed DHT effects. DHT suppresses oxLDL receptor (LOX-1) expression.

In conclusion, we demonstrated that MetS, and in particular dyslipidaemia is associated with prostate inflammation: fats could have a detrimental effect on prostate cells, boosting prostate inflammation, a key factor in the development and progression of BPH/LUTS. Beneficial effects of DHT in counteracting lipid- and insulin-induced prostatic alterations, suggest that testosterone – via its conversion into DHT – may have unexpected beneficial effects on prostate health. Clinical studies specifically addressing this point are urgently needed.

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Testosterone and Cardiovascular Health

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The most important cause of death as men age is cardiovascular disease and as men age so their androgen status declines. Studies have shown that lowering of endogenous androgen by deprivation therapy as treatment for prostate cancer increases the risk of cardiovascular death. In long term studies, low blood testosterone is associated with accelerated atherosclerosis as manifest in coronary, aortic and carotid vascular territories.

Animal models of hypogonadism with cholesterol feeding have also shown accelerated atherosclerosis, which replacement therapy (TRT) ameliorates. The mechanism is not understood but low testosterone in men, is associated with an adverse lipid profile, increased inflammatory activation, weight gain, insulin resistance and impaired glucose tolerance which is improved by TRT as proven in randomised controlled trials.

Testosterone has also been shown to be an arterial vasodilator, an effect mediated by blocking the L-type calcium channels in the vascular smooth muscle membrane. This may be the mechanism whereby it improves exercise capacity in men with low blood testosterone who also have angina or heart failure.

The disadvantage of a low blood testosterone level is not just related to accelerated atherosclerosis. There have been at least 4 epidemiological studies showing that a low serum testosterone is associated with an excess mortality, in populations of normal men without overt vascular disease. We recently reported a follow-up study of 930 men with coronary disease confirmed at angiography which showed that men with low levels of bioavailable testosterone had a highly significant increase in mortality (×2) compared with those with a normal bio-available testosterone over a follow-up period of 7 years.

Replacing testosterone levels to normal improves symptoms, functional capacity and

well being in men with heart failure and angina. This year Shores et al [JCEM 2012; 97: 2050–8] showed in an observational study that TRT halved mortality in men with testosterone deficiency.

ESU Course 1: Priapism, Penile Implants & Reconstruction and Other Surgical Problems in ED

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Supersizing the Penis Following Penile Prosthesis Implantation

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Question Following implantation of a penile prosthesis, some couples are dissatisfied with penile length, girth, shaft or glans engorgement. This may be delusional, due to the procedure per se or due to preexisting risk factors such as neglected priapism, Peyronie's disease, radical prostatectomy or overhanging suprapubic fat. In this work, we try to enhance penile size in patients dissatisfied with its dimensions following implantation of a penile prosthesis, using various augmentation techniques.

Methods 18 patients who have had penile prostheses implanted were enrolled in this study based on dissatisfaction with penile size. The complaint was relieved by counseling and administration of PDE5 inhibitors in 7 patients. Two patients had elongation, girth augmentation and glans injection, six had elongation and girth augmentation and two had elongation and glans injection.

Results Average preoperative length and girth were 7.87 cm and 11.62 cm respectively. Mean post-operative length and girth were 11.62 cm and 14.07 cm. The gain in length (47.6%) and girth (21%) were statistically significant ($p < 0.005$). All patients and partners were satisfied with the results following surgery except one who suffered graft loss.

Conclusion Implantation of a penile prosthesis may improve penile rigidity, yet may confound couple's satisfaction with penile size to variable degrees. Sex education may alleviate those concerns. In refractory cases, penile augmentation may enhance phallic size and increase patient/partner satisfaction.

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Assessment of Erectile Function after Surgical Treatment of Penile Fracture

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Introduction The purpose of this study was to evaluate erectile function in patients undergoing surgery for fracture of the penis.

Materials & Methods 74 patients were included in the study with isolated, combined and false penile fracture. Mean age was 32.9 (from 18–59) years. The international index of erectile function (IIEF-EF domain score) used for assessment of erectile function assessment of quality of life was conducted using a "Life satisfaction scale" (LSS). Hardness Erection Score (HES) used to quantify the degree of rigidity of erections during penile Doppler.

Results A time from occurrence of an injury to surgery ranged from 5–168 hours, with 76% of patients seeking medical help in the first 12 hours, 20% in the next 12 hours and only 4% at a later date. 6 (12.3%) patients were diagnosed with concomitant urethral injury. The right cavernous body was damaged in 85%. The size of the defect did not correlate with pain intensity or the presence of urethral injury. The average duration of hospitalization was 7.5 bed-days for isolated fracture of the penis. Only in 5 patients after surgery IIEF scores were less than 25 and LSS score were less than 20. Only one patient had HES score < 4. In other cases patients were referred to therapy with a psychotherapist-sexologist with positive results.

Conclusions Erectile dysfunction after surgical treatment of penile fracture has mostly psychosomatic genesis.

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Sexual Dysfunction after Surgical Treatment of Benign Prostatic Hyperplasia

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Introduction Benign prostatic hyperplasia (BPH) is a common condition in elderly men and is associated with a range of erectile function. One of the important problems of post-operative rehabilitation of patients with BPH is to restore sexual function. Despite the fact that the average age of patients with BPH is 75 years old, most men continue to experience sexual desire and difficulties in its implementation may damage the quality of life of these patients.

The aim of the study – evaluation of sexual function in patients with BPH after surgical intervention.

Methods In the study, 250 patients were examined with benign prostatic hyperplasia with severe symptoms of bladder outlet obstruction and LUTS. Average age of patients was 68.7 ± 11.5 years.

All patients underwent a full range of laboratory and instrumental diagnostics. The sexual function was assessed by questionnaire International Index of Erectile Function (IIEF). There have been 188 TURP (75.2%) and 62 prostatectomy (24.8%).

Results Afore the surgical treatment of BPH 131 patients (52.4%) assessed their sexual function as a part-time due to broken or weakened erection, seared or painful orgasm, 72 patients reported decreased libido. Sexual activity was completely absent at 119 patients (47.6%), in 48 of them libido was saved.

131 patients with preserved sexual function were performed 101 TURP (77.1%) and 30 prostatectomy. Among patients who underwent TURP deterioration of erectile function observed 49 patients (48.5%) of these 11 patients were marked by complete loss of sexual activity (8.4%). After prostatectomy 23 patients reported manifest sexual dysfunction (76.7%), 8 patients (26.7%) were marked by a full erectile dysfunction (ED).

Of the 119 patients with the initial sexual dysfunction we produced 87 TURP (73.1%) and 32 prostatectomy. After performed TURP 19 patients had intermittent erectile dysfunction.

Thus, during the postoperative rehabilitation ED of different severity was detected in 91 patients (36.4%); at 100 patients (40%) sexual function was completely absent from these 19 patients after TURP. 21 patients with completely preserved erectile function noted a blurring of orgasm, 48 patients did not have any complaints, and completing the questionnaire IIEF gained more than 21 points.

Mainly dominated complaints were intermittent, and weakened erection, which does not allow to complete sexual intercourse, caused discomfort during coitus and ejaculatory function disorders.

Conclusions

1. After surgical treatment of BPH the impairment of sexual function revealed an average of 36.4% of cases. The highest percentage of ED occurs after prostatectomy.

2. Among men's sexual dysfunction who underwent surgery for BPH dominated ED – 83.5%, while the ejaculatory dysfunction accounted for 47.2%.

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Erectile Function after Robot-assisted Laparoscopic Radical Prostatectomy with Complete Excision of the Neurovascular Bundles – Are all Patients Later on Impotent?

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Introduction & Objectives The objective of this study is to evaluate the erectile function of patients undergoing robot-assisted laparoscopic radical prostatectomy (RALP) with compete excision of the neurovascular bundles (NVB).

Material & Methods The records of n = 53 patients who underwent RALP with compete excision of the NVB from February 2006 to June 2011 were retrospectively reviewed. Preoperative potency was evaluated with the five item version of the International Index of Erectile Function (IIEF-5). According to their IIEF score, all of the patients were potent and had no signs of erectile dysfunction.

Postoperative potency was evaluated prospectively at 3, 6 and 12 months after surgery and than yearly. Potency was defined as erections sufficient for penetration with or without phosphodiesterase inhibitors. Once a patient was potent, he was considered potent on further analysis as well. After RALP was performed none of the patients underwent adjuvant therapy. The parameters analyzed included: age, prostate specific antigen (PSA), prostate size, clinical stage, preoperative Gleason score, pathologic stage, postoperative Gleason score, surgical margin status and potency.

Results The median preoperative IIEF-5 score was 23.8 (22–25). The median age of the patients was 63.3 years old (51–75 years old), the median PSA was 11.3 ng/ml (2–39 ng/ml) and the median prostate size was 38.4 gr. (23–130 gr.). The clinical stage was thought to be confined tumor in n = 46 patients (86.7%) and tumor with extracapsular extension in n = 7 patients (13.3%). The preoperative Gleason score was Gleason7 in n = 17 (32.2%). The pathologic stage exhibited confined PCa in n = 42 patients (79.2%) and extracapsular extension in n = 11 (20.8%). The surgical margins were negative in all cases. A Gleason7 in n = 17 patients (32.3%). After a median follow-up of 29 months (range 3–67) n = 11 patients (20.7%) had an erection sufficient for penetration.

Conclusions Our findings suggest that not all patients undergoing robot-assisted RALP with complete excision of the NVB will become impotent. As seen from our results approximately 20% of the patients exhibited an erection sufficient for penetration.

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Impact of Circumcision on the Sexual Function of Patients with Phimosis

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Introduction Circumcision is the treatment of choice for patients with phimosis, reducing significantly the risk of urinary tract infections and the number of episodes of acute urinary retention. Little information is available in the literature on the consequences of this procedure in the male sexual life.

Question To evaluate the impact of circumcision on the sexual life of patients with phimosis.

Methods Of the 109 patients who underwent circumcision for phimosis in C. H. V. N. Gaia/Espinho during 2011, 62 patients were selected (excluded patients aged < 18 years old, without sex life or with absence of sexual intercourse after surgery). We performed a telephone survey, by the same interviewer, regarding the prevalence of various sexual dysfunctions before and after surgery. McNemar's test was used for a matched pairs analysis of the frequency of sexual dysfunctions.

Results Some sort of sexual dysfunction was found in 74.2% of patients with phimosis. With circumcision we noticed an increase in the frequency of erectile dysfunction (9.7% vs 25.8% before and after surgery, respectively, $p = 0.002$) and delayed orgasm (11.3% vs 48.4%; $p < 0.001$) (although this latter dysfunction was perceived as not negative in 74.6% of patients). In patients with dyspareunia a significant symptomatic improvement was noticed (50.0% vs 6.5%; $p < 0.001$). No statistically significant differences were found regarding premature ejaculation (25.8% vs 24.2%).

Conclusion Apart from a relevant improvement in dyspareunia, circumcision seems to worsen erectile dysfunction and delayed orgasm. Therefore, patients proposed to circumcision should be fully informed on the consequences on sexual life.

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Erectile Function Improvement with Oral Sildenafil versus Placebo in Posterior Urethroplasty: Double blind Randomized Controlled Trial

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Introduction Traumatic injury to the pelvis is associated with both urethral disruption and erectile dysfunction (ED). The initial injury, not the reconstructive surgery, is responsible for most of the long-term problems with sexual function. However some argue that reconstructive surgery may impact negatively on sexual function. The aim of this double blind clinical trial is to investigate the efficacy of treatment with sildenafil citrate in comparison with placebo in improving erectile function of patients with pelvic fracture urethral distraction defects (PFUDD) who underwent urethroplasty.

Material & Methods Between 2008 and 2010, a total of 60 patients with urethral stricture who suffered from PFUDD and were candidate for perineal urethroplasty assessed for eligibility to enter the study. The exclusion criteria were systemic diseases that may cause ED, such as hypertension, diabetes mellitus, heart disease, and chronic liver disease. Patients using computer-generated simple random tables were randomly assigned to one of two groups according to the method of treatment: sildenafil citrate or placebo. The treatment was started 3month before the surgery in all patients. The sildenafil citrate and placebo, 50 mg once daily, were in capsule form and identical in appearance, size, shape and color. They were prepacked in the drug containing bags. A physician from a department not involved in this study received the drug containing bags according to the randomization schedule. The International Index of Erectile Function-5 was used as an evaluation tool. Assessments were made at three time points: three months before urethoplasty, the time of admission, and 3 months post-urethroplasty. Surgical team and physician who recorded data were not informed of the drug group assignment.

Results The incidence of moderate to severe ED following injury was 35% in all of the patients. There was no significant difference between the groups regarding any of the patients' age, previous history of internal urethrotomy, stricture length, any need for crural separation and inferior pubectomy. There was a significant increase in IIEF scores pre and post-treatment in sildenafil group in comparison with placebo ($p < 0.05$). There were neither adverse events reported as drug-related in sildenafil group nor in the placebo control.

Conclusion According to this RCT study, sildenafil citrate orally significantly increases erectile function postoperatively and could be useful in the drug treatment of ED in the PFUDD patients underwent urethroplasty.

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The Impact of Ventral Oral Graft Bulbar Urethroplasty on the Ejaculatory Function, Erectile Function and Sexual Life

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Objective To determine the effect on sexual function of the ventral oral graft urethroplasty for bulbar urethral stricture through the use of questionnaires.

Design, Settings, and Participants Between 2009 and 2010, 52 patients who underwent ventral oral graft bulbar urethroplasty were evaluated through questionnaires to ascertain sexual disorders before and after surgery.

Measurements All patients completed pre and postoperatively the validated Male Sexual Health Questionnaires and, postoperatively, the unvalidated but adapted Post-Urethroplasty Sexual Questionnaire.

Results & Limitations Before urethroplasty, most of the patients (84.6%) with urethral stricture complained about ejaculatory disorders influencing their QoL; many of them (34.6%) were afraid of a postoperative worsening of the quality of sexual life. Following urethroplasty, nobody reported a worsened erection, whilst most of them had an improved ejaculation in terms of force, volume and pleasure during ejaculation; 42.2% had experienced scroto-perineal disorders and 15.4% noticed aesthetic changes, but without impact on sexual life. There has been a significant improvement of sexual activity and desire, relationship with partner, and quality of sexual life. All reported an im-

provement of the QoL and were satisfied with the final result of urethroplasty. The limitation of the present study consists in analysing only ventral oral graft urethroplasty.

Conclusions Urethral stricture disease determines ejaculatory disorders which negatively impact on life. Patients confessed a marked anxiety tackling urethroplasty and claimed especially the fear of postoperative sexual complications. The minimally-invasive ventral oral graft urethroplasty showed to improve overall quality of life and sexual life, in particular the ejaculatory function.

ECA Plenary Lecture 2: Ageing

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The Ageing Testis

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It is now well established that advancing age in men is associated with a decline in androgen production, fertility and quality of spermatozoa. The age of the male partner is strongly correlated with a decrease in sperm motility and normal morphology as well as with decreased pregnancy rates and increased time to pregnancy. There is also mounting evidence that increasing paternal age is linked to chromosome damage and an increase in the incidence of conditions such as autism and schizophrenia among the progeny. However, many of the biological mechanisms that underlie these processes remain poorly understood.

Changes in sperm quality with advancing age are of societal concern with advancing age for paternity. Spermatogenesis is a complex multistep process whereby mature motile spermatozoa develop via a number of cell divisions from spermatogonial stem cells (SSCs). It is therefore important to keep the population of these stem cells healthy and in good supply and to ensure that the environment in which cells differentiate (niche, Sertoli cells) does not deteriorate.

Using the Brown Norway rat as a model of male reproductive aging, we have established that increased paternal age results in an increased rate of pre-implantation loss, decreased fetal weight and increased postnatal death rate. We have also found that spermatozoa from older males have increased chromatin damage and are less able to detoxify reactive oxygen species than spermatozoa from younger males. Analysis of gene and protein expression by pachytene spermatocytes isolated from young and aged rats revealed that mRNAs/proteins involved in base excision repair (BER) are downregulated. Furthermore there is an increase in 8-oxo-2'-deoxyguanosine (8-oxodG) immunoreactivity in germ cells from aged males and in the number of spermatozoa positive for 8-oxodG. Consequences of aging have been tracked back to SSCs. We have found that both the number and quality of SSCs declines with advanced paternal age; the SSC niche also changes with advancing age. The possible association of these changes with an increased mutation rate in germ cells and whether this is the cause for altered fertility and progeny outcome in older fathers remains to be elucidated.

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Genetic Changes in Spermatogonia of Older Men

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Advanced paternal age has been associated with an increased risk for spontaneous congenital disorders and some common complex diseases, but the mechanisms that mediate this effect have been poorly understood. A small group of disorders, including Apert syndrome (caused by FGFR2 mutations), achondroplasia (FGFR3), and Costello syndrome (HRAS), which we collectively term "paternal age effect" (PAE) disorders, provides a good model to study the biological and molecular basis of this phenomenon. Previous analyses involving the direct quantification of PAE mutations in sperm and testes have suggested that the common factor in the paternal age effect lies in the dysregulation of spermatogonial cell behavior, an effect mediated through the growth factor receptor-RAS signal transduction pathway. These studies implied that normal testes are mosaic for clusters of mutant cells: these clusters are predicted to have altered growth and signalling properties leading to their clonal expansion ("selfish spermatogonial selection"), but the DNA extraction methods used in previous studies eliminated the possibility to study such processes at a tissue level.

Using a panel of antibodies optimised for the detection of spermatocytic seminoma, a rare tumor of spermatogonial origin, we have found that putative clonal events are frequent within normal testes of elderly men. In some testes we could identify entire seminiferous tubules with a circumferentially altered immunohistochemical appearance that extended through serial sections that were physically contiguous (up to 1 mm in length), and exhibited enhanced staining for antibodies both to FGFR3 and a marker of downstream signal activation, pAKT. These findings support the concept that populations of spermatogonia in individual seminiferous tubules in the testes of older men are clonal mosaics with regard to their signalling properties and activation, thus fulfilling one of the specific predictions of selfish spermatogonial selection.

We hope that this work will provide a prelude to documenting these phenomena more broadly, for example asking how the frequency of such immunopositive tubules varies with age or position within the testis, and to what extent it reflects stochastic processes. In addition I will discuss results of our ongoing attempts to pinpoint the genetic correlates of these phenomena using microdissection of immunopositive tubules followed by targeted genetic analysis.

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The Natural History of Late onset hypogonadism – Implications for Clinical Management

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Serum testosterone (T) levels gradually decline with age in men. However, the clinical significance of this remains unclear. The concept of "late-onset hypogonadism" (LOH) as a geriatric syndrome defined by clinical and biochemical criteria remains controversial. To improve the specificity of the syndrome, we have recently proposed the minimum criteria for LOH which entailed the presence of three sexual symptoms (decreased sexual interest and morning erections, and erectile dysfunction) in combination with total T below 11 nmol/L and free T below 220 pmol/L.

Using these criteria, we have reported associations between LOH and a variety of end organ deficits suggestive of androgen deficiency. However, the clinical significance and the natural history of LOH remain largely undefined. This lecture will discuss new longitudinal data from the European Male Ageing Study (EMAS) on the natural history of LOH with respect to changes in testosterone and its predictors, increased all-cause and cardiovascular mortality and the changes in phenotypic and biochemical features. We conclude that LOH identifies men with poor cardiometabolic health and a greatly increased risk of dying but the condition is not irreversible.

ESU Course 2: Peyronie's Disease

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Surgical Treatment Methods of Peyronie's Disease

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Introduction The aim of our study was to evaluate the effectiveness and safety of various surgical methods of treatment of Peyronie's disease.

Materials & Methods For 92 patients the different methods of surgical treatment were performed.

For choice of surgical procedure we took into account the length of the penis, angulation, erectile capacity. Finally, 15 (16.3%) Nesbit's procedure; 50 (54.4%) autologous grafting; 8 (8.7%) autologous grafting + deep dorsal vein ligation were performed. Penile prosthesis implantation were perfomed for 17 (18.5%) patients.

Results 88 (95.6%) had a satisfactory outcome. 4 (4.4%) patients in this series had postoperative residual curvature (< 20º), but these patient had not difficulties for penetration. 6 from 8 patients after autologous grafting + deep dorsal vein ligation had increasing erectile function sufficient for coitus, 2 patients from this group had increasing erectile function after using PDE-5 inhibitors for sexual rehabilitation.

In early postoperative period 14 (15.2%) patients had decreasing sensitivity of glance penis, 6 (6.5%) patients had subcutaneous hàematomas of penis, 4 (4.3%) – oedema of penis. All of these complications were temporary and resolved without another surgical procedure.

Conclusion Surgical treatment of Peyronie's disease is effective and safe procedure. The using of the algorithm is effective option for this category of patients.

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Dynamic Contrast-enhanced MRI of the Penis in Tumescence before Surgical Treatment of Peyronie's Disease – Preliminary Results

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Introduction Preoperative diagnosis of Peyronie's Disease is based on the medical history, the palpatory evidence of plaques and normally a PGE1 injection test to evaluate an angulation and the penile length. The size of the plaque, the localization and even the evidence of calcifications is usually evaluated by ultrasonography. Unfortunately, the palpatory and the ultrasound results do not correspond in all cases. Furthermore, there are no possibilities to identify "inflammatory" lesions which are signs for an active disease. MRI techniques, especially after injection of PGE1 into the corpora cavernosa are suggested to provide further insights into the anatomical structure of the corpora cavernosa in this disease including a better localization of plaques and definition of inflammation. Unfortunately, until today due to lacking standardization there is no consensus to perform this technique preoperatively. Our study defines for the MRI evaluation in relation to the clinical findings.

Material & Methods One day before surgery 14 patients were examined at a 1,5T scanner with surface coil about 10–15 minutes after intracavernous injection of PGE1. 2 different MR protocols were elaborated containing high- resolution T2-turbo spin-echo (TSE) and T1-weight TSE sequences or short-tau inversion recovery (STIR), true-FISP and T1/2-TSE sequences. Both protocols contained T1-weight 3D gradient recalled echo sequences before and after intravenous injection of weight 0.5 mmol Gd-DPTA/kg body weight in five dynamic sequences over 420 seconds with subtraction-technique. Finally, fat suppressed T1-TSE sequences were acquired in both protocols.

Results There was a high variation in tumescence response. We found several varieties of Peyronie's disease from focal thickening of the tunica albuginea or simple plaques (most frequently dorsal) to complex hourglass configuration. In one case surgery was cancelled because of plaque localization at the penile septum, in another case a suspected deep dorsal vein thrombosis could be excluded. No patient showed signs of acute inflammation preoperatively. In only one plaque we found less contrast medium enhancement as a sign for increased perfusion.

Conclusion MRI is able to give an insight into the preoperative penile situs. It can be applied to localize plaques and to define the inflammatory activity of the plaque lesions. The ability to delineate plaques directly in relation to the anatomical structures of the penis allows a more detailed planning of the surgical approach.

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Surgical Treatment of Penile Curvature with the Tunica Albuginea Underlap Technique – Description of the Technique and Clinical Results

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Introduction Tunical plication procedures play and important role in the treatment of Peyronie's disease and congenital penile curvature. Several modifications of the classical Nesbit technique are part of routine clinical practice. We present our experience with the Tunica albuginea underlap technique, a new modification of the Nesbit procedure that we submitted for publication just recently.

Material & Methods Between 2008 and 2012, 54 patients were operated because of Peyronie's disease (40) or congenital penile curvature (14) in a single centre using the Tunica albuginea underlap technique. With this technique U-shaped flaps of tunica albuginea are freed from the corpus cavernosum instead of excising ellipsoids. The flaps are brought under the remaining tunica albuginea and are fixated with single absorbable sutures. So the correction of the abnormal curvature is achieved.

Pre- and postoperative evaluation included the Erection Hardness Score (EHS) and the IPP-SSC, a symptom score for Pyronie's disease that was based on a consensus of regional andrologists. Clinical data concerning the early postoperative outcome were analyzed retrospectively using standardized items.

Results Mean age was 59 years for patients with Peyronie's disease and 34 years for patients with congenital penile deviation. The mean follow-up period was 27 month. The major complication rate was 4%, overall satisfaction 86%. Intraoperative correction of the curvature was achieved in 100%. Significant relapse occured in 6%. The mean difference of pre- and postoperative IPP-SSC was 8.1 (95%-CI: 7.24–8.96). The mean difference of pre- and postoperative EHC was –0.03 (95%-CI: –0.16 to –0.09).

Conclusion Preliminary results obtained with the underlap technique show promising outcome with minimal morbidity. The new technique might have three main advantages: more flexible intraoperative correctability of the curvature, tighter sealing of the tunical defects and greater tensile strength of the plications.

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Radiotherapy is Safe, Clinically Effective and Well Accepted by Patients with Morbus Peyronie

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Introduction For M. Peyronie a number of conservative and surgical treatment options exist, depending on disease stage. However, patient benefit is often low, or these treatments have to be discontinued due to side effects. Radiotherapy has been applied for M. Peyronie since decades, but to date, clinical data on safety, effectiveness and patient satisfaction is scarce.

Question Aim of this study was to assess the safety, efficacy and patient satisfaction of soft X-ray therapy in patients with M. Peyronie.

Methods We performed an univariate retrospective analysis of 233 patients who underwent a radiotherapy between 1999 to 2008 with 8 fractions of 4 Gy over a period of 6 months. 83 patients (27%) responded to a comprehensive questionnaire, which covered patient characteristics, disease duration prior to radiotherapy, comorbidities, previous treatments, course of disease, treatment response, side effects and patient satisfaction.

Results Median age at the beginning of radiotherapy was 57.5 years. Patients were initially seen by urologists (61%), general practicioners (24%) or dermatologists (6%). 33.7% also suffered from one or more benign cutaneous fibromatoses, including M. Dupuytren, M. Ledderhose or knuckle pads, while no other disease-specific comorbidities were observed. In avergage patients saw a doctor 4.6 months after observing first signs of disease and 20% had received other treatments prior to initiation of radiotherapy at around month 8 of disease. Most prominent symptoms were penile induration, deviation, erectile pain and dysfunction. Disease progression stopped in 78.3% of patients with amelioration of erectile pain in 75.3%. 42.4% observed reduced penile curvature and 41.7% showed a reduction of plaque induration. Treatment satisfaction was rated with a median of 8 in a visual analogue scale of 10. Side effects included transient erythema in 38.6% of patients . 9.6% reported transient or chronic dryness. 12% of patients reported teleangiectasias in the irradiated field. No severe side effects were observed.

Conclusion Soft X-ray radiotherapy for early stage M. Peyronie is safe, clinically effective and has a high patient satisfaction rate. Patients should be evaluated for associated cutaneous fibromatoses.

ECA Session 1: Developmental Determinants in Male Reproductive Health

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Prenatal Determinants of Adult Male Reproductive Health

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Anogenital distance (AGD) is a marker for endocrine disruption. During sexual development, the immature genital precursors migrate via an androgen mediated pathway. Animal studies have demonstrated that abnormal genital lengths reflect aberrant genital formation with impaired testicular function. Recent evidence supports similar findings in humans. Studies in infants have shown abnormal AGD in boys with genital malformations. Studies in adults have shown that AGD is a measure of testicular function as assessed by sperm and testosterone production.

The clinical utilization of AGD has also been explored to assist with men with infertility as a novel metric to assess intrinsic testicular function. The determinants of AGD continue to be elucidated. Rodent studies have shown that endocrine disruptions at critical time points during gestation can lead to abnormal genital development including shorter AGD in males. Recent evidence supports that human AGD is also defined by both intrinsic and extrinsic factors which are at play during fetal life.

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Modifying Effects of Prenatal and Postnatal Lifestyle on Semen Quality and Fertility

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Western lifestyle has changed dramatically during the past 50 years and changes in lifestyle factors may contribute to the observed adverse trends in male reproductive health Lifestyle factors include BMI, smoking, caffeine and alcohol intake, diet and psychological stress. Obesity is reaching epidemic proportions worldwide, and the prevalence of smokers is high in many Western countries. Several studies of men from the general population and of infertile men have shown that obesity is associated with reduced semen quality. Smoking has also been found to impair semen quality, and a recent Danish study among men from the general population found a dose-response relationship between smoking on the one hand and sperm motility and total sperm count on the other. Maternal smoking during pregnancy has been found to have a negative impact on semen quality among the offspring, indicating that prenatal exposure is also important. Studies on the association between caffeine and alcohol intake and semen quality have been inconclusive, partly because they were conducted on highly selected groups of either infertile men or fertile men undergoing vasectomy.

Psychological stress has been implicated as a cause of idiopathic infertility. Most studies have been conducted among infertile populations making it difficult to differentiate between stress as a cause or consequence of infertility. In addition, the availability of a wide range of different tools to assess stress and other psychological conditions makes comparison across studies difficult. A recent Cochrane review suggested that treatment of the male partner with antioxidant supplementation may improve live birth and pregnancy rates for infertile couples undergoing infertility treatment, although no convincing effect on semen quality was found. A newly published study among 99 US men attending an infertility clinic found high intake of saturated fats was negatively related to sperm concentration.

We have recently conducted large studies among European, young men from the general population and fertile European and American men in which we have studied the effect of lifestyle factors on semen quality and serum reproductive hormones and data will be presented.

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In vivo Effects of *Eurycoma longifolia* (Tongkat Ali) Extract on Reproductive Functions in the Rat

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In South East Asia, the root extract of *Eurycoma longifolia* (Tongkat Ali; TA) is widely used in traditional medicine for its cytotoxic, antimalarial, anti-ulcer, antipyretic and aphrodisiac properties. It is even used to treat male infertility. Since no study was conducted to prove these pro-fertility effects in an animal model, this study aimed at investigating thein vivoeffects of TA on male reproductive functions in the rat.

A total of 42 male rats were divided into a control, low dose (200 mg/kg of BW) and high dose (800 mg/kg of BW) group, consisting of 14 animals per group. The animals were force fed the extract for 2 weeks and then sacrificed. The total body and organ weights of the liver, prostate, epididymides, testes, gastrocnemius muscle, and adipose tissue from the greater omentum were isolated and recorded. In addition, the following parameters were assessed: serum testosterone concentration, sperm concentration, motility, velocity, vitality, mitochondrial membrane potential (MMP).

While TA treatment significantly decreased total BW ($p = 0.0276$), omentum fat mass ($p = 0.0496$), testicular weight increased significantly ($p = 0.0042$). Epididymal weight ($p = 0.0513$) and testosterone concentration ($p = 0.0544$) increased markedly. Lean muscle weight of the gastrocnemius muscle also increased, yet not significantly.

Moreover, sperm parameters increased significantly; sperm concentration ($p < 0.0001$), motility ($p < 0.0444$), vitality ($p = 0.0156$) and sperm velocity ($p < 0.0298$). Improvement in MMP showed a clear trend ($p = 0.0765$).

In conclusion, TA enhances testosterone levels and thereby sperm parameters. It also changes metabolism from catabolic to anabolic state. Thus, TA appears to be suitable to treat male infertility and aging male problems.

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Why Androgen based Male Hormonal Contraception Lacks Efficacy: Evidence from a Mouse Model

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Introduction The basis of male hormonal contraception is the suppression of gonadotropins, leading to the disruption of the hypothalamic-pituitary-testicular axis. This can be achieved by high levels of androgens, alone or in combination with a gestagen or a GnRH analogue. The decreased testicular androgen production deprives developing sperm of the signal required for normal maturation, thereby leading to azoospermia or severe oligozoospermia and reversible infertility in men. Because the treatment involves the administration of testosterone (T), the question of an appropriate dose is essential. Our aim was to determine with our mouse model the threshold dose that would maintain peripheral androgen actions and anabolic effects without promoting spermatogenesis, which is the prerequisite of an effective hormonal male contraceptive.

Materials & Methods The experiments were carried out on LH receptor knockout (LuRKO) mice. The phenotype of the males includes low T levels, spermatogenic arrest at the round spermatid stage, and infertility due to underdeveloped sex organs. We explored the effect of T and its nonaromatizable metabolite dihydrotestosterone (DHT), administered for 90 days with subcutaneous silastic tube implants, on mating behaviour and spermatogenesis. Using various doses of T, we also determined the minimal dose that induced full spermatogenesis and restored fertility, as well as the maximal dose that induced mating behaviour without effect on spermatogenesis.

Results Both T and DHT implants led to the growth in external genitalia, testicular descent and restoration of full spermatogenesis. A dose of 5 mg T implant effectively restored full spermatogenesis and partial fertility, with good hormonal profiles. A 2.5 mg T implant retained azoospermia, or in some cases led to oligospermia, with enhanced sexual behaviour and development of reproductive and somatic tissues. Doses of 1.5 mg and less neither promoted spermatogenesis nor restored mating. Hence, only a narrow margin separated the T doses that activated peripheral androgen effects and spermatogenesis. Interestingly, DHT restored spermatogenesis, but the observed mating behaviour was lower than with T.

Conclusion The narrow margin in T doses required for peripheral androgen action and spermatogenesis may pose a problem for the development of effective male hormonal contraceptives. This may be an important reason why it is difficult to achieve uniform spermatogenic suppression in men upon hormonal contraceptive trials.

ECA Session 2: Epigenetic Mechanisms and Small RNAs

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Roles of Small Non-Coding RNAs in Male Reproduction

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Protein-coding genes only account for ~1% of the eukaryotic genome and > 95% of eukaryotic genome are transcribed into large or small non-coding RNAs. Increasing lines of evidence suggest that these non-coding RNAs play a critical role in fine-tuning the complex gene expression networks and thus can affect cellular functions. In general, non-coding RNAs function as sequence guides and their effects rely on the effector complexes that they recruit. Therefore, the genomic origin of these non-coding RNAs, the RNA or DNA sequences that they potentially target, and the effector complexes that they bind usually can give us a hint on their functional roles. Although several non-coding RNA species have been identified over the past decade, more and more novel non-coding RNA species continue to be identified and their functions turn out to be surprisingly diverse and complex.

In my talk, I will show you 2 novel classes of small non-coding RNAs and their physiological roles in male germline development.

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Post-Meiotic Male Genome Programming

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Portrait of a master regulator of spermatogenesis: is Brdt really a suitable target for male contraception?

Male germ cell differentiation is a highly regulated multistep process initiated by the commitment of progenitor cells into meiosis and characterized by major chromatin reorganizations in haploid spermatids. In a recently published work, Matzuk and collaborators have used a new molecule, JQ1, initially developed as an anticancer drug, to inhibit spermatogenesis, and propose that JQ1 could represent a prototype for the development of male contraceptive drugs [Cell 2012; 150: 673–84]. Their hypothesis is that JQ1, which is known to inhibit the acetyl binding domains (named "bromodomains") of proteins of the BET family, acts on Brdt, the testis specific member of this family. Our past and recent work [1–4] demonstrates that Brdt is an essential regulator of spermatogenesis, acting at several key steps of male germ cell differentiation, and indeed supports the hypothesis that a specific targeting of its functions should be an efficient way of blocking spermatogenesis. However our data also raise concerns regarding the possibility of specifically targeting Brdt's functions in a reversible manner and whether its inhibition should actually be considered as an appropriate strategy for male contraception.

Our recent and extensive exploration of Brdt's functions, using 3 complementary mouse models and genome-wide transcriptomic and epigenomic analyses of maturing male germ cells, demonstrates that Brdt, which is activated at onset of meiosis, is a master regulator of both meiotic divisions and post-meiotic genome repackaging. Indeed, in meiotic cells, Brdt initiates a histone acetylation-guided programming of the genome by activating essential meiotic genes and repressing a "progenitor cells" gene expression program, while at post-meiotic stages Brdt, via its first bromodomain, directs the genome-wide replacement of histones by transition proteins.

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UBE2B messenger RNA Mutations are Associated with Severe Oligozoospermia in Infertile Men

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Oligozoospermia is one of the most common semen deficiencies diagnosed in male infertility clinics. However, to date, very few genetic defects have been identified to cause

these conditions. Moreover, no molecular genetic diagnostic tests are available for patients with oligozoospermia in the andrology clinic. Based on numerous animal and expression studies of oligozoospermia, we know that several molecular pathways are disturbed in postmeiotic spermatozoa. One of the disrupted pathways of spermatogenesis is protein ubiquitination and cell apoptosis. A critical protein involved in this pathway is the ubiquitin conjugating enzyme 2B, UBE2B. It was shown that the absence of Ube2b in male mice is responsible for postmeiotic spermatogenesis arrest and increased apoptosis, leading to infertility. Previous human studies of UBE2B presented significant association between SNPs in the gene and oligozoospermia. To examine an association between UBE2B mutations and severe oligozoospermia ($0.1–10\times10^6$ cell/ml), we performed mutation screening of spermatozoal cDNA in 326 oligozoospermic patients and 421 normozoospermic controls. We identified UBE2B messenger RNA mutations in 20/326 (6.1%) oligozoospermic patients and no mutations in respective controls ($\chi^2 = 9.8$, $p = 0.001$). However, we detected some of the splicing defects at low expression levels in 11/421 (2.6%) controls. To test the statistical effect of their presence on association, we performed a chi-square test; statistics show UBE2B association, albeit with modest significance ($\chi^2 = 6.8$, $p = 0.02$). A follow-up study of the dbSNP database did not reveal the UBE2B alterations reported here. Our results corroborate previous findings from animal and human studies and suggest that UBE2B associates with the severe oligozoospermia phenotype, and perhaps with other unidentified semen defects (i.e., oligoteratozoospermia, morphology and/or low count defects). In addition, we demonstrate high utility of mRNAs as potentially powerful biomarkers of genetic defects in haploid male germ cells. We believe that advances in non-invasive genetic tests will be a vital future diagnostic tool for oligozoospermia and reproductive medicine in general.

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Differential Proteomic Distribution in the Chromatin of Human Sperm Cells

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Introduction The spermatogenic cell experiences an extremely marked chromatin transition during the last stage of spermatogenesis. Histones are disassembled and replaced by protamines, which are highly positive charged proteins that form tight toroidal complexes. However, this structural change of the sperm chromatin is not complete and, at the end, 85–95% of human sperm DNA is packaged by protamines (NP), while 5–15% remains associated with histones (NH). It is known that the differential chromatin distribution in the protamine- and histone-associated regions is not random. The NH domain is significantly enriched in important developmental genes, indicating a potential epigenetic role during the formation of the embryo. In a previous study from our laboratory, we have performed a proteomic analysis of the human sperm nucleus. Among the proteins characterized, zinc fingers proteins, transcription factors and several novel histones variants have been described. These results were unexpected in a cell supposed to be transcriptionally inactive. The aim of the present study was to contribute to a more detailed characterization of the human sperm nucleus, by establishing the distribution of the proteins in the NH and NP domains.

Material & Methods Sperm nuclei were isolated from a normozoospermic semen sample, which was previously purified from contaminating cells using 50% Percoll gradients and leukocyte depletion with magnetic beads. The chromatin was fractionated in NH and NP domains with a saline treatment, using a standard approach, and the proteins extracted from each fraction were then analyzed by liquid chromatography followed by tandem mass spectrometry (LC-MS/MS).

Results The proteomic results show the identification of 502 proteins, several of which characterized for the first time in the sperm cell. A preliminary analysis of our outcomes confirms, as expected, an enrichment in low abundant nuclear proteins. Moreover, and more interestingly, our data suggest that there is a differential protein pattern in the two sperm chromatin domains: 78.5% of the proteins were found exclusively in the NH fraction, 5.4% in the NP fraction and 16.1% were present in both fractions.

Conclusions These results open up the possibility of further characterizing these specific proteins and their associated genes, which likely have a potential epigenetic function.

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ESU Course 3: Varicocele

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Surgical Subinguinal Approach to Varicocele Combined with Antegrade Intraoperative Sclerosis of Venous Vessels

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Introduction Varicocele is treated by different surgical techniques, none of which is yet acknowledged as the "gold standard". Some of these, especially microsurgical techniques, are very time-consuming and thus expensive, and the treatment of varicocele still causes some complications and recurrences **(Fig. 1, 2)**.

Methods From September 2007 to December 2010 307 patients underwent sclaerembolization of the spermaic vessels. We modified and simplified the microsurgical technique of Marmar and Kim, using a subinguinal approach with intraoperative antegrade sclerotherapy of dilated veins. After the cord has been clamped, 1.5–3 ml. of 3% atoxysclerol mixed with 0.5 ml of air is injected.

Results Commonly minor complications can occur. The most common is transient penile lymphangitis, which cause is unclear. As the procedure allows selective sparing of the lymphatic vessels, it avoids hydrocele due to the performed procedure. Among the reported complications the most frequent was a penile lymphangitis, which occurred in 9 patients and regressed spontaneously after a few weeks; 3 patients complained of temporary orchialgia. In no cases did we observe formation of persistent hydrocele, which is a constant complication in other techniques (though with varying rates depending on the technique and statistics considered), nor were orchitis, orchiepididymitis, orchio-funiculitis or testicular atrophy observed in any case. Speaking about learning curve, any new operator took less than 20 cases to reach a considerable skill and a satisfactory surgical speed. We observed just one recurrence.

Conclusion This modified technique appears to be easy, safe and cheap. Considered the promising results in terms of complica-

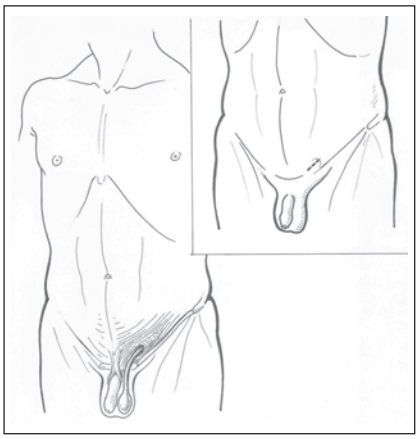

Figure 1. G. Bozzini et al. (Reprint with permission)

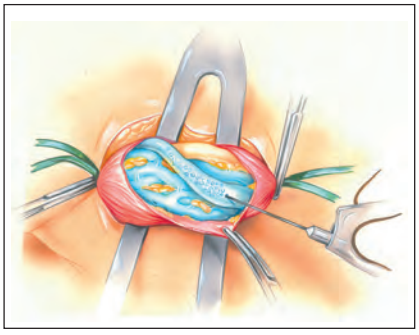

Figure 2. G. Bozzini et al. (Reprint with permission)

tions and persistence, the treatment appears to be a suitable fist-line approach for surgical treatment of varicocele.

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Low Ligation with Oral Treatment Improves Spermiogram in Infertile Men

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Objectives To evaluate the efficacy and safety of low ligation of spermotic vein with oral treatment (pampiniform plexuses) for the treatment of varicocele in infertile cases.

The efficacy parameters include: clinical, biochemical response (FSH, spermogram, pregnancy rate of partners).

Materials & Methods From 2006 until 2012 about 1000 patients in our clinic was seen, patient age 20 to 40 years with grade 2 to grade 3 varicocele were complain of scrotal pain and abnormal semen analysis, were included in the study.

Color Doppler ultrasound was used to measure testicular volume and vein diameter before the surgery. 3 months of post operation and follow-up visit, semen analysis result was obtained at the same time. The intervals are 30% where bilateral varicocele, 55% where left varicocele and ultrasound with no varicocele is in 15%.

Results All patients were treated surgically and medically (oral supplement). The mean of operation time was 20 minutes for unilateral and 40 minutes for the bilateral. In 3 months of post-operation showed hydrocele in 5% of all cases, pregnancy occurred 40% in other cases and at the earlier 3 months of post-operation 10% patients never come back for their follow-up.

Conclusion Low ligation and oral treatment had improve spermogram and it can save cost, theater time, hospitalizations, and patients time for work.

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Antegrade Scrotal Sclerotherapy for the Treatment of Varicocele in Adults, Childhood and Adolescence

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Since 1988 we performed antegrade sclerotherapy for varicocele in about 8000 adults, adolecences and children.

The recurrence and persistence-rate in adults was 8%, in children lower than 3%.

To avoid complications during the dissection of the pampiniform plexus detailed knowledge of the architecture of the spermatic cord is recommended. Phlebography of the internal testicular vein is mandatory. Sclerosing of the artery testicularis is strictly forbidden and is the reason for testicular destruction. Paravasation of sclerosing agent has to be recognized and treated immediately to prevent deleterious effects.

We never saw a hydrocele, therefore it is neither necessary to deliver the testicle, nor to identify the lymphatics passways by isosulfan blue.

Retrograde sclerotherapy is not possible if the spermatic veins cannot be probed selectively in 11-13% and have a recurrence-rate of 8%. Instead the antegrade sclerotherapy can be done in 99%.

The radiation exposure is 2–4 seconds, for the retrograde sclerotherapy 1–4 minutes (Porst, Wunsch).

It can be done in local anaesthesia on outdoor patients on a x-ray table.

We present follow-up results of 129 children and adolescences, who were operated by one surgeon. Follow-up was between 8–20 years. Recurrence rate was less than 3%.

In the hands of experienced surgeons the treatment is easy with a low complication rate; correction of varicocele might be carried out prophylactically in early puberty to prevent testicular hypoplasia and dysfunction.

The operating time and the economic effectiveness will be compared to the other treatments of varicocele.

The treatment is recognized as a low cost, safe, fast and effective management of varicocele. It seems to be the treatment of choice in treating the varicocele.

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Color Doppler Evaluation of Spermatic Vein Reflux predicts Sperm Quality Improvement following Varicocelectomy

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Introduction To date, impact of varicocelectomy on semen parameters is controversial probably because clear preoperative selection criteria of patients to be submitted to varicocele surgery are still lacking. In this study we considered the characteristics of venous reflux, detected with Color Doppler Ultrasound (CDU), as a criterion for selecting infertile men candidate to varicocelectomy.

Materials & Methods Since 1983 our group designed a prospective protocol on infertile patients affected with varicocele based on two preoperative semen analyses (basal and repeated three months later), a preoperative CDU in standing position to assess venous reflux along the spermatic and the pampiniform plexus veins, surgical varicocelectomy, a postoperative CDU (one month after varicocelectomy) for reflux recurrence exclusion, and 2 postoperative semen analyses (respectively 14 and 22 weeks after varicocelectomy). Semen samples were analyzed according to the WHO criteria in force at that specific time and sperm concentration, forward motility and morphology were recorded. According to the CDU, venous reflux, was classified as basal continuous when, in standing position, a spontaneous reflux independent from respiration and increasing during Valsalva manoeuvre was registered, and basal intermittent, when, under the same conditions, a discontinuous reflux synchronous with breath movements was documented. At the end, out of 1.775 infertile patients, 360 met all inclusion criteria and were considered for the study: 319 patients showed continuous reflux (group A), whereas 41 had intermittent reflux (group B).

Results Preoperatively, compared to the group A, group B showed both higher sperm concentration ($p = 0.03$) and morphology ($p < 0.0001$) without differences regarding sperm forward motility. Moreover, compared to the baseline, after varicocelectomy, neither sperm concentration, nor sperm motility, nor sperm morphology improved in the group B, whereas all sperm characteristics improved 14 and 22 weeks after varicocelectomy in the group A ($p < 0.0001$).

Conclusions Preoperative semen parameters were found worst in infertile varicocele patients with a basal continuous reflux, and varicocelectomy improved significantly sperm quality only in this group of men; based on our results, infertile patients with a discontinuous reflux on CDU should not be submitted to varicocelectomy.

ECA Session 3: Sexual Dysfunction and Erectile Failure

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Sexual Dysfunction and Erectile Failure: New Aspects from the Urological Point of View

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The PDE_5 inhibitors have completely changed the diagnostic and therapeutic approach of ED. For the non-responders, some maneuvers can improved the results: better education, testosterone substitution in hypogonadism patients and daily use. Chronic use of PDE_5-I also improves endothelial function of the entire vascular system by modifying the inflammatory process.

The treatment of prostate carcinoma could be at the origin of ED. First of all, erectile function evaluation is different before or after prostate biopsy or cancer announcement.

Delayed surgery in patients under active surveillance alters erectile function. Radical prostatectomy (RP) for localized carcinoma

in any form provokes ED and the results between the various techniques seem to demonstrate no difference in functional outcomes. More than the technique, the surgeon experience is important.

Penile rehabilitation has to be performed immediately after catheter removal. RP destroys NO production and therefore muscular relaxation but it increases Rho kinase system and muscular contraction.

For non-localized prostate cancer, testosterone deprivation could be administrated. However, in patients with cardiovascular problems, it could be deleterious and quickly after the treatment onset.

Peyronie's disease is due to poor cicatrisation of the tunica albuginea injuries. PDE inhibitors by increased NO inducible could be used as treatment mainly in the inflammatory process.

These different subjects will be discussed according with the new research studies in those fields.

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Sexual Dysfunction and Erectile Failure: New Aspects from the Andrological Point of View

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In past decade clinical andrology has witnessed a profound change in the attitude toward diagnosis and management of sexual dysfunctions. The unrevealing of the pathophysiology of all phases of the sexual act, on one side, and the acknowledgment of the impact of systemic diseases on erectile function, on the other, have forced a transformation of the andrologist into a physician dedicated to men's health.

A bidirectional process of integration of andrology into general and internal medicine has occurred. The role for androgen replacement therapy has widely expanded from 'just for libido' to a cardiovascular and metabolic modulator. Conversely, PDE5 inhibitors are now a standard treatment for pulmonary arterial hypertension. Our recent studies suggest that PDE5 inhibitors may also exert and anti-remodeling effect in diabetic cardiomyopathy and could have a role in the prevention of heart failure.

The advancement in the immunopathology of the prostate and accessory glands disclosed novel treatment strategies. Finally, the increased use of assisted reproductive technologies have generated novel psychological issues in the field of sexual dysfunction.

A novel PDE5i, avanafil, has been approved and the patent of sildenafil is about to expiry. The debate on chronic vs. on demand use of pro-erectile drugs continues, in search of a curative -rather than symptomatic- treatment for erectile dysfunction. The safety and efficacy of PDE5i in special populations, such as vardenafil in subjects with high cardiovascular risk, has been recently reviewed.

All these small revolutions contributed to the transformation and expansion of the figure of the clinical andrologists. A special focus will be given to the off-label use and novel indications for the andrological treatments.

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Efficacy of PSD502 (TEMPE) is similar in Lifelong and acquired Premature Ejaculation on Initial Dosing and in the Long Term

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Introduction & Objective TEMPE (PSD502) is a metered-doses, aerosolised eutectic-like mixture of lidocaine and prilocaine that has been shown to be effective in two phase III studies in premature ejaculation (PE) [1, 2]. As the entry criteria were based on the ISSM definition, only patients with lifelong PE were included in the analysis. One of the primary endpoints was intra-vaginal ejaculatory latency time (IELT) although there was good concordance with data captured from the index of premature ejaculation (IPE) questionnaire. It is expected that the majority of patients presenting to the physicians' offices will have lifelong (LL) PE, but a substantial number are likely to have acquired (Acq) PE; it is obviously important that the impact of any novel potential therapeutant is evaluated in this patient sub-set.

Methods In one of the protocols, data from a number of patients with acquired PE was captured and is presented in this abstract.

Results At baseline the IELT (seconds) was similar for all groups (Placebo LL 29.4; Acq 34.3; PSD502 LL 31.4; Acq 40.2). Likewise, at 3 months the IELT observed in both subsets was similar for both placebo (LL 43.6; Acq 38.9) and active (LL 111.7; Acq 104.6). Quantitatively similar changes in the domains of the IPE were also noted for PSD502 with placebo only producing marginal changes.

Conclusions Although the number of patients in the acquired group was relatively small, it can be concluded that in both lifelong and acquired PE, placebo produced only marginal changes in IELT whereas in both forms of PE, PSD502 produced clinically significant changes in IELT. In general the changes in IELT were mirrored in the changes in the satisfaction, control and distress domains of the IPE. Overall, it is likely that the response to PSD502 will be similar in men with lifelong or acquired PE. This would be consistent with the proposed mechanism of action of reducing penile hypersensitivity while leaving the „normal" ejaculatory reflex intact. [3]

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Sexual Dysfunctions Induced by Stress of Timed Intercourse and Medical Treatment

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Introduction Male sexual dysfunction is highly prevalent and is one of the most common health complaints reported by men. Erectile dysfunction (ED) is a major problem, and ejaculatory dysfunction (EjD), including premature ejaculation (PE), delayed ejaculation (DE), anejaculation, and retrograde ejaculation, is common. Treating ED with tadalafil, a phosphodiesterase type-5 inhibitor (PDE-5i), has been shown to be effective. The hypothalamic-pituitary-adrenal axis instigates a cascade effect that eventually results in cortisol production that may play causative roles in the ED induced by stress. Timed intercourse (TI) during the fertile window of a woman's menstrual cycle has been widely adopted and is frequently prescribed by fertility specialists to assist couples attempting to conceive. Men represent one half of each couple endeavoring to conceive naturally. The impact of impending TI on the psychological well-being and sexual dysfunction of male partners has not been thoroughly investigated, however.

Methods This study consisted of 439 men and was conducted during a 3-year period between July 1, 2008 and June 30, 2011. Various characteristics were evaluated, including newly acquired ED, EjD, anxiety levels (using the Beck Anxiety Inventory), self-reported aggression (using the Buss Perry Aggression Questionnaire), hormone levels (such as follicle-stimulating hormone [FSH], luteinizing hormone [LH], testosterone [T], prolactin [PRL] and estradiol [E2]), and semen parameters.

Results 188 of the men (42.8%) and 26 of the men (5.92%) experienced ED and EjD, respectively. Additionally, anxiety, anger, hostility, and aggression intensified as the number of TI episodes increased. The numbers of men with ED or EjD also increased with TI ($p < 0.0001$). LH, T and E2 were significantly lower in the men with ED ($p < 0.05$). The men who required high doses of tadalafil (10 mg) had significantly higher scores on both the BAI and the BPAQ subscales ($p < 0.0001$). TI imposes a great deal of stress on male partners, potentially causing ED and EjD. TI also elevates anxiety levels, which leads to aggression.

Conclusions Physicians and clinicians should acknowledge the potentially harmful effects of TI on men. Furthermore, both fe-

male and male patients should be cautioned about the increased likelihood of ED, EjD, and elevated levels of anxiety, anger, hostility, and aggression as the number of incidents of TI increases.

ECA Session 4: Pick One and Inject – Will any Old Sperm do for ART?

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Current Status and Unresolved Issues in ICSI

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Thanks to ICSI it became possible to obtain offspring in couples with severe male factor infertility and even azoospermia and this insemination technique is now increasingly used even in patients with borderline semen parameters. But although introduced 20 years ago, still some patient categories will show poor outcome after ICSI, i. e. patients in which only senescent sperm available, patients with acrosomeless spermatozoa and patients presenting with immotile spermatozoa. Limited progress has been made to alleviate the poor ICSI outcome. Treatment of non-obstructive azoospermia by ICSI too is not invariably successful because chances to recover testicular spermatozoa are limited as are the chances for a pregnancy after ICSI itself. Finally, follow-up data in many subgroups of after ICSI are insufficient to reassure candidate-parents that this approach is absolutely safe.

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Sperm Biology Function Tests in Relation to Assisted Reproductive Techniques (ART)

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ARTs represent the only option for many cases of male factor infertility. It is well known that the rate of malformation recorded at ART cycles is higher respect to spontaneous pregnancies [Hansen et al, 2004]. A large body of evidence in the literature however demonstrates that the risk is not due to the procedures but rather to factors related to gametes. It has been recently reported that the risk of major malformations is higher in case of ICSI children from subfertile couples even after adjustment for confounders such as maternal age and other risk factors [Davies et al, 2012]. As ICSI is mostly applied in case of severe male factor infertility, these results pointed out the need for tests assessing sperm quality to add to poorly informative routine semen analysis. In particular, evaluation of sperm DNA/chromatin quality (DNA strand breaks, base oxidation, chromosomal aberrations, methylation, extent of protamination, status of sulfhydril groups) may be of help in these cases as all these damages can be transmitted to the progeny. In addition, there is now evidence of presence of RNA and miRNA in spermatozoa, whose significance is presently unknown. At the same time, sperm functional tests may be of help for the clinician to choose the more appropriate technique (IVF or ICSI) and thus to increase the chance of ART success. Virtually, each step of the fertilisation process can be monitored both by evaluating the expression of the proteins specifically involved in each step, as well as by functional tests evaluating sperm ability to encompass it. In particular, sperm ability to undergo capacitation, to develop hyperactivated motility, to respond to stimuli inducing acrosome reaction and to fuse with oolemma, can be all assessed by functional tests with known predicting values of IVF outcome.

Promises and pitfalls of such evaluations will be discussed.

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Use of In vivo Monitoring Techniques to Select the best Sperm for ICSI

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Despite recent technological and analytical advances in the field of reproductive medicine, fertility clinics worldwide are still confronted with the large disparity between the high fertilisation rates now achievable by modern ART techniques and the concomitantly low pregnancy and/or take home baby rates. The introduction of the many and varied, modern techniques has significantly increased the amount of information available to scientist and clinician alike, however the ability to non invasively and non destructively assess, then select a homogeneous sperm population, all of which are capable of successfully achieving a pregnancy and a healthy baby remains elusive. Numerous methods aimed at identifying and selecting the best sperm for ICSI have been suggested over the years, amongst others: intracytoplasmic morphologically selected sperm injection (IMSI), the use of hyaluronic acid, zona-binding, zeta potential, birefringence, hyper osmotic swelling (HOST), surface markers with magnetic activated cell sorting (MACS). As well as these more established procedures there have been promising technological developments such as confocal Raman microspectroscopy and microfluidic "labs on chips". The purpose of this presentation is to describe the various sperm selection methods, discuss their advantages and disadvantages review the available studies on their efficacy and appraise their potential clinical use.

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The Impact of RNA Expression in Human Spermatozoa on Male Fertility

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Introduction Our aim is to identify markers on a molecular level for improved prediction of IVF treatment success.

Human spermatozoa express a spectrum of different types of RNA, among them messenger RNA (mRNA), ribosomal RNA (rRNA), micro RNA (miRNA), PIWI-interacting RNA (piRNA). We suppose that they influence male fertility not only individual but also in concert. Our aim is to investigate the expression of these RNA species in the same ejaculates to determine the impact of their interaction on male fertility.

Material & Methods Ejaculates were pelleted after liquefaction by centrifugation. In case of studying pure spermatozoa ejaculates were fractionated by Percoll gradient centrifugation before. Total RNA was isolated by RNeasy Plus Micro Kit. Small RNAs were enriched by raising ethanol from 35% to 60% in the flowthrough of the RNA binding column. Microarrays were hybridized with amplified RNA. Ratios of intact ribosomal RNAs (28S/18S rRNA) were determined by calculating the corresponding peak areas of electropherograms (Bioanalyzer). Ribosomes were investigated in sections of spermatozoa with transmission electron microscopy (TEM).

Results

1. mRNA: Gene expression profiles of ejaculates from 25 donors with normozoospermia [WHO guidelines, 2010] showed a high degree of individual heterogeneity with low correlation to fertilisation rates and pregnancy rates of IVF treatment. However, filtering with ANOVA revealed a group of genes which separate ejaculates into three groups according to the outcome of IVF treatment (pregnant, not pregnant, no fertilisation of the oozyte).

2. mi RNA: Human ejaculates contained a high amount of small RNA species (< 200 nt). Besides a fraction of mi/piRNA, they mainly contained small rRNA (5S, 5.8S) and transfer RNA (tRNA). In purified spermatozoa, rRNA and tRNA were nearly absent while the portion of the pi/miRNA fraction largely increased. We developed a method to isolate total RNA and small RNA from the same preparation, which enabled us to study gene expression and miRNA profiles by microarray hybridization in parallel.

3. Ribosomes, rRNA: Electropherograms of total RNA preparations of pure spermatozoa revealed a remarkably reduced ratio of intact 28S and 18S rRNA molecules of 1:20 compared to the expected ratio of 1:1 in ribosomes. However, ribosomes were identified in the cytoplasm of many spermatozoa by TEM.

Conclusion Individual heterogeneity of gene expression profiles of human ejaculates superimposes the effects on fertilisation outcome. Currently, we are investigating gene expression in combination with expression profiles of miRNA, rRNA and the ultrastructure of ribosomes to identify correlations to the fertilisation potential of ejaculates.

ECA Plenary Lecture 3: Non-obstructive Azoospermia

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Non-obstructive Azoospermia

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Non-obstructive azoospermia may affect up to 1% of the male population and 10% of men who seek fertility treatment.

There are a number of causes for non-obstructive azoospermia including hypogonadotrophic hypogonadism, Sertoli-cell only, maturation arrest and hypo-spermatogenesis.

This lecture will outline the diagnosis, investigation and management of men with non-obstructive azoospermia.

Non-obstructive azoospermia is caused by impairment of spermatogenesis and can be due to a number of causes. Importantly genetic causes including Klinefelter's syndrome and Y-deletions in particular AZFa, b and c microdeletions need to be excluded in men presenting with non-obstructive azoospermia. As part of normal evaluation the patient should undergo ultrasound scanning of the testes combined with hormonal assay. Pending investigations to exclude AZFa and b deletions, men with AZFc microdeletions and Klinefelter's have the ability to father children by testicular sperm extraction. A number of methods have been described to obtain sperm by testicular sperm extraction in men with NOA, although in the majority of men, a microdissection sperm retrieval is performed.

Sperm retrieval rates of approximately 50% are achieved in men with non-obstructive azoospermia, although this would depend upon the primary aetiology of their NOA. There is some argument as to whether hormone manipulation prior to TESE may be of use in men with NOA, and in particular patients with Klinefelter's.

The original description of microdissection sperm retrieval was described by Schlegel from New York, but has more recently been developed by other investigators. Sperm can be retrieved in even those men with Sertoli-cell only.

The long-term effects of testicular biopsy on testicular function are largely unknown although there is an intial reduction in serum Testosterone which is reversible in most patients. However, there is a significant risk of hypogonadism which patients would need to be aware of when counselled for microdissection.

This talk will focus on the investigation, and in particular the technique and outcome of microdissection sperm retrieval in men with non-obstructive azoospermia.

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Imaging of the Male Genital Tract: From Ultrasound to MRI and Beyond

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Objectives Radiographic imaging is integral to the evaluation of male infertility. Techniques of scrotal and transrectal ultrasonography and vasography constitute the backbone of imaging technology in male reproductive medicine. However, a need exists for more quantitative and physiologic assessments of reproductive tract function to improve the accuracy and precision of infertility diagnoses.

Methods This lecture will review the imaging procedures used to assess the male reproductive tract. The discussion is based on review of Medline and Pubmed online databases in the English literature. Among identified citations, papers were selected on the basis of quality and clinical relevance.

Results A case-based presentation of relevant imaging techniques in reproductive medicine. A 28-year old man presents with primary infertility and a left scrotal mass. His semen analysis reveals low volume azoospermia and serum FSH and testosterone levels are normal. What imaging studies are indicated in this patient? What are the most common abnormalities found on scrotal ultrasound for male infertility? What is value of treating subclinical varicoceles? How sensitive and specific is transrectal ultrasound for ejaculatory duct obstruction? What is the meaning of testicular microlithiasis in the setting of male infertility? Does an assessment of testis tissue perfusion improve sperm retrieval rates with non-obstructive azoospermia? What emerging technologies in metabolomic or physiological imaging are on the horizon and how might they be better than current diagnostic tests?

Conclusions Radiographic imaging offers abundant information about the anatomic aspects of male factor infertility. However much of this information is irrelevant or incidental to the problem, currently limiting its routine use in the infertility evaluation. Physiologic or metabolomic technologies may offer more dynamic and potentially more precise and relevant information for this diagnosis.

ECA Session 5: Klinefelter's Syndrome

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The Muenster EXAKT Project: The epigenetic Phenotype of XXY

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Klinefelter's syndrome (47,XXY; KS) is a very common chromosomal disorder, affecting 1:500 men and leading to hypergonadotropic hypogonadism as well as an increased incidence of metabolic syndrome. However, our knowledge on the functional role of the supernumerary X chromosome itself and to which extent its origin contributes to the observed pathophysiology is still very limited. Recently we started the EXAKT (Epigenetics, X-Chromosomal features and clinical Applications in Klinefelter's syndrome Trial) project which is a Muenster-based prospective study involving Klinefelter patients (n = 130), and their parents assessing a wide area of biochemical, physiological and genetic parameters in comparison to age-matched healthy male and female controls (2 × n = 50).

The aim of the genetic and epigenetic part of the EXAKT project is to obtain information on the paternal or maternal origin and the meiotic disjunction events leading to the presence of a supernumerary X chromosome, the inactivation of the second X-chromosome by the non-coding RNA XIST and the expression of X-linked genes which escape X inactivation.

Determination of the origin of the X chromosome by microsatellite analysis in KS and their parents revealed a nearly equal distribution between the paternal (56%) and maternal (44%) origin of the second X chromosome. Methylation analysis of the XIST promoter displayed similar methylation patterns in KS patients and women, indicating grossly normal X inactivation in KS. Analysis of several escapee genes such as KDM6a and SMCA1 in blood RNA samples of KS revealed expression levels comparable to levels detected in women, but significantly higher when compared to normal men.

The first genetic and epigenetic analyses of KS within the EXAKT project revealed a normal X chromosomal inactivation status, but elevated expression patterns of escapee genes indicate a pathophysiological role of the supernumerary X chromosome.

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Klinefelter's Syndrome: an Update

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Klinefelter's syndrome (47,XXY) is the most frequent chromosome disorder.

Patients with Klinefelter's syndrome share numerous clinical characteristics, although marked variability exists betgween patients.

Consequently, only 25% are ever diagnosed. Phenotypic characteristics include increased learning disabilities, height, truncal fatness, osteopenia, small testes, hypoandrogenaemia and azoospermia. Updates results regarding phenotype and current treatment options will be discussed.

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Number and Protein Expression of Sertoli Cells is Altered in 41,XX^{Y*} Mice, an Animal Model of Klinefelter's Syndrome

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Introduction The male genetic disorder Klinefelter's syndrome (KS) is characterized by a supernumerary X-chromosome. In the majority of patients apart from hypergonadotropic hypogonadism, complete germ cell loss is found after puberty indicating problems in the maintenance of the spermatogonial stem cell (SSC) population. However, only little information is available on the fate and development of the somatic Sertoli cells (SC) which are also essential for germ cell survival in the disturbed testicular environment. Therefore, we analyzed the SC population histologically and immunohistochemically during testis development using our 41,XX^{Y*} mouse which is a sufficient animal model for KS, resembling endocrine, cognitive and testicular phenotype associated with this sexchromosomal condition.

Material & Methods Immunohistochemical stainings against Anti Muellerian Hormone (AMH) as a maturation marker and 5 methylcytosin (5meC), *de novo* methyltransferase (Dnmt) 3a and 3b reflecting important regulatory functions of SC were performed in testes of different developmental stages (1, 3, 5, 10, 14, 21 d pp and 30 wks pp; n = 3 each) of males with a 41,XX^{Y*} karyotype, 40,XY* littermate and 40, XY C57bl/6 controls. SC number was assessed using unbiased optical disector stereology on samples from 30 weeks old adult (n = 4) and 90 week old aged (n = 3) mice.

Results In adult mice, stereology revealed significantly, almost 2-fold higher numbers of SC per volume unit in XX^{Y*} mice. However, correlated to the organ size, SC number per testis in these animals was approximately 3-fold lower compared to controls (SC hypoplasia), a finding which was even more pronounced in aged mice but here also the number per volume unit was already lower. AMH expression was detected in controls up to day 10 pp. Whilst no AMH expression was seen in these controls on day 14 pp, it was still present in 41;XX^{Y*} mice, indicating a delayed maturation of SC during postnatal development. Interestingly, in a single case of focal spermatogenesis found in one 41, XX^{Y*} mouse (aged 14 d pp), in the few tubules that contained differentiating germ cells, AMH expression was not longer present. No differences were found between the groups in the expression patterns of 5meC and Dnmt3a. However, on day 21pp Dnmt3b was expressed only in SC of 41, XX^{Y*}, but not in the control mice.

Conclusion SC number, maturation and physiology is altered in 41, XX^{Y*} male mice, indicating that the disturbed karyotype of the animals also affects SC which might therefore contribute to the germ cell loss, the structurally disturbed testicular morphology and the endocrine phenotype observed in KS. Observations in tubules with focal spermatogenesis suggest the SC - germ cell communication to be involved.

The study was supported by the DFG (Grant No. WI2723/4-1).

ECA Session 6: Andrological Implications of Genital Tract Infections

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Regulatory T Cell (TREG) is a critical Physiological Mechanism against Testicular Autoimmunity

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Introduction A popular paradigm for the control of testis autoimmunity depends on local mechanisms including the Sertoli cell barrier (SCB) that sequesters male meiotic cell antigens (Ag). Herein, we test the novel hypothesis that: (1.) Some meiotic cell Ag are not sequestered; they maintain physiological Ag-specific Treg function, (2.) Ag specific Treg normally prevent autoimmune response and autoimmune orchitis (AO) by continuously suppressing pathogenic T cell response to autologous meiotic cell Ag; and (3.) the sequestered meiotic cell Ag are not protected by systemic tolerance. We therefore explore Ag-dependent mechanism operating outside testis, and new mechanisms of AO.

Material & Methods Treg markers are IL2 receptor (CD25) and the transcription factor Foxp3. 98% Treg are depleted for 1 wk from the DEREG mice (diphtheria toxin [DT] receptor-Foxp3 transgenic B6AF1 mice) by DT. 60% of Treg are depleted for 5 wks from wild type B6AF1 mice by CD25 mAb. Unilateral vasectomy (uni-vx) is ligating one vas. EAO (orchitis) and EAE (encephalomyelitis) are induced by testis Ag and brain Ag injected with adjuvant. Germ cell Ab is detected by ELISA and immunofluorescence (IF); and T cell response by Ag specific proliferation. We studied recombinant LDH3 and zonadhesin (ZAN) antigens. Testis injury is determined by testis wt, histology (orchitis, IC detection, spermatogenesis), epididymal sperm, SCB integrity and analysis by immunoblot and IF.

Results These findings support our hypotheses. First, Testis cell specific antibody (including LDH3) and severe AO develop rapidly in DEREG mice treated with DT; AO disrupts germ cell production, by massive immune complex (IC) that invades and ablats SCB integrity. Second, uni-vx mice are resistant to subsequent induction of EAO but not EAE, indicative of testis Ag-specific tolerance; however, Treg depletion by CD25 mAb in uni-vx mice results in T cell-mediated bilateral AO, autoAb to Zan but not LDH3. Third, LDH3 egresses outside the SCB of normal mice and form ICs with iv LDH3 Ab; unlike LDH3, Zan is sequestered. Fourth, in mice immunized with testis homogenate, female surpass male mice in response to LDH3 but they respond equally to ZAN.

Conclusions Treg continuously protect the testes from autoimmunity. This critical physiological Treg function is maintained by non-sequestered meiotic cell Ag. Two major mechanisms of spontaneous autoimmune orchitis are defined. The first is post-vx AO, triggered by sequestered Ag for which systemic tolerance is lacking. The second is AO triggered by non-sequestered Ag in mice with defective or low Treg capacity.

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Prostatitis and Andrological Implications

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Introduction The prostatitis syndrome is a frequent disease affecting men in their reproductive age. Population assessed prevalence of the prostatitis syndrome is up to 12%. The prostatitis syndrome is classified according to the NIH definition in acute and chronic bacterial prostatitis, chronic pelvic pain syndrome and asymptomatic prostatitis. The WHO definition of male accessory gland infection in men does not differentiate between prostatitis, epididymitis, and other inflammatory alterations of the urethral compartment. The NIH definition is therefore used. Andrological implications might encompass fertility issues, sexual dysfunctions and endocrinological alterations in an unknown percentage.

Material & Methods A medline search using the terms prostatitis AND andrological implication, fertility, sexual dysfunction or endocrinology was performed. References were augmented according to personal experience.

Results Acute bacterial prostatitis and andrological implications have not been adequately addressed. Beeing a severe acute

infection andrological implications might not play a predominant role.

Patients with chronic bacterial prostatitis and chronic pelvic pain syndrome have been investigated in several studies evaluating sperm parameters. Some studies showed impaired sperm parameters. In chronic bacterial prostatitis half of the patients reveal significant bacteriospermia with still debatable deleterious effects on sperm quality. Few interventional studies have addressed fertility issues in those patients. Antiinflammatory treatment perhaps might have a positive impact on sperm parameters. Functional sperm disorders, such as acrosomal dysfunction have been attributed to chronic prostatitis syndromes, but are not well defined.

Sexual dysfunction can be described by different components such as erectile, ejaculatory, orgasmic and sexual desire dysfunctions. While well described, the pathophysiology of these sexual dysfunctions has not been well studied. Sexual dysfunction in chronic prostatitis adds to the number of positive symptom phenotypes and correlates therefore with increasing symptom scores in patients with chronic prostatitis syndromes. Prospective interventional studies on the role of sexual dysfunctions are however missing.

Endocrinological factors might interact with the inflammatory regulation in the prostate. Hormones have been found to alterate the inflammatory response via different receptors.

Conclusion Andrological implications are heterogenous and frequently described in patients with chronic prostatitis syndrome. Usually andrological factors have not been addressed as primary variables in the different studies. Further research in interventional studies is therefore needed.

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HMGB1 Modulates Immune Responses in a Cell-Specific Manner in the Rat Autoimmune Orchitis

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Introduction High mobility group box protein 1 (HMGB1) is a nucleosomal protein. Under inflammatory conditions HMGB1 is secreted from activated immune cells or released from necrotic cells and acts as an endogenous danger signal. Despite its immune privileged status, testis is prone to inflammatory infertility. In this study we analyzed the role of HMGB1 in mediating autoimmune responses in experimental autoimmune orchitis (EAO) – a rodent model of chronic testicular inflammation.

Materials & Methods EAO was induced in WKY rats by immunization with testicular homogenate in complete Freund's adjuvant. HMGB1 localization in testis was analyzed by immunofluorescence staining. Isolated testicular macrophages (TM), Sertoli cells (SC) and peritubular cells (PTC) were stimulated with recombinant HMGB1 and cytokine mRNA levels were measured by qRT-PCR. Testosterone production was evaluated using RIA assay in HMGB1 treated Leydig cells (LC). Activation of NF-κB (p65), MAPK (p38, ERK1/2) and Akt pathways were investigated in isolated testicular somatic cells by western blot. Level of HMGB1-TLR4 binding was investigated in isolated testicular cells using Duolink PLA assay.

Results EAO testes showed translocation of HMGB1 from the nucleus into the cytoplasm. Concentrations of IL-6 and TNF-α were increased 50 days after first immunization in EAO testes, whilst HMGB1 levels increased 80 days post-immunization. RAGE was highly expressed in SC as well as PTC and to a lower level in TM. HMGB1-TLR4 binding was higher in TM than in PTC ans SC. HMGB1 activated phosphorylation of p38 and p65 in TM. However, HMGB1 treated PTC and SC activated ERK1/2. HMGB1 induced an increase in mRNA expression of TNF-α and IL-6 in TM. HMGB1 induced a significant increase in testosterone production by LC.

Conclusions Under chronic inflammatory conditions HMGB1 is released into the testicular extracellular milieu. In contrast to IL-6 and TNF-α, an increase in HMGB1 levels in testis was observed only at later stages of the disease. Late phase of action makes HMGB1 an interesting therapeutic target as orchitis is asymptomic at earlier stages. We propose that the effect of released HMGB1 on different testicular somatic cells is mediated via their individual receptor expression profiles. HMGB1-RAGE binding in SC, PTC and presumably LC can induce local tolerance due to ERK1/2 and subsequently autophagy activation and increased testosterone production. However, HMGB1 binding to TLR4 promotes inflammation by inducing secretion of proinflammatory cytokines such as TNF-α and IL-6 which triggers chronic inflammatory responses at later stages of EAO which aids in the pathogenesis of autoimmune orchitis.

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Protective Effect of Probiotic Lactobacilli against Sperm Damage exerted by Soluble Factors from *Escherichia coli*: Focus on Lipopolysaccharide

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Introduction Unidentified soluble factors secreted by *E. coli* have been reported to inhibit mitochondrial membrane potential (ΔΨm), motility and vitality of human spermatozoa. In this study we investigated whether the endotoxin lipopolysaccharide (LPS) released by Gram-negative bacteria could account for these effects, as LPS receptor, Toll-like receptor4 (TLR4) has been recently identified in human sperms. Furthermore, as strains of lactobacilli have been reported to produce soluble factors interfering with TLR4 signalling, we also investigated whether a mix of 3 selected strains of vaginal lactobacilli (*L. brevis* CD2, *L. salivarius* FV2, and *L. plantarum* FV9) could exert protective effects.

Material & Methods Sperm motility was evaluated with CASA and sperm vitality with the eosin exclusion staining under light microscope. Sperm ΔΨm was assessed at flow cytometry with JC-1, which emits red or green fluorescence when ΔΨm is high or low, respectively. In order to avoid direct contact between motile sperms and bacteria, coincubations were carried out in a Transwell system, where two independent compartments are delimited by a 0.4 mm pore membrane.

Results When compared to untreated samples, sperm suspensions coincubated for 1 h with *E. coli* (1.5×10^6 CFU/mL), exhibited dramatically lower percentages of viable spermatozoa (19.6 ± 3.2% vs 70.4 ± 11.4%, p = 0.007), with consequent drop in motile sperms (1.2 ± 1.2% vs 63.6 ± 10.8%; p = 0.0004) and sperm ΔΨm. All these effects were completely prevented by the concomitant addition of the probiotic mix (1×10^8 CFU/ml), while no preventive effects was observed using UV-inactivated lactobacilli. Sperm exposure to LPS (100 ng/ml) inhibited ΔΨm, as indicated by the decrease in sperm % emitting red JC-1 fluorescence (47.4 ± 6.2% vs 75.7 ± 2.9%, p = 0.02), throughout 6 h incubation, without affecting sperm motility and vitality. The LPS-induced ΔΨm inhibition was also prevented by probiotics.

Conclusions LPS cannot account for the adverse early effects exerted by soluble products of *E. coli* on sperm motility and viability. The protective effect of probiotic lactobacilli against the loss of motility/vitality exerted by soluble factors from *Escherichia coli* is not related to a possible competition for LPS binding to TLR4. Mechanisms of this dramatic protective effect, remain to be elucidated.

ESU Course 4: Obstruction of the Seminal Pathways

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Ultrasonographic Determination of Caput Epididymidis Diameter is Strongly Predictive of Obstruction in the Genital Tract in Azoospermic Men with Normal Serum FSH

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Introduction The relationship between epididymidis ultrasonography (US) and infertility is poorly defined probably due to lack of objective and reproducible criteria of US evaluation.

Materials & Methods Here we evaluated US size of testes, caput and of corpus epididymidis in infertile men: 165 with total sperm count ≥ 39×10^6, 187 with total sperm count 6 and 75 azoospermic men. Blood levels of FSH and of total testosterone were also evaluated. US measures obtained using a high-frequency (12 MHz) linear array transducer, included the mean value of bilateral testicular volumes (mL) (Testes-M), of bilateral longitudinal diameter of caput epididymidis (mm) (Caput-M), and of the bilateral antero-posterior diameter of the corpus measured on a longitudinal scan (mm) (Corpus-M). Testicular histology of azoospermic men was obtained and the percentage of seminiferous tubules with elongated spermatids (%T) was used to classify cases with normal spermatogenesis (obstructive azoospermia) (n = 17; %T ≥ 80), or with deranged spermatogenesis (n = 58; %T ≤ 33).

Results Caput-M was correlated with Testes-M (p = 0.0003; r = 0.17) and with FSH serum levels (p = 0.024; r = –0.14), but not with semen parameters. Caput-M but not Corpus-M values resulted greater in obstructive azoospermia compared to other groups but difference was not significant. Cut-off values of Testes-M, Caput-M and of FSH correctly classified cases of obstructive azoospermia (AUC > 0.5). A patient with FSH ≥ 7.8 UI/mL, which represented the cut-off value with the highest combination of sensitivity (100%, CI 76.8–100%), and specificity (84.9%, CI: 72.4–93.3%), had no probability of being affected by obstructive azoospermia, while a patient with FSH < 7.8 UI/mL only had 63.6% (CI: 40.1–83.2%) probability of being affected by obstructive azoospermia. US Caput-M ≥ 10.85 mm, which represented the cut-off value with the highest combination of sensitivity (58.8%, CI: 32.9–81.6%) and specificity (91.4%, CI: 81–97.1%) applied in cases with FSH < 7.8 UI/mL, increased the probability for obstructive azoospermia from 63.6% up to 92.3% (CI: 76.5–98.8%).

Conclusions The US caput epididymidis diameter determination represents a new valuable step in the evaluation of the infertile man, mostly in case of azoospermia and normal level of serum FSH. On the contrary it has not provided any relevant information in non-azoospermic men **(Fig. 3, 4)**.

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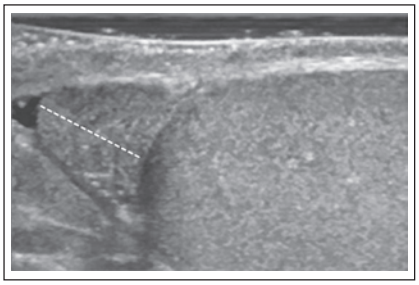

Figure 3. A. Pezzella et al. Longitudinal ultrasonographic image of caput epididymidis appearing as a pyramidal structure above the upper pole of the testis. The maximal diameter is measured from the top to the base of the pyramid (line).

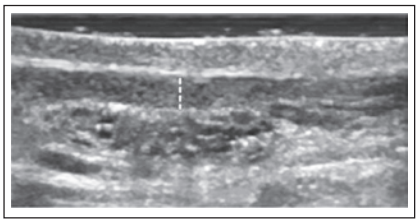

Figure 4. A. Pezella et al. The maximal antero-posterior diameter of the corpus is measured at its middle portion on a longitudinal scan (line).

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Vasoscopy – A new Diagnostic and Therapeutic Tool in Andrology?

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Introduction Micro-Endoscopy has been performed in the bile ducts, in the breast ducts and in the nasolacrimal ducts. Up to now, the endoscopy of the vas deferens has not yet been performed successfully because of the small lumen (1.24 mm) and the narrow internal inguinal ring. Therefore the aim of our study was to successfully perform vasoscopy using a new prototype of a micro-endoscope.

Material & Methods In a pre-clinical randomized study first the vasa deferentes of transsexual men were investigated ex vivo after surgery. In a second step the vasa deferentes of men within 24–48 hours after death were investigated *in situ* conditions. A semi-rigid micro-endoscope of 0.6 mm outer diameter (covered with Nitinol and with the possibility of insertion of 0.4 mm tools) with integrated fiberoptic (0.9 mm, 10000 pixels) and a depth of field from 3–20 mm was used.

Results Antegrade and retrograde views of the inner lumen of the vasdeferenswere achieved. Using a working channel a biopsy forceps and a laser fiber could be introduced into the inner lumen allowing to obtain probe material and to close or open the lumen by coagulation and vaporization, respectively.

Conclusions Vasoscopy might be used as a valuable tool for the assessment of a site-specific ejaculatory function (e. g. in varicocele patients), for sperm revovery in case of ejaculatory dysfunction by washout (e. g. in patients with spinal cord injuries) or for treatment of obstructive azoospermia (e. g. by application of a laser or by dilatation). Furthermore vasoscopy for the first time enables to obtain material for histological and microbiological investigations or to directly apply drugs for therapy.

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Seminal Plasma Biomarkers and Congenital Bilateral Absence of the Vas Deferens: Relationships with the Anatomical Urogenital Phenotypes and Place in the Diagnostic Strategy

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Introduction The clinical diagnostic of the congenital bilateral absence of the vas deferens (CBAVD) is critical since it implies a specific support for the infertile couples including a genetic counseling. However this diagnostic is puzzled by the wide spectrum of phenotypes and involves a complex combination of clinical, ultrasonographic, biological and genetic investigations. Various anatomical abnormalities of the accessory glands have been reported in CBAVD patients suggesting that biochemical markers of the seminal plasma should have a high contributive value. However, the relationship between seminal plasma biomarkers and the anatomical urogenital phenotypes of CBAVD has been scarcely addressed in the andrological literature.

Material & Methods 82 male patients with azoospermia for whom definitive diagnostic of CBAVD was assessed by scrotal and transrectal ultrasonography were retrospectively selected. According to their urogenital anatomy, patients were divided into three groups: complete absence of both vas deferens (G1, n = 36), partial absence of both (G2, n = 28) and complete/partial absence (G3, n = 18). For each patient, clinical and ultrasonographic data, sperm analysis results were recorded. 9 biochemical markers were assayed: citrate and zinc (prostate), fructose, choline and total proteins (seminal vesicles), total carnitine, acylcarnitine, alpha-glucosidase and glycerophosphocholine (epididymidis). For each group, numerical data were reported as mean, median, standard deviation, 5th and 95th percentiles.

Results For G1, G2 and G3 patients and according to WHO procedures, median sperm volume and semen pH were below the

reference ranges (respectively 0.77, 1.14 and 1.04 mL for volume; 6.8, 7.1 and 6.9 for pH). However, despite a CBAVD with azoospermia, 25 patients (34%) presented a normal pH and/or volume. Strikingly, normal pH and volume could be found despite a bilateral absence of seminal vesicles. For the three groups, total concentrations of epididymal and seminal vesicles markers were very low but prostatic markers remained within the normal ranges. Interestingly, total amounts of fructose were below < 13 µmol/ejaculate in 94% of G1, 85% of G2 and 100% of G3 patients.

Conclusion Whatever the anatomical urogenital phenotype of CBAVD, total amount of epididymal and seminal vesicles biomarkers in ejaculates are very low. Thus, assessment of biochemical markers should be an imperative first-line investigation for azoospermia.

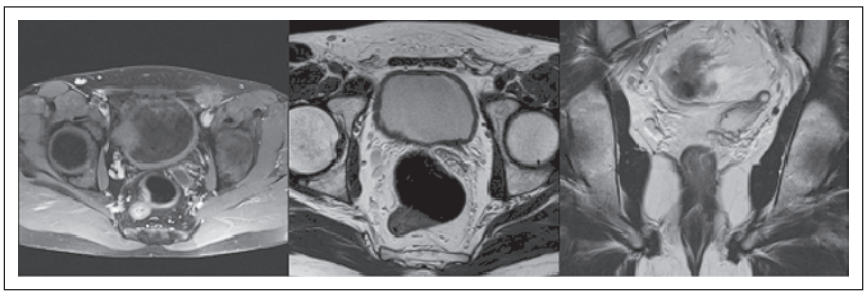

Figure 5. R. Amorim et al.

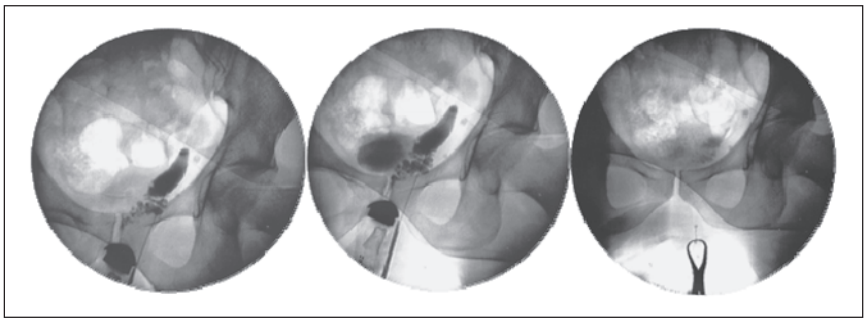

Figure 6. R. Amorim et al.

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Male Infertility – a rare Complex Case of Abnormal Development of the Seminal Pathways

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Vas deferens agenesy can be uni- or bilateral and in this case is frequently linked to Cystic Fibrosis. When there is also aplasia/agenesy of the seminal vesicle(s), we are probably facing abnormal embryologic development of the Wolff structures, implying imagiologic study of renal system.

We present a case report of a 34 year old male with primary infertility, having severe male factor (azoospermia). The 35 year old female had no gynecologic abnormality.

Physical examination showed left side palpable vas deferens and impalpable on the right, with normal volume testicles. The ejaculate confirmed azoospermia, low volume (0.5 mL) and low pH (6.8). The FSH, LH and testosterone levels were normal. Karyotype and Y chromosome microdeletions showed no abnormalities. Cystic fibrosis transmembrane conductance regulator (CFTR) gene mutation testing was negative.

The transrectal ultrasonography (TRUS) showed right seminal vesicle absence and left seminal vesicle enlargment. Pelvic magnetic ressonance imaging (MRI) confirmed right seminal vesicle absence.

Suspecting malformation of the Wolf duct resultants we performed a abdominal ultrasound (US) that demonstrated right renal agenesy.

The sperm count evaluation guided us to a disgnostic hypothesis of ejaculatory duct obstruction so we performed a vesiculography under general anesthesia, in order to proceed to transurethral ejaculatory duct ressection. The aspiration of the left seminal vesicle showed no spermatozoa and vesiculography test showed normal left ejaculatory duct. The anterograde deferentography showed a 3 cm length left vas deferens ending outside inguinal canal. We explored the left vas deferens and confirmed its blunt ending (histological exam with inspecific changes).

Diagnostic Testicular Sperm Aspiration (TESA) retrieved intact spermatozoa and the couple was oriented to medical assisted reproduction procedures (ICSI).

Our case report illustrates the variability of embryologic changes regarding internal genital system and the relation with the development of renal system (insults before the 7th gestational week).

In conclusion the observation of an azoospermic male must include a complete medical history and physical examination in order to seek for obstructive causes. When several wolffian structures are absence the renal system should be evaluated **(Fig. 5, 6)**.

ECA Session 7: Normal and Abnormal Development of the Male Reproductive Tract

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Cryptorchidism – Clinical perspective

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Introduction Cryptorchidism, i. e., incomplete descent of one or both testicles into the scrotum at birth, is the most common abnormality in newborn boys. In European countries this disorder affects 3–5% of all boys, although there are considerable regional differences. Spontaneous descent occur in most cases but a substantial number (~1%) remains unilaterally or bilaterally cryptorchid after 3 months of age. Since undescended testis has been associated with infertility and testis cancer in young adult life it has been important to develop optimal treatment to prevent future infertility and possibly also testis cancer. This presentation reviews recent developments in the management and treatment of cryptorchidism, focusing on testicular function.

Material & Methods Presented data are from the literature and from a Swedish ongoing randomized controlled study comparing the outcome of surgery for cryptorchidism performed at 9 months and 36 months of age, with follow-up of testicular and endocrine parameters. Ultrasonography and ruler measurements were used to determine testicular volume. Testicular biopsies (> 200) taken at orchidopexy were investigated morphometrically. Reproductive hormones were also analyzed.

Results revious studies have indicated that surgical treatment of cryptorchidism should be recommended in most cases rather than hormonal stimulation therapy. Orchidopexy at younger age is associated with a more favorable outcome than later treatment. In the ongoing study, a significant depletion of somatic cells and germ cells was found at 3 years compared with 9 months in cryptorchid testes, and the testicular volume was also smaller. There was good correlation between testicular volume and testicular cell counts. Endocrine analyses did not correlate with testicular morphometry at any of the investigated ages.

Conclusions Curative treatment of cryptorchidism should be offered at an early age,

preferably before 1 year of age, to prevent degenerative changes of the affected testis. Surgical rather than hormonal treatment is to be recommended. Testicular volume is a good proxy for testicular cell numbers, reflecting both somatic and germ cell counts. Reproductive hormones do not reflect cellular parameters of the cryptorchid testis, nor the tendency to spontaneous descent.

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Delayed Puberty

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Puberty in humans is a remarkable postnatal developmental process marked by accelerated skeletal growth, the acquisition of secondary sexual characteristics, and psychosocial changes that culminates in reproductive capacity. Both clinicians and scientists have long been intrigued by puberty, yet the mechanisms regulating it remain largely unknown. Initiation of puberty occurs with the secretion of pulsatile gonadotropin-releasing hormone (GnRH) by a specialized network of hypothalamic neurons. As such, human disease models of GnRH dysregulation in which the timing of pubertal onset is altered have provided valuable insight into this fundamental biological problem. This lecture will provide an overview of the clinical presentation of delayed puberty in the male, the genetic basis of delayed puberty focusing on the most severe cases of congenital GnRH deficiency, and the use of whole-exome sequencing in gene discovery efforts as well as the impact these developments may have on our understanding of delayed puberty.

ECA Session 8: Testicular Cancer

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What's New in the Pathogenesis of Testicular Cancer?

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Human germ cell tumors are a heterogeneous group of neoplasms, in which five entities are recognized based on patho-biological and clinical characteristics. Within the testis, the Type I (teratomas and yolk sac tumors), Type II (seminomas and nonseminomas) and Type III (spermatocytic seminomas) can be identified. These have their specific chromosomal constitution, expression profile, both on mRNA, miRNA and protein level. These data are informative from a diagnostic point of view. The rare type III germ cell tumors originate from a spermatogonia/spermatocyte. They are characterized by gain of chromosome 9 and expression of DMRT1. In contrast, the type II germ cell tumors originate from a primordial germ cell/gonocyte, and show gain of the short arm of chromosome 12p upon invasive growth. OCT3/4, NANOG are absolute markers for the diagnosis of the precursor lesion (carcinoma in situ). In addition, these markers are also positive in seminoma and embryonal carcinoma. In contrast, CIS and seminoma are positive for the transcription factor SOX17 and embryomal carcinoma for SOX2. This allows a defined set of diagnostic markers. Besides immunohistochemistry, these can be visualized using the quantitative Tagman Protein Assay (TPA), based on PCR-amplification. In addition, c-KIT and KITLG SCF) are found to be of value for diagnosis of the earliest stage of malignant germ cells. This is of particular interest in the context of recent Genome Wide Association studies (GWAS) results. The different types of germ cell tumors, in addition, show a specific pattern of mRNA and miRNA expression. Especially the pattern of miRNAs is of interest because of its impact in biology in general, and of the germ cell lineage specifically. miRNAs related to embryonic stem cells, i. e. 371-3 and 302 clusters, are indeed highly expressed in the CIS/seminoma/embryonal components, found to be intrinsically related to their phenotype and behavior. The consistent expression pattern initiated investigation of the value of these miRNA to be used as serum markers in these patients, both in the context of primary diagnosis and follow-up. An update of the results will be presented.

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Non-Invasive Diagnosis of Testicular Carcinoma *in situ* in Ejaculates – Perspectives in Relation to Current Routine Methods

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Testicular cancer (TC) is usually diagnosed due to an overt tumor. Tumor formation is preceded by a pre-invasive and asymptomatic stage, carcinoma *in situ* (CIS) testis, except for very rare subtypes. The CIS cells are located within seminiferous tubules but can be exfoliated and detected in ejaculates with specific CIS markers.

We have built a high throughput framework involving automated immunocytochemical staining, scanning microscopy and in silico image analysis allowing automated detection and grading of CIS-like stained objects in semen samples [1]. Investigation of semen samples from subfertile men with a contralateral testicular biopsy performed during clinical work-up revealed a test sensitivity of 0.67 and a specificity of 0.98. In addition, ejaculates from patients with clinical signs of an overt TC were investigated and yielded a slightly lower sensitivity, possibly due to obstruction.

Current golden standard for identification of CIS is a testicular biopsy, but it is performed routinely only in few countries. Despite its high sensitivity, a biopsy also occasionally yields false negative results. The semen test may hence substitute the biopsy in those centres where this invasive procedure is not performed. Among subfertile men and men with a history of cryptorchidism, who are at higher risk of developing TC, the semen test represent an attractive TC screeining solution. However, currently the test represents a substantial added workload to a standard semen analysis, which may only be feasible for large centres to handle.

Reference:
1. Almstrup K, et al. Screening of subfertile men for testicular carcinoma in situ by an automated image analysis-based cytological test of the ejaculate. Int J Androl 2011; 34: e21–e31.

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Long-term Follow-up using Testicular Sparing Surgery for Leydig Cell Tumor

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Purpose We performed a long-term evaluation of conservative surgical treatment of benign Leydig cell tumor.

Material & Methods A multicentre retrospective clinical study was performed at 6 European centres. Case files of all patients diagnosed with Leydig cell tumor and treated with conservative surgery were examined. Patients underwent physical examination, hormone and tumor marker assays, scrotal and abdominal ultrasound, chest x-ray and endocrinological examination.

Results From 1987 to 2006, 22 patients with Leydig cell tumor underwent conservative surgery. Patient mean age was 35 years (range 5–61). Mean follow-up was 179.78 months (range 77–290). No local recurrence or metastasis was observed. Patients presented either with a palpable testicular nodule (3 patients, 13.7%) or a nodule diagnosed by ultrasound (15 patients, 68.2%), gynecomastia (2 patients, 9.1%), precocious pseudopuberty (1 patient, 4.5%) or scrotal pain (1 patient, 4.5%). Diagnosis after frozen section examination was Leydig cell tumor in 20 of 22 cases (91%). Mean histological size of the nodule was 1.11 cm. Follow-up was conducted for all patients every 3 to 6 months with physical examination, tumor markers, scrotal and abdominal ultrasound, chest x-ray. All patients underwent CT scan. No local recurrence or metastasis were observed. 100% of patients are still alive with a 100% free disease survival.

Conclusions When diagnosed early Leydig cell tumors present a favorable follow-up even its potential metastatic behaviour. In

these cases sparing surgery proved to be a feasible and safe choice and could be regarded as the first line therapy.

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Is Preservation of Dysgenetic Gonads Until Adulthood Justified?

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In patients with Y chromosome and ambiguous genitalia disorder of sex development (DSD) is recognised in childhood. Usually streak gonads are resected, while dysgenetic testes preserved. In patients with female external sex organs DSD is recognised considerably later, during the period of expected puberty. The aim of our study was to evaluate adult or pubertal patients with gonadal dysgenesis and answer a question if preservation of dysgenetic testes is a correct procedure.

We evaluated 42 patients with gonadal dysgenesis (GD), aged 15–46 years, with 46,XY (78,6% of cases), 45,X/46,XY, 45,X/47,XXY, 45,X/47,XYY or 45,X/marY karyotype. Serum levels of FSH, LH and testosterone were determined. Ultrasonography of gonads was performed. Preserved gonads were biopsied or removed. In total 83 gonads were histologically evaluated, including immunohistochemical reaction with monoclonal antibodies against placental like alkaline phosphatase (PLAP), a marker of neoplastic germ cells. Morphometry of testicular structures was achieved with the use of image analysis software.

In all patients serum FSH level was increased above 10 IU/L (mean 63.7 ± 38.3 IU/L) and volume of gonads diminished (5.1 ± 3.9 nmol/L), LH increased above 10 IU/L (22.1 ± 12.7 IU/L) and they had clinical symptoms of hypogonadism. Streak gonads on both sides (pure GD) were recognised in 54,8% of cases, a streak gonad on one side and an underdeveloped testis on the other (mixed GD) in 26.7% and underdeveloped testicular structure on both sides (partial GD) in 18.6%. Germ cell neoplasia was found in 30.1% of gonads. Among them overt germ cell tumors were revealed in 10.8% of cases. In gonads with testicular structure intratubular germ cell neoplasia predominated (47.2%), while in the streak gonads gonadoblastoma (26.7%) was the most frequent. In one case spermatozoa were found, in one spermatogenesis was arrested at pachytene spermatocytes level and in another one at the level of spermatogonia. Sertoli cell only syndrome was found in 5 gonads with testicular structure (26.3%) and in one gonad seminiferous tubules were totally degenerated. Testicular structure revealed features of poor organogenesis: diminished tubular diameter, increased thickness of tubular membrane and increased intertubular spaces.

In conclusion, dysgenetic gonads preserved until adulthood have poor growth and minimal probability to produce complete spermatogenesis. In turn they exhibit high risk of germ cell neoplasia. They have also poor hormonal activity, thus most of patients develop hypergonadotropic hypogonadism and need supplementation with testosterone. Because of these resection of dysgenetic gonads is recommended as soon as diagnosis is available.

ESU Course 5: Vasectomy and Sperm Retrieval

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Sperm Retrieval General Considerations

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This lecture will cover general considerations prior to the techniques of sperm retrieval used for both non-obstructive and obstructive azoospermia. In particular it will focus on the investigation and pre-operative management of patients with azoospermia. The causes of azoospermia include both obstructive and non-obstructive causes and the management pathways are different.

A number of non-invasive techniques are used for treatment of non-obstructive and obstructive azoospermia or alternatively reconstructive procedures can be undertaken after counselling of the patient.

In non-obstructive azoospermia the gold standard of treatment is sperm retrieval in the form of microdissection sperm retrieval, although this still remains controversial.

Prior to treatment patients should undergo full evaluation including both hormonal assay, ultrasound imaging combined with genetic profiling. There are a number of important genetic causes of both obstructive and non-obstructive azoospermia.

The role of hormonal manipulation in men with non-obstructive azoospermia prior to biopsy will also be discussed.

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Results of a Combined Trifocal TESE plus M-TESE in a Series of 75 "Low Chance" Non-Obstructive Azoospermia (NOA) Patients: Experience from 2 Centres

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Background There is still no agreement regarding which is the best surgical technique for Testicular Sperm Extraction (TESE) in patients with "low chance" NOA.

Objective To report the surgical sperm retrieval rates and spermatogenic scores in a two centre study (Santiagao, Giessen) of 75 "low chance" NOA patients (testicular volume < 8 ml, FSH > 12.4 IU/ml) using a trifocal TESE (biopsy in upper, medial and lower pole) plus Microscope assisted TESE (M-TESE) in a middle extended incision [Marconi et al, European Urology, 2012].

Patients & Methods 75 patients with "low chance" NOA were prospectively recruited for the study. All patients underwent Trifocal plus M-TESE bilaterally (three biopsies per side) (e. g. Technique Presented As Video Sequence). Success was defined as the presence of at least one elongated spermatid that could be used for an ICSI procedure in the retrieved tissue. We report the surgical sperm retrieval success rates and compare the spermatogenic scores between the different biopsy sites.

Results Using the combined TESE + M-TESE approach sperm were retrieved in 48 patients (64%). A significant (p = 0.04) difference was observed between the different areas of the testis regarding spermatogenic scores (quantity of elongated spermatids retrieved), confirming the patchy distribution of spermatogenesis in the testis of patients with NOA. In the follow-up no serious complications were observed (i.e. testicular infarction), 2 patients needed additional testosterone supplementation therapy 6 months after surgery.

Conclusions Combined trifocal TESE + M-TESE is an efficient surgical technique for sperm retrieval in patients with "low chance" NOA. This may be explained by the heterogeneous distribution of spermatogenesis in patients with NOA and by the advantage that the microscopic approach offers for this subgroup of patients with a bad prognosis. The transfer of the technique to a different centre after training the surgeon has been realized providing high retrieval success rates without severe complications.

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Multimodal MRI of the Testes for Characterization of Intact Spermatogenesis in Non-Obstructive Azoospermia: A Pilot Study for Establishing Functional MRI of the Testes

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Introduction Non-Obstructive Azoospermia (NOA) is encountered in 10% of infertile males. TESE for sperm retrieval is the golden standard for therapy. Unfortunately, there are no preoperative parameters to predict successful or not successful sperm retrieval in the individual case. The goal of this study was to develop a functional MR imaging protocol for characterization of the testes to

provide hints for areas of intact spermatogenesis.

Material & Methods MRI was performed at a 3 T scanner. In 5 subjects within 7 sessions the imaging protocol was developed. Morphology was assessed by a T2 turbo Spin-Echo sequence (TR/TE 4000/101ms, FA 150°, slice thickness 3 mm, FOV 20 × 20 cm^2, matrix 310 × 320, coronal and transversal). Spectroscopy was obtained with a single voxel spin echo sequence (voxel size = 12 × 12 × 12mm^3, TE 30 and 135ms, NSA 80/128) and evaluated at an offline workstation. Diffusion weighted imaging was performed with EPI: b-values 0, 100 and 800 s/mm^2, ADC-map, TR/TE 4500/93ms, slice thickness 3,6 mm, FOV 22 × 26 cm^2, matrix 160 × 102. Perfusion of the testes was assessed after application of 0.05 mmol Gd-BOPTA/kg body weight, by a T1 3D gradient echo sequence (75 dynamics, interval 4.3 sec, TR/TE 4.8/1.9 ms, FA 12°, slice thickness 3.6 mm, FOV 26 × 26 cm^2, matrix 192 × 130). After the protocol was established, until now 4 patients with NOA (mean age 34 years) and 2 additional patients were investigated.

Results Image quality was good in all cases. The testes were of normal size in the subjects and in 3 patients. One NOA patient had atrophy. In one patient with NOA a unilateral varicocele was detected. In a case of epididymitis there was slight enlargement and oedema. Perfusion could be measured in all patients and one subject. It was decreased in a patient with trauma and unilateral in a patient suffering from inflammation. Spectroscopy revealed a mean ratio of 10.41 between choline and creatine at TE = 30 ms and 7.93 at TE = 135 ms (n = 5). The mean ratio of ADC for a healthy subject was 0.64 left, 0.54 right.

Conclusion T2-w imaging provides detailed information of morphology, relevant for assessment of atrophy, scars within the parenchyma, tumors or inflammation. Diffusion weighted imaging is a measure of extracellular free water content and cellularity of the testes. Perfusion is a biomarker for the quality of microvasculature and blood flow in the parenchyma. Perfusion might be decreased in injury and structural changes of the capillary bed. Choline as a supposed indicator for normal spermatogenesis could be measured and a ratio of this metabolite and creatine was established to evaluate spermatogenesis.

Our first results demonstrate that multimodal MRI may be a new promising non-invasive technique for comprehensive assessment of the testes, providing hints for normal spermatogenesis **(Fig. 7, 8)**.

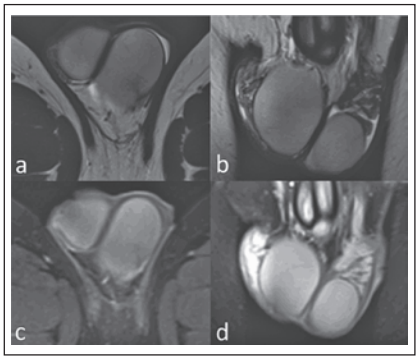

Figure 7. S. Irrle et al. T-weighted turbo Spin Echo (TSE) images (**a:** axial, **b:** coronal) and T1-weighted TSE images (**c:** axial, **d:** coronal) obtained after intravenous injection of contrast medium. Morphologic details are visible, both testes enhance homogenously after injection of contrast medium.

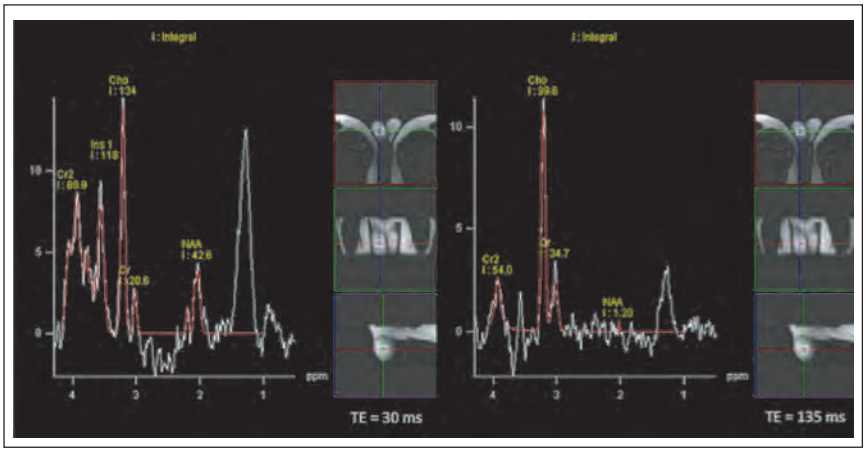

Figure 8. S. Irrle et al. Single voxel spectroscopy obtained with TE of 30 ms **(left)** and TE 135 ms **(right)** in the same position.

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What Predicts the Success of Sperm Retrieval in Adolescents with Klinefelter's Syndrome?

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Objectives To identify predictive factors for the success of microsurgical testicular sperm extraction (mTESE) or sperm retrieval in semen from adolescent patients with Klinefelter's syndrome.

Methods The clinical, ultrasound and laboratory data of 18 patients with Klinefelter's syndrome, a pubertal Tanner stage 4–5, aged 13.6–19.8 years, and who intended to undergo mTESE for sperm retrieval were analyzed with respect to factors that may influence spermatogenesis (i. e. pre-treatment with testosterone, testicular volume, pre operative serum levels of LH, FSH, testosterone, free testosterone, and inhibin B). All adolescents were asked to provide a semen sample for analysis prior to the operation. The sperm retrieval rate was compared to a cohort of 29 adult Klinefelter patients aged 20–47 years.

Results 8/18 adolescents were able to provide semen for analysis with all but one having azoospermia. In 10/18 (56%) of the adolescents, sperm could be retrieved and cryostored for future artificial reproductive treatment. Of these, in 9 elongated spermatids were found in the mTESE samples whilst the remaining patient had cryptozoospermia (> 0,1mill/ml). In the adult group, in 8/29 (28%) mTESE was successful (p = 0,055, χ^2-Test; RR for sperm retrieval [adolescents vs adults]: 2.01 [95%-CI: 0.98–4.14]. A pretreatment with exogenous testosterone had been performed in 4/18 patients and stopped 6 months prior to the operation. In 2 patients this was replaced by hCG injections. Three of the treated group had sperm retrieved by mTESE. Neither the adolescent patient's ejaculate volume, nor testicular volume determined by ultrasound (median 5,5 ml in sperm-positive vs 5ml in negative; range 2–11 ml), nor hormones **(cf. Tab. 3)** were predictive of mTESE outcome.

Inhibin B was above the detection limit of the assay (>10pg/ml) in only 5/17 patients (29%). Medians and maxima for those with sperm retrieved versus those that did not were 19.3 vs 23.9 pg/ml and 24 vs 32 pg/ml. Levels were very low when compared to those of healthy adolescents aged 14–18 years (125–330 pg/ml).

Conclusions At an adolescent age patients with Klinefelter's syndrome have higher chances to collect sperm either from semen or by mTESE. The other parameters assessed did not predict success. Pre-treatment with testosterone, if stopped 6 months prior to the operation and replaced by hCG did not affect mTESE outcome.

Table 3. J. Rohayem et al.

Sperm retrieval	LH IU/1 (normal 2–10)	FSH IU/1 (normal 1–7)	T nmol/l (normal > 12)	Free T pmol/l (normal > 250)
Successful range (median)	5.3–29 (9.8)	19–50 (27.8)	7.5–19.3 (9.6)	132–348 (212)
Unsuccessful range (median)	4.8–41 (10)	15–99 (23.1)	2.8–13.7 (10.7)	59–277 (173)

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Outcome of Microdissection Testicular Sperm Extraction in Patients with Non-Mosaic Klinefelter's Syndrome

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Introduction Klinefelter's Syndrome (KS) occurs in 1:500–1:1000 of male newborns and is the commonest chromosomal cause of non-obstructive azoospermia. The aim of this study was to evaluate TESE-ICSI treatment in patients with non-mosaic Klinefelter syndrome.

Material & Methods We retrospectively evaluated the MD-TESE performance in 117 patients with classic KS referred to Royan Institute between 2009 and 2011. The patients were divided into two groups according to MD-TESE outcome. Several factors including patient age, level of Follicle Stimulating hormone (FSH), Luteinizing hormone (LH) and Testosterone were compared between the two groups.

Results In 35 patients sperm was successfully retrieved. Intracytoplasmic sperm injection with cryopreserved sperm was performed for 17 out of 35 patients and resulted in 3 clinical pregnancies. The mean age of KS patients with successful sperm retrieval of spermatozoa was significantly lower when compared with those with unsuccessful retrieval of spermatozoa (30.2 ± 4.1 vs 34.5 ± 5.9 year, p = 0.02). Comparison of laboratory parameters between the 2 groups showed that the level of testosterone was significantly higher in patients with successful sperm retrieval (3.39 ± 2.97 vs 2.05 ± 1.26 ng/ml).

Conclusions This study of sperm recovery and ICSI outcome in men with KS shows that MD-TESE/ICSI is a successful intervention for the majority of men with Klinefelter's syndrome. Testosterone level and age could be two important factors to predict success of sperm retrieval.

ECA Session 9: INYR Session – Genetics of Male Infertility

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Sex Chromosome Linked Genetic Factors in Male Infertility

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Both sex chromosomes are enriched with genes prevalently or exclusively expressed in the testis. While data in the literature clearly indicate that Y chromosome linked CNVs removing genes affect spermatogenesis, the exact function of single AZF genes in spermatogenesis is still unknown. The only reported isolated mutations occurred in the AZFa region and affect the *USPY9* gene. The phenotypic expression of the *USP9Y* deletion is highly variable suggesting a relatively marginal role for this gene in spermatogenesis. Similarly, mutational screening of 7 X-linked spermatogenesis candidate genes did not lead to the identification of clinically relevant mutations. While mutations of the AR gene are clearly involved in androgen resistance, they have been rarely found in unselected infertile men. The long-lasting debate on the role of CAG repeats of the AR gene as genetic risk factor for impaired sperm production is still ongoing and has been further questioned by the discordances of in vitro studies.

The clinically most interesting data concern the sex chromosome-linked copy-number variants (CNVs). CNVs are structural variations of the genome including large insertions and deletions and have been shown to play a role in a number of complex diseases. One of the most relevant example in medicine of a clear cut cause-effect relationship between a CNV and a pathology are the AZF deletions. AZF deletions are the most frequent molecular genetic causes of severe impairment of spermatogenesis. Beside these "classic" deletions, removing entire AZF regions (from 0.8Mb to 7.7Mb), novel CNVs inside the AZFc region and in the *TSPY1* array have been also linked to spermatogenic efficiency. Among the AZFc rearrangements, gr/gr deletion is considered a significant risk factor for oligozoospermia, whereas the role of partial AZFc duplications is still poorly investigated. An interesting feature of the partial AZFc rearrangements and the *TSPY1* array is that their clinical effect is largely dependent on the Y chromosome haplogroup i.e. ethnicity. According to various meta-analyses, gr/gr deletion in Caucasians is the only proven genetic risk factor for male infertility.

By using an X chromosome specific high resolution array-CGH, we found an X chromosome-linked deletion burden in men with impaired sperm production. A similar phenomenon was observed in an other study focusing on SCOS patients. These findings raise question about genetic instability in some infertile men with potential implications also on their general health. Our follow-up study on selected CNVs revealed a clinically relevant deletion on Xq with a cause-effect relationship with oligo/azoospermia. We expect that similarly to the Y chromosome, also X-linked CNVs will turn out to be responsible for a portion of male infertility.

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Potential Biomarkers of Non-Obstructive Azoospermia identified in Gene Expression Analysis

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Introduction Idiopathic non-obstructive azoospermia (NOA), which may be genetically based, constitute a significant number of male infertility cases. Potential biomarkers could help to determine the stage of spermatogenetic failure and support classic clinical diagnosis.

Material & Methods We analyzed 31 testicular biopsy samples with Affymetrix Human Gene 1.0 ST microarrays. 27 of them were obtained from patients with various types of NOA and 4 with normal spermatogenesis. R/Bioconductor analysis of microarray data led us to selection of biomarkers differentiating between subtypes of NOA and normal testis tissue.

Set of selected genes was evaluated with quantitative real-time PCR and validated on an independent set of microarray samples (data base; [Spiess et. al., Human Reprod 2007]). Validation data were obtained from patients with azoospermia (20 samples) and full spermatogenesis (5 samples) hybridized on Affymetrix HGU133 Plus 2 microarrays.

Results The comparative analysis of gene expression profiles in infertile and control groups resulted in selection of 650 differentially expressed genes with Student's t-test p-value < 0.001. We successfully verified and validated 6 of them: UBQLN3, CAPN11, GGN, SPACA4, SPATA3 and FAM71F1. All of them were down-regulated in infertile patients group.

Additionally, global analysis of gene expression profiles shown that azoospermic patients formed two cluster subgroups. The samples with maturation arrest at postmeiotic stage formed one cluster and samples with maturation arrest at premeiotic stage and Sertoli cells only syndrome – another one. Meiotic arrest samples could not be easily classified to any of those groups. The comparative analysis between two cluster subgroups led to identification of 5 up-regulated genes: WBSCR28, SPATS1, TMEM225, FSCN3 and GSG1. All of them were verified with qPCR and validated with independent set of microarray samples.

Conclusions Expression of 6 biomarker genes was significantly down-regulated in infertile group what suggests they may be closely related to male fertility. 5 other, up-regulated genes detected in men with late maturation arrest are proposed as spermato-

genetic failure indicators. Molecular profil of meiotic arrest samples requires further, more accurate genomic investigations. The set of selected genes can be used to create the molecular diagnostic platform to determine degree of spermatogenic impairment of infertile men with idiopathic non-obstructive azoospermia.

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Human Sperm Tail Proteomics: New Insights on Endogenous Metabolic Pathways

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Introduction Although a lot have been done in the sperm proteomics field, more detailed descriptions are expected to clarify additional cellular and molecular sperm attributes. The aim of this study was to characterize the subcellular proteome of the human sperm tail, and hopefully identify less concentrated proteins (not found in whole cell proteome studies). Specifically, we were interested in characterizing the sperm metabolic proteome and add new insights to the sperm metabolism issue.

Material & Methods Sperm were isolated from normozoospermic semen samples; tail fractions were obtained by sonication and sucrose-gradient ultracentrifugation and their purity was confirmed by various techniques. Isolated sperm tail peptides were analyzed by liquid chromatography tandem mass spectrometry (LC-MS/MS).

Results We have identified 1049 proteins, more than half of which had not been previously described in human sperm. The categorization of proteins according to their function revealed two main groups: proteins related with metabolism and energy production (26%) and proteins related with sperm tail structure and motility (11%). Interestingly, a great proportion of the metabolic proteome (24%) were constituted by enzymes involved in lipidic metabolism, including enzymes for the mitochondrial beta-oxidation of saturated and unsaturated fatty acids, and for the utilization of ketone bodies. Unexpectedly, we have also identified various peroxisomal proteins, some of which known to be involved in the beta-oxidation of very long chain fatty acids. Analysis of our data usingReactomesuggests that both mitochondrial and peroxisomal pathways might indeed be active in sperm.

Conclusion The use of fatty acids as fuel may be more preponderant in sperm than previously thought. Notably, and contradicting a common concept in the literature, we suggest that the male gamete may have the capacity to obtain energy from endogenous pools, and thus to adapt to putative exogenous fluctuations.

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A Genome-Wide DNA Methylation Study in Azoospermia

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Introduction In infertile men, the incidence of azoospermia is 15–20% and affects 1% of the general male population. We can distinguish non-obstructive azoospermia (NOA) which has been defined by the failure or dysfunction of spermatogenesis and obstructive azoospermia (OA) which results from an obstruction at some position along the genital tract. A "therapeutic" solution could be proposed, called testicular sperm extraction (TESE), not systematically positive, in order to realize an assisted reproduction techniques based on intracytoplasmic sperm injection (ICSI). Furthermore, in NOA, failure of spermatogenesis cannot be explained, but epigenetic alterations such as DNA methylation defects have been proposed to impact it.

Question The aim of this study was to define DNA methylation profiles in testicular tissue by comparing OA patients with normal spermatogenesis with NOA patients. These data may speed progress towards understanding the mechanism(s) that underlie NOA.

Methods This study included 94 azoospermic patients, subdivided according to biological criteria in three groups: 29 OA patients (controls); 26 NOA patients with spermatozoa retrieved by testicular sperm extraction and 39 NOA patients with no sperm retrieved after TESE. The methylation profile was analyzed thanks to an Illumina Infinium® Human Methylation27 BeadChip DNA methylation array (which analyses 27578 CpG sites genome-wide, spanning 14 495 genes). A relative M-value ($\log_2$ ratio of methylated and unmethylated probe intensities) was defined for each CpG sites, comparing OA and NOA patients.

Results The NOA and OA groups had significantly different DNA methylation profiles, with differences at over 9000 CpG sites (M-value > 1). Among them, 212 CpG sites (corresponding to 195 genes) had a relative M-value higher than 3. These results highlighted 11 genes that were specifically expressed in testicular tissue. Patient clustering with respect to these 212 CpG sites corresponded closely to the clinical classification (i. e. OA vs. NOA). The DNA methylation patterns showed that in the NOA group (TESE+ and TESE- pooled), 78 of the 212 CpG sites undermethylated and 134 overmethylated relative to the OA group.

Conclusion The OA and NOA groups had markedly different DNA methylation profiles. Azoospermia could be classified as OA or NOA by considering the 212 CpG sites. Furthermore, we identified genes that may provide insight into the mechanism of idiopathic NOA.

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New Insights into Sperm DNA Methylation: Intra- and Inter Individual Stability and the Methylation Status of piRNA Genes

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Introduction Human semen is peculiar for the heterogeneity of its sperm population presenting a number of different qualitative features including metabolic and chromatin differences. Whether such differences between sperm subpopulations also reflect modifications in the DNA methylation pattern has not been addressed so far. Similarly, the question about what we could consider as a "normal" sperm DNA methylome and whether it is stable among normozoospermic individuals is still largely unexplored.

Materials & Methods 8 normozoospermic men belonging to the upper normal range were selected. From each individual, 3 sperm subpopulations were analyzed: 1) whole sperm population; 2) "up" fraction; 3) "down" fraction. The high resolution Infinium 450K methylation array was used to compare DNA methylomes of spermatozoa and for the comparison with those of somatic and cancer cells

Results Our study, based on the largest number of subjects ever considered for such a high amount of CpGs (n = 487,517), provided clear evidence for i) a highly conserved DNA methylation profile among normozoospermic men; ii) a stable sperm DNA methylation pattern in different quality-fractioned sperm populations of the same individual. We found some major differences between DNA methylation profiles of spermatozoa and somatic/cancer cells: i) highly polarized sperm DNA methylation profile; ii) association of histone-enriched hypomethylated loci with embryonic development. Then, we observed a clearly distinct genomic and functional organization of hypo- versus hypermethylated loci. Our array allowed the characterization of a total of 2,591 unique piRNAs. In spermatozoa, we found a significantly higher proportion of piRNA-linked CpGs within the total of hypomethylated loci compared to those found within the hypermethylated loci (p = 1.585E–05). The preferential hypomethylation of piRNAs was evident also in comparison with two other cell types.

Conclusions The rationale behind sperm selection before ART is mainly related to a predicted higher functional competency and a higher genomic integrity of selected spermatozoa. Our finding, for the first time in the literature, indicates sperm methylation is stable in different quality-fractioned sperm subpopulations of the same individual i.e. sperm methylation is not altered in "poor" quality spermatozoa of normozoospermic men despite that these cells are clearly different from a metabolic and DNA integrity point of view. In addition, we provide both confirmatory and novel data concerning the "normal" sperm DNA methylome, including its peculiar features in respect to somatic and cancer cells. Our data, with special focus on hypomethylated piRNAs-linked genes, represents a solid basis for future basic and clinically oriented research.

ESU Course 6: Vasectomy, Sperm retrieval and Spermatic Cord Denervation

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Microsurgical Spermatic Cord Denervation for Chronic Testicular Pain

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Introduction Microsurgical spermatic cord denervation has become a consented procedure for the treatment of Chronic Testicular Pain (CTP) in specialized centres. In previous reports success rates concerning loss of pain range from 71% to 100%. Surgical approach can be either performed using a microscope or surgical loupes.

Objective To evaluate the results of Microsurgical Spermatic Cord Denervation (MSCD) in patients with CTP, using either a loupe assisted approach (magnification 3.5×) or a surgical microscope (magnification 20×).

Patients & Methods 25 patients with CTP were prospectively enrolled in the study. In order to be a candidate to MSCD all patients had a positive response to a spermatic cord block test with bupivacaine and no response to a placebo injection (saline solution). Pain severity was measured using an Analog Visual Pain (AVS) scale for the last 30 days (range 0–10). 13 patients were operated using surgical loupes and 12 using a microscope (e.g. Technique Presented As Video Sequence). Patients were controlled 1 week, 3 and 6 months after surgery symptomatically (AVS) and with a color doppler ultrasound of the testis to evaluate testicular perfusion.

Results A total of 27 testicular units were operated. The mean duration of surgery was 70 minutes (range 50–90 minutes). No intraoperative complications were observed. No testicular units were lost and the color doppler ultrasound was normal in all cases. 3 and 6 months after surgery 21 (84%) patients significantly reduced pain (AVS: median score 0, range 0–2) with no need of further oral analgesia. 3 patients persisted with intermittent testicular discomfort (AVS: median score 4, range 2–4) that could be managed with on demand paracetamol. One patient had no variation in AVS after surgery (AVS: 7). No statistically significant differences ($p > 0.05$) were observed between patients operated using loupes or microscope regarding operation time and success rate.

Conclusion Microsurgical spermatic cord denervation is a procedure with a high success rate without severe complications. The loupe assisted approach is comparable to the approach using a microscope.

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Micro-TESE Option in NOA Management

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Introduction Azoospermia, defined as the complete absence of spermatozoa in the ejaculate after centrifugation, is found in 1–3% of the male population and in approximately 10% of the infertile males. Obstructive azoospermia(OA) is treated with surgical reconstruction or surgical sperm retrieval (SSR) with excellent success rate. Treatment of non obstructive azoospermia (NOA) on the other hand, is yet to be standardized and its success lags behind normal and OA males. Microdissection TESE (micro TESE) is been tried as an alternative method of sperm retrieval in NOA with encouraging results. On these lines, we introduced micro TESE in our centre for NOA.

Materials & Methods 35 patients were recruited and had detailed clinical and endocrinological evaluation along with karyotyping and y microdeletion testing. Micro TESE was done with operating microscope using up to 25× magnification and removing only dilated tubules when they were found. Otherwise random tubules of comparatively larger sizes were taken. Here, we are presenting SSR data with their preliminary ICSI outcome.

Results SSR was successful in 50% of men with retrieval of motile sperms in 58%. Average operative time was 2 hours, fertlization rate of 65% and cleavage rate of 55% was achieved and 48% transferable and cryopreservable embryos were seen on day 3. There were no major complications in any of the patient.

Conclusion Micro TESE is an effective modality of SSR in NOA men. It is associated with qualitatively and quantitatively good sperms available for ICSI with minimal complications. It gives a opportunity to couples to become biological parents as it gives a valid alternative for donor sperm or adoption which these couples had been otherwise offered.

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Probe-based Confocal Laser Endomicroscopy (pCLE) – a New Diagnostic Tool in Andrology

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Introduction Probe-based confocal laser endomicroscopy (pCLE) allows an in vivo and in situ histological evaluation of tissues up to 20 µm depth. Up to now, it is used for diagnostic purposes in gastroenterology, pulmonology and ophthalmology. The aim of our study was to for the first time evaluate the use of pCLE as a diagnostic tool in andrology. Thus, we wanted to optimize sperm retrieval rates in patients undergoing testicular sperm extraction (TESE) for assisted reproduction by localization of vital spermatozoa in the testis. Additionally we wanted to characterize testicular alterations by pCLE.

Material & Methods In a pre-clinical randomized study the testes of transsexual men as well as human testes removed by surgery after diagnosis of a tumor were investigated by pCLE using the Cellvizio® confocal microprobe ProFlex™ S1500 (Mauna Kea Technologies, France) after incubation in 1) 0.01% fluorescein isothiocynate (FITC), 2) 5% cresyl violet solution (CV), 3) 0.01% Fluorescein Alcon 10% (FA) and 4) 0.04% acriflavine (AF). The images obtained were correlated to the results of fluorescent confocal laser microscopy.

Results FITC and FA clearly marked spermatozoa, spermatocytes and spermatogonia in the Tubuli seminiferi contorti. CV specifically marked the intercellular matrix in the tubules, whereas AF stained the nuclei of all cells during spermatogenesis. The endothelial cells of vessels including capillaries of 7 mm diameter were strongly stained thus being a marker for vascularisation which was distinctly enhanced e.g. in embryonic carcinomas.

Conclusions Probe-based confocal laser endomicroscopy might be used as a valuable tool for the determination of areas in the testis with effective spermatogenesis thus enabling to optimize the positive sperm retrieval rates in TESE patients. Additionally pCLE can provide efficient and fast in situ information on cellular morphology and vascularisation of testicular tumors.

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ICSI from "fresh" TESE in Absolute Asthenozoospermia: An Option or the Solution?

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Introduction Absolute asthenozoospermia (i. e. total sperm immotility) is rare (1:5000 men) and has a strong negative impact on

ICSI outcome. Il can be due either to ultrastructural genetic defects of the sperm tail, or to necrozoospermia secondary to genital infections, oxidative stress, cryopreservation, antisperm antibodies, metabolic disorders affecting the ATP production, exposure to environmental pollutants, delayed epididymal transport or long lasting sexual abstinence.

Case Report An infertile couple, where the 38-years-old female partner, previously submitted to uterine septum surgery, had regular ovulatory cycles, and the 39-years-old male partner, previously submitted to varicocelectomy, was usually cryptozoospermic with a completely absent sperm motility, as documented by 4 consecutive semen analyses. The last and best semen analysis showed a severe oligoasthenoteratozoospermia (according WHO, 1999) (4.4 spermÍ10^6/ml, 100% immotile sperm, 3% normal forms and severely low vitality [3%] by eosin-nigrosin test). Both the testes (8 ml) were correctly located into the scrotum, with a normal sonographic feature and no varicocele recurrence. All reproductive hormones fell into the normal range, and neither genetic abnormalities nor seminal tract infections could be detected. A transmission electronic microscopy (TEM) sperm analysis showed structural defects (apoptosis, immaturity and necrosis) in 100% of the sperm. The couple had been already submitted to one ICSI cycle with fertilisation failure (4 oocytes injected) and, on the basis of TEM findings, three other IVF centres discouraged them from further ICSI attempts. An informed consent was obtained, and an ICSI with timed testicular sperm retrieval („fresh“ TESE) the same day of oocytes pick-up was carried out. Very few motile sperm were retrieved from the testis: one out of the three injected oocytes was fertilized and the embryo was transferred. A full term pregnancy was achieved with a healthy male baby delivery.

Discussion In case of absolute (crypto)-asthenozoospermia, the results of the traditional ICSI are very poor because of the difficulty of distinguish the vital and potentially injectable sperm among immotile ones. Thus, several techniques have been proposed to select immotile, but viable sperm, all of them of unproven efficacy, or very expensive as Laser-Assisted Selection (LAISS) and microscopy birefringence. The use of motile sperm retrieved by TESE, in this case, was effective and, after the signing an appropriate informed consent, may be a feasible option, confirming previous data reported in literature.

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Is it Worth Doing Spermatozoa Retrieval in Azoospermic Non-Mosaic Klinefelter's Syndrome Patients?

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Introduction Patients with non mosaic Klinefelter's Syndrome (KS) are in general azoospermic. In many medical assisted procreation institutions these couples are oriented to reproductive procedures using donor sperm. Neverthless some testicular biopsies retrieve spermatozoa and there are published cases of unassisted pregnancies in KS patients.

Methods Our centre experience in non mosaic azoospermic KS patients seeking for reproductive assistence was retrospectively analyzed, from 2002 to 2011.

These patients were evaluated including medical history, physical examination and hormonal analysis (FSH, LH and testosterone).

We performed a TEsticular Sperm Extraction/IntraCitoplasmatic Sperm Injection (TESE/ICSI) in the day of the oocyte retrieval. In those couples where it was impossible to retrieve spermatozoa we offered the possibility of using donor sperm.

Results We performed 35 TESE. All biopsies were unilateral, accomplished under local sperm cord block, in our Medical Assisted Procreation (MAP) laboratory, allowing immediate embryologist evaluation.

The male age ranged from 25 to 38 year, all with atrophic testis (volume under 6 mL), high FSH and LH measurement, eleven showing normal testosterone levels. The retrieved fragments varied from three to eight, until the embryologist could find intact spermatozoa.

There were no complications in the TESE/ICSI procedures. Spermatozoa were successfully retrieved in 17 patients (48.6%), some of them taking several hours. We found the positive results to be independent of patient's age and hormonal measurements and testis volume.

Since 2004 we began doing unilateral biopsy in order to maintain existing testicular hormonal prodution. The fertilisation rate was 35%.

All the newborn were tested for peripheral karyotype analysis that showed no numeric chromossomal abnormality.

Conclusions Patients with KS should be encouraged to seek a biological paternity. A simple surgical procedure performed under local anesthesia can provide in this patients the same spermatozoa retrieval rate as the other non-obstructed azoospermic patients. The patient must be offered an open biopsy at the same time as the oocyte harvest since ICSI should be done using fresh tissue.

The most important prognostic factor to the success of the TESE was the time spent in the procedure by the urologist and the embryologist. The normal newborn karyotype corroborates previous papers conclusion that this disease is not passed on to the offspring.

ECA Plenary Lecture 4: New Horizons in Andrology

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Genetic Models of the Future – Making the Most of the Available Technology

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Our understanding of the genetic basis of male infertility has been hindered by the difficulty of identifying the responsible genes. Genetic mapping in infertile men is more challenging than for other clinical conditions as these men do not have children, making pedigree analysis problematic. Furthermore, many cases of idiopathic male infertility likely result from de novo mutations in individual men, or inheritance of multiple contributing loci that shift the balance from fertility to infertility. As we move towards the era of personalised medicine, where patient genomes can be rapidly sequenced and comparisons across populations will permit identification of underlying genetic lesions, the development and application of genetic models to understand the roles such genes play in male fertility becomes ever more important; only by understanding the function of these genes can we hope to develop effective treatments.

Over the past 20 years, the development of knockout mice has revolutionised our understanding of the genetics underlying male reproductive development and fertility. This has been supported by the relatively recent development of conditional gene-targeting where the role of specific genes can be localised to a single cell-type, or time of development, and recent efforts using mutagenesis "phenotype-driven" screens, which have taken models of male infertility and used genetic mapping to identify genes underlying the infertility. Now we are moving to a new horizon, the era of whole genome sequencing, in vivo genome editing and gene therapy are providing new possibilities to increase both our understanding of the genetics underpinning male infertility, and also our ability to treat such conditions. This presentation will describe current and emerging genetic technologies, and describe how they can and are being used in the context of andrology research.

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Sperm Chemotaxis – Throwing Surprises

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Introduction How does a sperm find the egg? In many species, sperm are guided by the chemical cues provided by the egg – a process called chemotaxis. Most of our knowledge on sperm chemotaxis originates from the study of marine invertebrates. However, much less is known about chemotaxis of mammalian sperm; wherein, sperm has to find its way through the complex chemical milieu inside the female reproductive tract? The identity of the chemoattractants, their receptors, and the underlying signalling pathways are the subjects of debate and intense investigation [1].

The recent discovery of sperm-specific CatSper channels (cation channel of sperm) [2, 3] and technical improvements in imaging and electrical recordings of sperm have paved ways for improved understanding of chemotaxis of mammalian sperm. CatSper channels control the intracellular Ca2+ concentration and thereby the swimming behavior of sperm. Progesterone and prostaglandins – factors of the oviduct – directly activate human CatSper channels and stimulate Ca2+ entry [4, 5].

Surprisingly, CatSper does even more: the channel serves as a polymodal sensor and thereby translates multiple chemical cues of the female reproductive tract into a behavioral response of sperm [6]. Moreover, our findings also show that human sperm do not smell after all.

Material & Methods Purification of human sperm, Ca2+ measurements, patch-clamp recordings, and cAMP-RIAs were performed as described [5–7].

Results

- The CatSper channel is promiscuously activated by a variety of odorants: they enhance CatSper currents and stimulate Ca2+ entry via CatSper channels.
- Odorants, as well as several other ligands for G-protein-coupled receptors, do not activate a cAMP-signalling pathway.
- Activation of CatSper by odorants does not involve a metabotropic pathway.
- Analogues of cyclic nucleotides activate human CatSper through an extracellular site.

Conclusions Human sperm lack functional transmembrane adenylate cyclases as well as a signalling pathway like the one in olfactory sensory neurons.

Intracellular rise in cyclic nucleotides fails to activate Ca2+ channels in human sperm. This differs from the observations in sea urchin and bovine sperm making the physiological differences between sperm of different species further evident.

CatSper may function as a polymodal, chemosensory Ca2+ channel that translates a complex environment in the female reproductive tract into a behavioral response of sperm.

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Ejakulationsstörungen

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Allgemein Ejakulationsstörungen sind:

- Vorzeitige Ejakulation (Ejaculatio praecox)
- Verzögerte/fehlende Ejakulation (Ejaculatio retarda/absentia)
- Retrograde Ejakulation
- Ejakulatvolumenstörungen (Hypospermie/Aspermie)
- Schmerzhafte Ejakulation

Anderweitige seltene Ejakulationsstörungen sind:

- unwillkürliche Ejakulationen (Pollutionen)
- anhedonische Ejakulationen (fehlendes Lustgefühl)
- asthenische Ejakulationen (reduzierte/fehlende Ejakulationskraft)
- postejakulatorisches/orgastisches Erschöpfungssyndrom (schwerer Erschöpfungszustand mit diffuser klinischer Symptomatik)

Bei der Ejaculatio praecox unterscheidet man die lebenslange oder primäre („kongenitale“), partiell genetisch bedingte Ejaculatio praecox von der erworbenen, sekundären Ejaculatio praecox, welche meistens durch andere Erkrankungen wie z. B. einer Erektilen Dysfunktion (ED), einer oder Schilddrüsenfunktionsstörungen (Hyperthyreose) hervorgerufen wird.

Für die Therapie der Ejaculatio praecox steht die topische Applikation von Lidocain-/Prilocain-haltigen Medikamenten (z. B. Emla®) oder aber die orale Medikation mit Substanzen aus der Stoffklasse der selektiven Serotonin-Re-uptake Inhibitoren (SSRI) bzw. der trzyklischen Antidepressiva zur Verfügung, wobei alle diese Therapien im Off-label-Use eingesetzt werden.

Dapoxetin (Priligy®), ein kurz wirksamer SSRI, ist derzeit das einzige, offiziell zur Behandlung der EP in Deutschland zugelassenen Medikament. Bei über 6000 in die Dapoxetinstudien eingeschlossenen Patienten kam es unter Dapoxetin zu einer zwischen 3–4-fachen Zunahme der IELT. Dapoxetin war sowohl bei lebenslanger als auch bei erworbener Ejaculatio präcox vergleichbar gut wirksam **(Abb. 9)**.

Dapoxetin (Priligy®) ist in 30- und 60-mg-Tabletten im Handel und stellt derzeit die First-line-Therapie der E. praecox dar. Typische Nebenwirkungen von Dapoxetin sind Übelkeit, Kopfschmerzen und Schwindel, diese führten aber in den Studien nur selten (3–8 %) zum vorzeitigen Absetzen der Prüfsubstanz.

Andere Substanzen wie Tramadol, ein zentral wirksames Opioidanalgetikum, Alphablocker wie Phenoxybenzamin, Terazosin, Tamsulosin und zuletzt Sildosin haben in kleineren Studien gewisse Wirksamkeiten gezeigt, sollten aber als Ausnahmemedikation betrachtet werden.

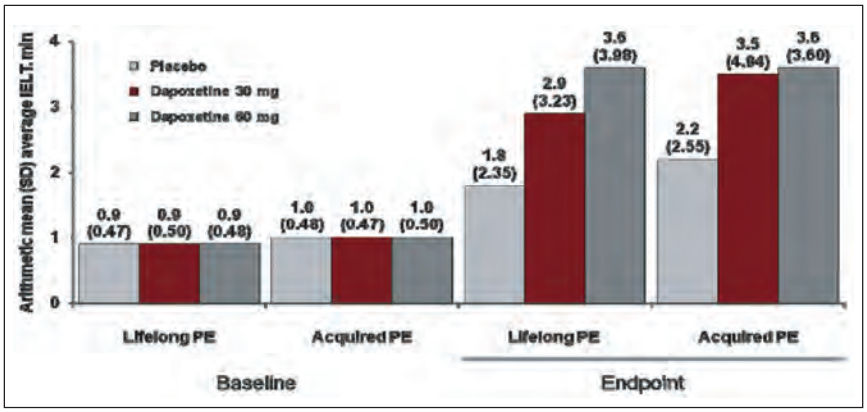

Abbildung 9. H. Porst. Gepoolte Ergebnisse der Dapoxetinstudien bei lebenslanger und erworbener E. praecox. Nachdruck mit Genehmigung aus: [Porst H et al. Baseline Characteristics and Treatment Outcomes for Men with Acquired or Lifelong Premature Ejaculation with Mild or No Erectile Dysfunction: Integrated Analyses of Two Phase 3 Dapoxetine Trials. J Sex Med 2010; 7: 2231–42].

PDE-5-Hemmer zeigten bei reiner E. praecox eine widersprüchliche Wirksamkeit. Gemäß den Richtlinien von EAU und ISSM/ESSM sollte mit PDE-5-Hemmern nur bei der Kombination ED + EP begonnen werden und, falls noch erforderlich, durch eine spezifische E. praecox-Medikation (Emla®, Priligy®) ergänzt werden.

Die Ejaculatio retarda/absentia kommt bei bis zu 30 % der älteren Männer vor, wobei mit dem Alter physiologischerweise die Reizschwelle bis zum Auslösen der Ejakulation ansteigt. Hier ist die Aufklärungsarbeit des Arztes gefragt, die u. a. auf eine Anpassung der sexuellen Stimulationstechniken zuhause abzielen sollte (Siehe z. B. „Lost penis Syndrom“ bei weiter Vagina bei Multipara). Medikamentös zeigten im Einzelfall folgende Substanzen Wirkung: Yohimbin, Midodrin, Pseudoephedrin, Imipramin, Amantadin Bupropion, Cyproheptadin, Levodopa und Oxytocin-Spray 24 U intranasal. In schweren Fällen einer E. retarda/absentia haben sich auch die Vibrator-/Elektroejakulation mit Ferticare® bzw. Viberect® bewährt.

Die retrograde Ejakulation kann iatrogen-chirurgisch oder durch Affektionen des autonomen Nervensystems bedingt sein. Bei Fertilitätswunsch muss der postmasturbatorische Urin entsprechend aufbereitet werden.

Hypospermie und Aspermie können hormonell (Hypogonadismus,Hyperprolaktinämie) bzw. medikamentös (Alpha-Blocker, 5-Alphareduktase-Hemmer) bedingt sein und werden durch Behebung der Ursache (T-Substitution, Prolaktinhemmer) bzw. Veränderung der Medikation (Alfuzosin statt Tamsulosin oder Silodosin) beseitigt.

Poster Presentations

Postersession 1: Andrological Implications of Genital Tract Infections

P1

Review of Management of Cases of Acute Epididymo-Orchitis: How Can We Improve and are our Urological Guidelines in Need of Updating?

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Question Epididymo-orchitis is a common diagnosis among urological admissions. Patients with epididymo-orchitis classically present with characteristic unilateral scrotal pain and swelling of relatively acute onset, which can masquerade as torsion. Complications include abscess formation, testicular infarction, testicular atrophy, development of chronic epididymal induration, and permanent occlusion of the epididymal ducts, resulting in infertility.

Despite the seriousness of the condition, recent studies have demonstrated that many urologists and general practitioners are falling short of the recommended European Association of Urology guidelines for managing epididymo-orchitis. Therefore, the aim of our study was to assess the current literature concerning epididymo-orchitis, and to determine if the current practice at our teaching hospital could be improved.

Methods All Patients assigned ICD codes for Orchitis, Epididymitis and other unspecified disorders of the male genital organs were identified over a nineteen month period. Those with alternate diagnoses such as testicular torsion, hydrocele and postoperative complications were excluded from the study. For those remaining, information regarding demographics, whether or not a sexual history was taken, investigations performed and treatment initiated was recorded.

Results 118 cases were identified, of which 49 were < 35 years old. 54% of all patients were discharged without seeing a member of the urology or genito urinary teams.

Discussion A number of key areas were identified for development, including better identification of the primary agent of infection. Indeed, the authors believe the mainstay of improvement will come from a greater awareness by all specialities of current sexual health guidelines, and the knowledge of how to implement these regarding epididymo-orchitis. Currently, many patients with epidydimo-orchitis have commenced antibiotics prior to referral to urology or genitourinary medicine, making diagnosis of sexually transmitted disease difficult and further contact screening impossible, unless symptomatic re-infection occurs.

We have produced a set of recommendations of how these improvements can be met, and hope they will enhance current treatment pathways.

P2

Leukocytes are Associated With Apoptosis in Human Spermatozoa

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Introduction Leukocytes are the major source of reactive oxygen species (ROS) in the ejaculate and an excessive number of leukocytes in the ejaculate contribute to up to 35% of all cases of male infertility [1]. ROS have been associated with markers of apoptosis such as sperm DNA fragmentation [2], externalization of phosphatidylserine [3], and caspase activation [2]. Thus, this study aimed at investigating the impact of seminal leukocytes on the induction of apoptosis in human spermatozoa.

Materials & Methods Semen samples were obtained from 60 men consulting for fertility problems at the Tygerberg Academic Hospital and Vincent Pallotti Hospital (Cape Town, South Africa). To investigate the relationship between seminal leukocytes and sperm apoptosis, the following parameters were measured: oxidative status in the ejaculate by evaluating the concentration of leukocytes in the ejaculate (Peroxidase test), ROS production in the ejaculate (Luminol test), generation of superoxide ($O_2^{-\cdot}$) (DHE test) and hydrogen peroxide (H_2O_2) by spermatozoa (H_2DCFDA: 2,7-dichlorofluorescein diacetate), and the activation of reduced glutathione (GSH) in sperm (GSH-Glo™); sperm apoptotic markers by measuring mitochondrial membrane potential ($\Delta\Psi$m) disruption (DePsipher™), caspase-3/7 activation (Caspase-Glo™), and DNA fragmentation (TUNEL).

Results Leukocyte concentration had a significant positive correlation with ROS production in the ejaculate ($\rho = 0.378$; $p = 0.0064$), sperm $O_2^{-\cdot}$ production ($\rho = 0.336$; $p = 0.0098$), and caspase-3/7 activation in sperm ($\rho = 0.527$; $p < 0.0001$). ROS production in the ejaculate, besides the correlation with the concentration of seminal leukocytes, was positively correlated with GSH activation ($\rho = 0.577$; $p < 0.0001$), caspase-3/7 activation ($\rho = 0.487$; $p = 0.0005$), and DNA fragmentation ($\rho = 0.331$; $p = 0.0171$). Sperm $O_2^{-\cdot}$ production, which was associated with seminal leukocyte concentration, had a significant positive correlation with sperm $\Delta\Psi$m disruption ($\rho = 0.261$; $p = 0.0446$), and caspase activation ($\rho = 0.457$; $p = 0.0055$).

Discussion Excessive ROS production in the ejaculate, mainly a consequence of seminal leukocytes, is not only linked to the internal generation of $O_2^{-\cdot}$ by spermatozoa, but has a significant association with early markers of apoptosis; disruption of sperm $\Delta\Psi$m and the activation of effector caspases-3 and -7. This suggests that an increased concentration of seminal leukocytes, is not only associated to elevated ROS production in the ejaculate and by spermatozoa, but may be linked to the induction of apoptosis in human spermatozoa.

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P3

Testicular Function in HIV-positive Patients Under Stable Antiretroviral Therapy

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Introduction & Objective With the availability of effective antiretroviral therapy (ART) for HIV-positive patients and subsequent improvements in life expectancy, aspects of sexual function and family planning issues are of increasing importance. However, the testicular function in patients under stable ART has not been systematically investigated with respect to HIV surrogate parameters. We present a prospective study to assess sex hormones and semen parameters in HIV-positive patients.

Methods Since May 2011 we enrolled 97 men in a prospective study* for semen analysis (median age: 44 years; range: 24–64 years) and determined clinical parameters as well as sex hormones. Exclusion criteria were being not on stable ART, ongoing testosterone therapy, a history of vasectomy, and co-infection with hepatitis B or C.

Results The following median (range) semen parameters were determined in 64 patients: Volume 2.4 ml (0.2–6.0); sperm concentration 53.4 Mio/ml (0.01–425); progressive motility 44% (0–69); normal morphology 3% (0–10); peroxidase + leukocytes 0.1 Mio/ml (0.0–15.7); elastase 77 ng/ml (10–2500). The median (range) testicular volume measured by ultrasound was 12.6 ml (3.3–25.6). The median (range) clinical parameters documented were: knowledge of HIV infection 6 years (0–28); duration of ART 5 years (0–17); current CD4+ cells 582/µl (98–1297); nadir CD4+ cells 233/µl (3–762); viral load in blood below detection limit 59/64 (92.2%). Univariate analysis revealed no significant correlations between semen pa-

rameters/sex hormones and any HIV surrogate parameter. In contrast, both sperm concentration and progressive motility were significantly correlated with FSH and testicular volume (in all cases: $p < 0.01$).

Conclusions Semen quality in HIV-positive patients under stable ART is not impaired. Our data provide evidence, that semen quality is not associated with any HIV surrogate parameter, but rather an individual feature.

* Supported by LOEWE (excellence initiative of the state government of Hessen, Germany) focus group MIBIE (Male Infertility during Infection and Inflammation) project B2.

P4

Testicular Atrophy after Acute Epididymitis/Epididymo-orchitis: Fact or Fiction?

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Introduction & Objective Male genital tract infections are suggested to be an important cause for male infertility. However, the frequency of testicular atrophy resulting from acute epididymitis/epididymo-orchitis is unknown. We present an ultrasound-based prospective follow-up study to assess testicular size in patients suffering acute epididymitis with and without concomitant orchitis.

Methods Since May 2007 we enrolled 80 men (median age: 49 years; range: 18–83 years) with acute unilateral epididymitis/epididymo-orchitis receiving a throughout conservative therapy. Testicular volume and perfusion on both sides was determined by ultrasound at first presentation, after 2 weeks, and after 3 months in all patients. At first presentation, the patients were allocated to subgroups according to the intratesticular perfusion of the affected testis compared to the contralateral side: 1) isolated epididymitis (normal perfusion) or 2) epididymo-orchitis (increased perfusion). The investigation was conducted by a single urologist experienced in scrotal ultrasound, with identical equipment in all cases. Statistical analysis was performed using the Friedman test with follow-up pairwise comparisons.

Results Epididymitis without testicular involvement was evident in 37 patients, while 43 suffered from epididymo-orchitis. In patients with isolated epididymitis, the median testicular volume on the affected side was 14.3 ml at first presentation, 13.7 ml after 2 weeks, and decreased to 12.9 ml after 3 months ($p < 0.01$). In patients with epididymo-orchitis, the median testicular volume on the affected side decreased from 16.6 ml at first presentation, to 13.7 ml after 2 weeks, and finally to 11.6 ml after 3 months ($p < 0.001$). However, the median testicular volume on the healthy contralateral side was initially 12.6 ml, 12.8 ml after 2 weeks and 13.3 ml after 3 months, and thus not significantly different over time ($p = 0.121$). Compared to the contralateral testis, no significant differences regarding the testicular volumes were evident after a period of three months in patients with isolated epididymitis as well as those with concomitant orchitis ($p = 0.925$ for epididymitis; $p = 0.063$ for epididymo-orchitis).

Conclusions We provide first-time evidence, that acute epididymitis and epididymo-orchitis do not result in testicular atrophy as measured by ultrasonographic volumetry. In fact, acute epididymitis/epididymo-orchitis is associated with initial elevated testicular volumes and subsequent normalization in the course of time compared to the non-affected side. A longer follow-up as well as a detailed investigation of testicular function is required to further elucidate the impact of epididymitis on male reproductive health.

Supported by a start-up funding of the medical faculty (A.P.).

P5

Correlation of Seminal Inflammatory and Sperm Quality Parameters in Patients with CP/CPPS and Infertility – Is diagnostic Value of Seminal Cytokine Interleukin-8 (sIL-8) Superior to Elastase or Peroxidase Positive Leukocytes?

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Question Male genital tract (MGT) infections/inflammations are considered causative factors for infertility in up to 20%. The effect of Chronic Prostatitis/Chronic Pelvic Pain Syndrome (CP/CPPS) and silent MGT infections/inflammations on male infertility is a matter of debate. Diagnostic value of different seminal inflammatory parameters is controversially discussed in the literature. Recent studies suggest that sIL-8 might be a good biomarker for these disorders. The aim of our study was to compare seminal inflammatory and fertility parameters in a series of male patients with infertility and patients with CP/CPPS evaluating the correlation of inflammatory parameters in the ejaculate (Peroxidase Positive Leucocytes-PPL, Leucocyte-Elastase-LE and seminal Interleukin-8 – sIL-8) and basic parameters of semen quality (sperm density-D, motility-Mot, morphology-Mor) with special focus on expected diagnostic value of sIL-8.

Methods Inflammatory parameters in the ejaculate (PPL – n/mL, LE – ng/mL and sIL-8 – pg/mL) and sperm quality parameters (D – n/mL, Mot – a+b in % and Mor – normal in %) using WHO 4. Edition reference levels were determined in 77 infertile patients and 174 patients with CP/CPPS (34 NIH IIIA, 140 NIH IIIB) and correlation was statistically evaluated. For analysis all variables were dichotomized because of their distributions. Results of localization studies (2 glass test) and NIH-CPSI scores were compared to seminal inflammatory and fertility parameters.

Results In patients with CP/CPPS IIIA and IIIB median PPL were 230,000 and 40,000, LE 187 and 95, sIL-8 2,968 and 1,558 respectively. After dichotomizing variables, only IL-8 ($p = 0.034$) was significantly higher in patients with CP/CPPS IIIA compared to IIIB. Median D was 47.8×10^6 vs 46.5×10^6/mL, Mot a+b 57 vs 56.5%, Mor 3 vs 4% normal, respectively. NIH-CPSI median pain score was 11 vs 10, total score 23 vs 22, respectively.

In infertile patients median PPL were 7,000, LE 91, sIL-8 1,602. Median D was 4.9×10^6/mL, Mot a+b 24% and Mor 2% normal. There was no significant correlation between inflammatory parameters in the ejaculate and sperm quality parameters in patients with CP/CPPS and infertile patients and no significant correlation between inflammatory parameters in the ejaculate and NIH-CPSI scores in patients with CP/CPPS.

Conclusion Inflammatory parameters in the ejaculate, if dichotomized, are not correlated with sperm quality parameters in patients with CP/CPPS and infertile patients. In our series of patients sIL-8 was not found to be a sensitive and reliable marker of MGT infections/inflammations. Determination of specific and sensitive seminal markers for MGT infections/inflammations in male infertility with validated break points would be of great importance.

P6

Alpha-Haemolysin Differentially Modulates Innate Immune Responses in the Experimental Murine Epididymitis Model

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Introduction Infection and inflammation of the urinary tract are aetiological factors contributing to infertility in men. Epididymitis is often the consequence of ascending bacterial infection into the urinary tract. Specific strains of Uropathogenic *E. coli* (UPEC) produce the toxin alpha-haemolysin (hlyA), which has been shown to manipulate host immune responses. The aim of this study was to elucidate consequences of hlyA production on innate immune responses in the UPEC induced murine experimental epididymitis model.

Methods & Materials To evaluate effects of hlyA on cytokine production in vitro, RAW 267.4 cells or single epididymides dissected from C57BL/6N mice were left untreated, treated with LPS (100 ng/mL) or treated with clinically relevant pyelone-

phritic α-haemolytic (UPEC CFT073) or the non-haemolytic *E. coli* strain NPEC 470. Cell supernatants were collected and cytokine production analyzed using the BD™ Cytometric Bead Array. Establishment of the murine experimental epididymitis model was achieved by injection of 40,000 UPEC CFT073 or NPEC 470 in total, into both left and right vas deferens of C57BL/6N mice. PBS injections were used as a sham control for in vivo experiments. Following 3 days infection, epididymides were dissected and snap frozen. qPCR analyses was performed to quantify pro-inflammatory gene expression. Immuno-fluorescent staining was used for the detection of macrophages (F4/80) and T-cells (CD3). Statistical analysis was performed by One-way ANOVA. $P \leq 0.05$ is considered statistically significant.

Results We show that α-haemolytic UPEC significantly reduced secretion of pro-inflammatory cytokines TNF-α, IL-6, IL-1 and IFN-γ in epididymal organ cultures and RAW 267.4 cells following infection. Conversely, in vivo qPCR analyses revealed expression of early response genes TNF-α and IL-6 were significantly increased by day 3 following UPEC infection. Interestingly, RANTES/CCL5 and IFN-γ gene expression were differentially modulated dependent on the hylA status of the *E. coli* strain. Our findings illustrate hlyA production significantly enhances RANTES expression following UPEC infection in contrast to PBS and NPEC infected mice. Conversely, IFN-γ gene expression in UPEC infected mice was comparable to PBS mice, whereas NPEC significantly increased IFN-γ expression. In addition, a decrease in the number of F4/80 and CD3+ cells within the epididymal interstitium of UPEC infected mice was observed in comparison to NPEC infected mice.

Conclusion Overall these finding suggest that hlyA produced by UPEC attenuates host innate immune responses in the initial phases of infection for successful colonisation within the epididymis. Furthermore, the down-regulation of IFN-γ attenuates maturation of adaptive immune responses evading elimination and contributing to persistence.

P7

Characteristics of *Staphylococcus aureus* and *Escherichia coli*, Collected from Infertile Men's Urethra

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Methods Clinical and microbiological examination of 77 men from infertile couples was implemented. It included bacteriological research of separable urethra.

Results From clinical point examined men had the following symptoms: frequent and painful urination (85%), nycturia (15%), stomachache (44%), perineal pain (32%), skin rash (18%), hyperaemia (98%), edema of urethra's mucous membrane (23%). Prostatitis and vesiculitis were revealed in 82% of cases as a result of ultrasonic diagnostics. Microbiological research showed 75,3% of gram-postive bacteria strains, 22,1% of gram-negative bacteria strains and 23,6% of *Candida* fungus. Gram-positive microorganisms included genus *Staphylococcus sp.* (75,9%), *Streptococcus sp.* (19%) and *Corynebacterium sp.* (5,1%). In 56,8% of cases staphylococci were coagulase-positive (76% St. aureus, 24% St. intermedius). Gram-negative microorganisms were represented with Enterobacteriaceae in 76,5% of cases and *Neisseria* in 23,5% of cases. *E. coli* were singled out in 76,9% of cases, Citrobacter diversus – in 7,7% of cases, *Edwardsiella ictaluri* – in 7,7% of cases, *Klebsiella sp.* – in 7,7% of cases. Monocultures were singled out in 42,2% of cases (50% *St. aureus*; 25% *St. haemolyticus*; 16,7% *St. intermedius*; 8,3% St. epidermidis). 66,7% of *St. aureus* were resistant to oxacillin, their quantity was more than 10^5 CFU/cottonwool tampon. These strains were resistant to at least 5 antibiotics in 75% of cases. *St. aureus* in quantity less than 10^4 CFU/cottonwool tampon were oxacillin-sensitive, resistance to at least 5 antibiotics was fixed in 50% of cases. *St. aureus* strains resistant to at least 3 antibiotics were singled out in 16,7% of cases. *E. coli* was founded in monoculture in 31,6% of cases (83,3% of them had haemolytic characteristics). At least 10^5 CFU/cottonwool tampon of *E. coli* were singled out in 66,7% of cases, haemolytic characteristics had 75% of them. 75% of at least 10^5 CFU/cottonwool tampon were resistant to not more than 3 antibiotics. No more than 10^4 CFU/cottonwool tampon of *E. coli*, in one half of cases were resistant to at least 5 antibiotics. Other half was resistant to no more than 3 antibiotics. In 9,1% of cases *E. coli* strains were picked out with *St. aureus*.

Conclusion Therefore our research shows that urethra of infertile man more frequently has staphylococci and enterobacteria – less frequently. However their presence influences greatly on the flow of inflammatory process.

P8

Clinical and Etiological Features of Nonspecific Male Urethritis

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Question Increase of unit weight of conditionally pathogenic microflora in etiological structure of infections inflammatory diseases of urogenital tract is noted nowadays. Aim of this research is to examine clinical and microbiological features of men with nonspecific urethra.

Methods 45 men with inflammatory urogenital diseases were inspected. Clinical and laboratory inspection included anamnesis collection and bacteriological study of separated urethra's part.

Results Clinically examined men had the following symptoms: frequent and painful urination, nycturia, perineal pain, skin rash, hyperaemia and edema of urethra's mucous membrane. Prostatitis and vesiculitis were revealed in 82% of cases as a result of ultrasonic diagnostics. During microbiological study of urethra's separated part 77 microorganisms strains were figured out. 75,3% of them were gram-positive bacteria strains, 22,1% of them were gram-negative bacteria strains, and 23,6 were *Candida* fungus. Gram-positive microorganisms included genus of *Staphylococcus sp.* (75,9%), *Streptococcus sp.* (19%) and *Corynebacterium sp.* (5,1%). In 57% of cases staphylococci were coagulase-positive. Coagulase-negative staphylococci were presented with *St. haemolytics*, *St. saprophyticus* and *St. epidermidis*. Among streptococci appeared *Str. viridans* in 45,4% of cases, *Str. mitis* – in 9,1% of cases, *Str. sanginis* – in 9,1% of cases. All figured out corynebacteria were presented with nonpathogenic species of natural normal flora. Gram-negative microorganisms were represented with Enterobateriaceae in 76,5% of cases, and *Neisseria* in 23,5% of cases. *Escherichia coli* were singled out in 76,9% of cases, *Citrobacter diversus* in 7,7% of cases. *Neisseria* was represented with *N. lactamia*, *N. sicca*, et al. All figured out yeast-like fungi were represented with *Candida albicans*.

Conclusion Therefore our research shows that urethra of men with nonspecific urethritis more frequently has staphylococci. Enterobacteriaceae and yeast-like fungi appear less frequently in the case of nonspecific urethritis. However their presence influences greatly on the flow of inflammatory process.

P9

Does HPV have an Impact on the Male Idiopathic Infertility?

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Introduction & Objectives In 25% infertile men this condition is considered as idiopathic. Role of HPV in men infertility has not been determined.

Material & Methods A series of 49 infertile patients, aged 25–40 years, without overt cause were reviewed. Evaluation of ejaculate was performed in according to WHO criteria (2010). For microbiological analysis urethral swab and ejaculate were used. Virus detection in ejaculate and urethral swab using analysis of amplified DNA sequences was performed. Considering a possibility of viral infection contamination during taking of urethral swab and ejaculate, a control group was assessed (n = 16). The urethral swab and ejaculate evaluation were made in the different days.

Results In all patients of both groups in ejaculate HPV was found. However, in urethral swab HPV was found in 79.5% and 68.7% patients in Group 1 and Group 2, re-

Table 4. K. Shiranov et al.

Incidence Viral type	Ejaculate	Urethra	p
6.11	32.6%	22.4%	< 0.05
16	79.5%	30.6%	< 0.05
18	71.4%	42.8%	< 0.05
31	12.2%	10.2%	> 0.05
33	57.1%	34.7%	< 0.05

Table 5. K. Shiranov et al.

Pathospermia	Viral type	Ejaculate	Urethra
Oligo-zoospermia	6.11	8.2%	8.2%
	16	10.2%	–
	18	12.2%	6.1%
	31	–	–
	33	6.1%	–
Astheno-zoospermia	6.11	24.4%	2%
	16	22.4%	16.3%
	18	26.6%	20.4%
	31	8.2%	10.2%
	33	20.4%	14.3%
Terato-zoospermia	6.11	–	–
	16	32.6%	14.3%
	18	20.4%	10.2%
	31	4.0%	–
	33	16.3%	20.4%
Oligoastheno-zoospermia	6.11	–	–
	16	14.3%	10.2%
	18	–	6.1%
	31	–	–
	33	14.3%	–

spectively **(Tab. 4)**. In Group 1, in ejaculate and urethral swab HPV types 16, 18 and 33 were predominated, while the less frequent was type 31. In Group 2, the most common viral type was 16. In Group 2, the only one viral type was seen in 16.3% cases, and in 83.7% cases viral associations was found, while in Group 2 only viral associations were seen.

Comparative analysis of pathospermia types and viral in ejaculate/urethra is shown in **Table 5**.

Conclusions In patients with idiopathic infertility in ejaculate HPV types 16, 18, 33 are often diagnosed. Incidence of viral in ejaculate and urethral swab is the highest in asthenozoospermia and teratozoospermia. Analysis of amplified DNA sequences results of urethral swab and ejaculate were similar in 20.5% men. Viruses are seen more frequently in ejaculate, than in urethral swab. Causative role of HPV in men with idiopathic infertility will be needed to determine.

P10

Uropathogenic *E. coli* inactivate host Survival AKT Signalling Pathways in Testicular Cells

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Uropathogenic *Escherichia coli* (UPEC) are a major cause of acute and chronic ascending bacterial genital tract infections in men which ultimately can result in impaired spermatogenesis. UPEC can modulate host survival pathways to attenuate host inflammatory responses and escape from host immune response. Here, we have shown that in isolated rat Sertoli cells (SC) and peritubular cells (PTC) the UPEC virulence factor alpha hemolysin dephosphorylates AKT, also know as Protein Kinase B (PKB), in order to inactivate it. AKT dephosphorylation leads to activation of several downstream targets by dephosphorylation, two of which are FOXO (Forkhead box class of transcription factors) and glycogen synthase kinase (GSK)-3β. GSK-3β is inactivated by Akt-mediated phosphorylation at serine 9 (S9). We have observed dephosphorylation of phosphorylated GSK-3β at S9 following treatment with UPEC in SC and PTC. In addition we have observed the activation of FOXO1 transcription factor by dephosphorylation at serine 256 in SC. Following dephosphorylation at serine 256, FOXO1 can translocate to the nucleus and execute diverse cellular function such as cell cycle arrest, apoptosis, reactive oxygen species detoxification and DNA repair. In SC and PTC, we have not observed any significant change in the expression of cyclin D1, a regulator of cell cycle progression. Similarly we have not observed any visible change in the expression of SOD (sodium dismutase, a detoxification enzyme of reactive oxygen species). However, we have observed increased expression of sirtuin 3/4 (histone deacetylase) by Western blot analysis. These results indicate that upon translocation to the nucleus FOXO1 may be deacetylated by sirtuins, consequently inhibit FOXO function. Taken together these results suggest that UPEC can evade host immune cell response by manipulating host surviving signalling pathway. Moreover suppression of AKT signalling pathway may lead to damage of Sertoli cells which could impair spermatogenesis and germ cell death.

P12

Lipopolysaccharide Inhibits Mitochondrial Membrane Potential in Human Spermatozoa through the Activation of Cannabinoid Receptor-1 by Sperm-Generated Anandamide

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Introduction Gram-negative bacteria, frequently involved in urogenital tract infections, release the endotoxin lipopolysaccharide (LPS), and its receptor, Toll-like receptor-4 (TLR4), has been recently identified in human spermatozoa. It has been recently reported that sperm exposure to LPS affects sperm motility and activates sperm apoptotic processes, although the underlying signal transduction remains to be clarified. In macrophages, LPS induces the generation of the endocannabinoid Anandamide (AEA) from its membrane phospholipid precursors, through the activity of the calcium-dependent NAPE-hydrolyzing phospholipase D (NAPE-PLD). In human spermatozoa, which exhibit a completely functional AEA-related endocannabinoid system (including NAPE-PLD), the activation of cannabinoid receptor-1 (CB1) inhibited sperm mitochondrial membrane potential (ΔΨm). In this study we tested the hypothesis of a contribution of CB1 activation by sperm-generated AEA in the adverse effects exerted by LPS on human spermatozoa.

Material & Methods Sperm motility was evaluated with CASA and spermatozoa exhibiting an average pathway velocity > 5 μm/sec were categorized by the software as motile sperms. Sperm mitochondrial membrane potential (ΔΨm) was assessed at flow cytometry with JC-1, which emits red or green fluorescence in the presence of high or low ΔΨm, respectively.

Results The exposure of motile sperm suspensions for 6 h to LPS (100 ng/ml) produced a significant decrement in sperm ΔΨm, as indicated by the lower percentage of spermatozoa emitting red JC-1 fluorescence with respect to untreated samples (43.1 ± 9.4% vs 65.0 ± 9.1%; p = 0.007); this effect was not associated to decreased sperm motility (motile sperms: 55.8 ± 18.9% vs 55.5 ± 21.3%). The LPS-induced inhibition of ΔΨm was prevented by the selective CB1 cannabinoid receptor antagonist, SR141716 (0.1 μM), which preserved high percentages of spermatozoa with red JC-1 fluorescence (60.6 ± 13.0%).

Conclusions These data suggest that the activation of CB1 by AEA generated by calcium-dependent NAPE-PLD activation could account for the adverse effect of LPS on sperm ΔΨm. However, it is not here confirmed that LPS affect sperm motility. Actually, ΔΨm depression cannot induce sperm immobilization in a standard medium containing glycolysable sugars, as glycolysis ac-

tively compensates for any lack of ATP production by mitochondria in maintaining sperm motility.

P13

The effects of Kerack used in Iran on Sperm Parameters and Testis Structure in Adult Mice

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Introduction Kerack is emerging illicit substance which its use is rising up in Iran. It has harmful effects on body organs. The aim of this study is to investigate the effects of Iranian Kerack on sperm parameters and testicular structure of mice.

Methods In this study, 25 male mice (Balb/C) were divided into 5 groups (control **[Fig. 10]**, sham and 3 experimental). Experimental groups of Kerack-dependent mice (received ascending dose of Kerack for 7 days twice daily) were divided into three categories, experimental I, II and III. Experimental I was given Kerack at a dose of 5 mg/kg, experimental II 35 mg/kg and experimental III 70 mg/kg, intraperitoneally twice a day for a period of 35 days **(Fig. 11)**. The sham group received normal saline and lemon juice (2.6 µl/ml) whilst the control group just received water and food. Mice were then scarified and sperm removed from cauda epididymis and analyzed for sperm count, motility, morphology (normal/abnormal) and viability. Testes were also removed, weighed and processed for light microscopic studies.

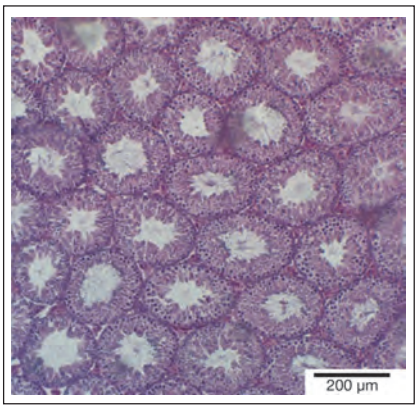

Figure 10. M. Amini et al. Cross Sections of normal Mouse Testes staining with H & E (Control Group).

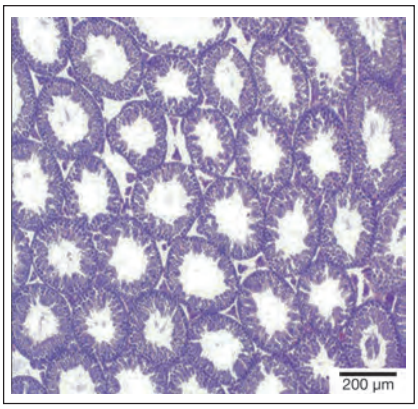

Figure 11. M. Amini et al. Cross sections of Mouse Testes staining with H & E treated with Kerack used in Iran at 70 mg/kg boy weight twice a day for 42 days (Experimental Group III).

Results The results showed that epididymal sperm parameters were significantly decreased in experimental groups (dose-dependent) compared with sham and control groups ($p \leq 0.01$). Gonadosomatic index and diameters of seminiferous tubules were significantly reduced with high dose Kerack (70 mg/kg) injected in comparison with control testes.

Conclusion It is concluded that Kerack used in Iran has the destructive effects on reproductive system in male mice.

P14

Involvement of Testosterone in the Regulation of Inflammatory Responses in Testicular and Immune Cells

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Introduction An increasing body of evidence indicates that beside their spermatogenic function androgens also play a role in the modulation of autoimmune disease and contribute to suppression of inflammatory/autoimmune response.

In our recent study, we have shown that substitution of reduced testosterone levels in a rat model of chronic testicular inflammation – experimental autoimmune orchitis (EAO) – has led to a significant amelioration of disease characteristics by inhibition of inflammatory responses in the testes. This was documented by a strong decrease of elevated macrophage and CD4+T cells numbers in the interstitial space concomitant with significant increase of "anti-inflammatory" regulatory T cells (CD4+CD25+Foxp3+) as well as significantly lower mRNA levels of TNF-α, IL-6 and MCP-1 as compared to EAO controls. Anti-inflammatory effects of testosterone supplementation during chronic testicular inflammation prompted us to investigate a putative direct influence of androgens on the generation of regulatory T cells and immune response in testicular macrophages (TM) and Sertoli cells (SC), both important contributors to immunological balance in the testis.

Material & Methods Leydig cells were isolated from adult rat testis by Percoll gradient and testosterone production was stimulated by addition of hCG. Collected conditioned media were used for cultivation of isolated splenic T cells. Differentiation of regulatory T cells was estimated by measurement of expression of Foxp3 transcription factor by FACS and secretion of IL-10 and TGF-β by ELISA. In another approach, isolated TM and SC were pre-incubated with different concentrations of testosterone before induction of inflammatory response by LPS. IL-6, IL-10, TNF-α and MCP-1 mRNA expression was investigated by quantitative real-time RT-PCR.

Results Testosterone produced by conditioned media from Leydig cells stimulated the expression of Foxp3 transcription factor in splenic CD4+ T cells as well secretion of IL-10 and TGF-β by these cells.

In TM inflammatory response induced by LPS was inhibited by pre-incubation with increasing concentrations of testosterone. Significant reduction of IL-6 and TNF-α mRNA levels were achieved in the presence of 1000 nM of testosterone. A similar effect was visible in SC for TNF-α mRNA. LPS-induced MCP-1 transcripts in TM were significantly downregulated after pre-incubation with 100 and 1000 nM of testosterone. The anti-inflammatory effect of testosterone in TM and SC was abolished by use of the androgen antagonist flutamide.

Conclusions In view of the high intratesticular testosterone levels under normal conditions and their decrease under inflammatory conditions, our data point to a role of androgens in the immune homeostasis of the testis. Androgen action could be mediated by direct effect on SC and TM as well as on thede novogeneration and functional differentiation of regulatory T cells.

P15

Screening Urin Analysis for Diagnosis of Prostatitis and Urogenital Tuberculosis

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Introduction Abnormal urin analysis can be seen in up to 90% of urogenital tuberculosis (UGTB). The diagnosis of prostatitis by 4-glass Meares-Stamey test leads to misdiagnosing of UGTB.

Material & Methods 177 patients were enrolled. Patients were randomized in 4 groups. 1st group (33 men) was examined by 4-glass test Meares-Stamey. 2nd group (87 men) was examined with 2-glass test: in 2-a (42 patients) urin analyses were performed before digital rectal examination (DRE); in 2-b (45 patients) DRE preceded urin analyses.

3rd group (57 patients) was examined with our technique. The urine was investigated in 3 consequent portions during urine voiding without interruption. DRE performed after urin analysis, expressed prostatic secretion (EPS) was investigated. Efficiency of tests was evaluated by comfortability and by percent of false-positive results (FPR).

Results Most comfortable test was in 2-b subgroup, and 4-glass test was worse of all. Comfortability of examination in both 2nd and 3rd groups were similar and significantly.

Conclusions Specificity and sensitivity of 3-glass test with followed DRE for diagnostic of chronic prostatitis is superior and does not allow overlooking UGTB.

P16

Clinical Features of Male Genital Tuberculosis in Siberia

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Introduction Extrapulmonary tuberculosis (EPTB), in spite of a few number of new-revealed patients, plays significant role in pathophysiology.

Material & Methods We retrospectively analyzed 131 history cases of Urogenital tuberculosis (UGTB patients), who were revealed in Novosibirsk in 2009–2011.

Results Among 131 patients in 88 (67.2%) the isolated kidney TB was diagnosed, in 33 (25.2%) – male genital TB; 10 (7.6%) men had generalized UGTB – combination of renal and genital TB, all of this ten had policavernous renal TB. Prostate TB was diagnosed in 19 patients, all were older 20 years: 21–39 yrs: 5 pts (21.0%), 40–59yrs: 8 pts (42.1%), ≥ 60 yrs: 7 pts (36.8%). Most common complaints were perineal pain – 6 pts (31.6%), dysuria (also 6 pts: 31.6%). Hemospermia was revealed in 5 pts (26.3%); erectile dysfunction – in 2 pts (10.5%). Prostate TB had latent asymptomatic course in 2 pts and was revealed pathomorphologically after prostate biopsy because of benign prostatic hyperplasia and high level of PSA (9.3 ng/ml). MBT in prostate secretion / ejaculate was found in 2 pts by PCR (10.5%).

TB orchiepidydymitis was diagnosed in 14 pts, all were older 20 yrs: 21–39yrs: 3 pts (21.4%), 40–59 yrs: 7 pts, ≥ 60 yrs: 4 pts (28.6%). The debut was with increasing of the testis size in 8 patients (57.1%), among them 5 had severe local pain (35.7%) and high body temperature. Hemospermia was in one man (7.1%), dysuria – in 5 patients (35.7%). Acute onset presented 5 patients. MBT was not found in this group.

Generalized urogenital tuberculosis (simultaneous lesion urinary tract and male genitals) was revealed in 10 patients, all were older 20 years again: 21–39 yrs: 2 pts, 40–59 yrs: 7 pts, and one was ≥ 60 yrs. Half of the patients complained of dysuria, 3 patients presented flank pain and 2 hematuria. One patient had hemospermia, another one perineal pain. In 2 cases toxicity was found. In 3 patients the debut of the disease was with acute orchiepidydymitis. Mycobacteriuria was found in 4 patients (40%) – both by culture and PCR.

Conclusion Most common form among UGTB is kidney TB (74.8%, including 7.6% with generalized UGTB). The onset of TB orchiepidydymitis was in 35.7% of patients, hemospermia was in 7.1%, dysuria – in 35.7%. Most common complaints for prostate TB were perineal pain (31.6%), dysuria (also 31.6%), hemospermia (26.3%). MBT in prostate secretion/ejaculate was revealed in this group in 10.5%.

UGTB has no specific symptom, even sterile pyuria meets only in 25% only. All urogenital tract infections should be suspected on UGTB in patients living in the region with high incidence rate, who had contact with TB infection, who has recurrent course of the disease, resistant to standard therapy.

P17

Efficiency of Uro-Vaxom in Prophylaxis of Relapse of Chronic Prostatitis

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Introduction Urogenital tract infections (UTIs) mostly are caused by *E. coli* and about 60% have recurrent course. One of optimal methods for prophylaxis of relapses is immunoprofilaxis with *Escherichia coli* extract Uro-Vaxom.

Material & Methods 127 pts with recurrent UTIs: 23 chronic bacterial prostatitis, 75 recurrent cystitis and 29 chronic pyelonephritis. Relapses of UTI: 3.4 ± 0.8 per year.

All were treated for 1 month with 1 capsule daily of *Escherichia coli* extract Uro-Vaxom. Just after one more UTI episode occurring, the second course of Uro-Vaxom was prescribed. Follow-up: 2 years.

Results After first course of Uro-Vaxom 86.7% had no relapse of UTI for 6 months. After second course 29.2% had a "cold period" for 6 months, 48.8% for 9 months and 22.0% had no recurrence during a year. The frequency of UTI episodes decreased from 3.4 ± 0.8 to 0.4 ± 0.2 per year, QoL increased from 4.7 ± 1.0 up to 1.3 ± 1.1 score.

Conclusion The number of recurrence of cystitis, prostatitis and pyelonephritis was significantly lower after 2 consecutive courses of Uro-Vaxom. Immunoprophylaxis of relapses of UTIs with Uro-Vaxom is high effective and good tolerant in patients with chronic prostatitis as well as for another Urogenital tract infections.

P18

Male Urethral Condylomata: Our Experience

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Aim To analyze clinical behaviour and treatment of men presenting urethral condylomata.

Method Clinics and treatment of men who consult for genital's area condylomata and also with urethral lesions. Treatment of choice in patients for visible meatal lesions was cryotherapy and gel instillation of 5% fluorouracil in affected urethral. Follow-up's frequency was every 7–15 days and urethroscopy controls 6 months after the patients is free of lesions. Urethroscopy before treatment was applied only in 4 patients with dysuria. Serology screening was performed to all, except three with known HIV+.

Results A total of 359 men consulting for genital condylomata, 45 (12.5%) presented urethral lesions. Four patients were loosed of follow-up. For 24 patients (58.5%) first consult was because the meatus exophytic lesion. 11 (26.8 %) were not aware of having urethral lesions, 4 (9.7%) consulted because of dysuria, 1(2.4%) because of urethrorrhagia, and 1(2.4%) for stinging upon voiding. The primary treatment performed was cryotherapy. Urethroscopy was performed in 4 patients previous treatment and warts verified beyond the navicular fossa. In those, treatment was 5% fluorouracil without having complications. All control urethroscopies post treatment were negatives.

Conclusions Meatal/urethral condylomata are infrequent and misleading condition in cases of genital warts. Nevertheless it is to be advice an active investigation to find them. Endoscopic manipulation is recommended only in cases of urinary symptoms. Cryotherapy is simple and easy in cases of accessible lesions. When urethral involvement, 5% fluorouracil gel instillation seem to be a good initial therapeutic option.

P19

Testicular Inflammation in Infertile Men

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Introduction Infections and inflammation of the genital tract are major causes of male infertility. Chronic, asymptomatic orchitis, however, is difficult to diagnose by means of clinical parameters. Notably, testicular biopsies of infertile patients frequently show impaired spermatogenesis associated with focal or diffuse interstitial inflammatory infiltrates. The infiltrates are dominated by T-lymphocytes, macrophages and mast cells. Our knowledge concerning specific subpopulations of testicular immune cells, e.g. T-helper cells and Th17-inducing antigen-presenting cells, is still rather limited.

Material & Methods In this project*, testicular inflammatory infiltrates were characterized immuno-histochemically in order to identify T-lymphocytes (CD3+/45R0+/CD45RA+), B-cells (CD20+/CD45RA+), macrophages (CD68+), dendritic cells (CD11+/S100+) and mast cells (tryptase+). In addition, the pattern and degree of spermatogenic impairment was analyzed in areas affected by inflammatory reactions compared to non-inflamed tissue. With regard to putative functions of participating immune cells, gene expression profiles of cytokines were determined using qualitative and quantitative RT-PCR after RNA extraction from de-paraffinized tissue sections and cDNA synthesis.

Results Testicular biopsies obtained from infertile men with focal or multi-focal inflammatory lesions revealed high numbers of T- and almost absence of B-lymphocytes, typically in peritubular and/or perivascular localization. Affected or adjacent seminifer-

ous tubules showed partial or complete loss of germinal epithelium, thickening of the lamina propria, or complete tubular fibrosis. Few dendritic cells could be identified in the proximity of the lymphocytic infiltrates, whereas the occurrence of macrophages and mast cells was not restricted to inflammatory areas. Analysis of gene expression profiles including both pro- and anti-inflammatory cytokines unraveled significant expression of TNF-α in inflammatory lesions compared to non-affected areas of the same testis. Similarly, expression of interleukin (IL)-1β, IL-6, IL-12 (p35), and interferon-γ could be detected.

Conclusion Pattern analysis of testicular inflammatory reactions seen in infertile men provides a perspective for "functional" assessment of the participating immune cells. Especially cytokine expression profiles could help to elucidate, whether lesions are of bacterial, viral, or autoimmune origin.

* Supported by LOEWE (excellence initiative of the state government of Hessen, Germany) focus group MIBIE (Male Infertility during Infection and Inflammation).

P166

Necrosis is the Dominant Cell Dead Pathway in UPEC Induced Epididymo-orchitis Model and is Responsible for Structural and Functional Damage of Rat Testis

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Introduction Bacterial infections of the male genital tract result from ascending canalicular infections of the male excurrent ducts and thus could cause male infertility. Uropathogenic *Escherichia coli* (UPEC) is a relevant pathogen in urogenital tract infection.

Method & Materials To explore how testicular defenses react against UPEC infection, epididymo-orchitis model was established by injecting UPEC CFT073 into vas deference in close proximity to the epididymis. Mimicking an infection ascending from the urinary tract, in the testis UPEC bacteria were found exclusively in the interstitial space 7 days post infection. Tracer experiments revealed that the integrity of the blood-testis and blood epididymis barrier was intact 7 days post-infection indicating that evasion of bacteria occurs probably intracellularly. In the testis, UPEC infection resulted in impairment of spermatogenesis by germ cell loss, damage of testicular somatic cells, a decrease in sperm numbers and a significant increase in TUNEL (+) cells. To investigate potential mechanism of germ cell death, hall mark steps of apoptosis were investigated. Activation of caspase-8 (extrinsic apoptotic pathway), caspase-3/-6 (intrinsic apoptotic pathway), caspase-1 (pyroptosis pathway) and the presence of 180 bp DNA fragments, all of which serve as indicators of the classical apoptotic pathway and pyroptosis, were not observed in infected testis. Notably, electron microscopical examination revealed degenerative features of Sertoli cells (SC) in UPEC infected testis. Furthermore, the passive release of high mobility group protein B1 (HMGB1), as an indication of the necrosis, was observed in infected testis.

Conclusion In summary, the findings indicate that UPEC infection causes the induction of an organized self-destruction cascade termed programmed necrosis in the testis, which may ultimately be the major mechanism contributing to impairment.

Postersession 2: Free Communications

P20

Eurycoma longifolia (Tongkat Ali) as a Potential Herbal Supplement for Physically Active Male and Female Seniors

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Tongkat Ali (*Eurycoma longifolia*; TA) is known to increase serum testosterone levels and alleviate aging males' symptoms. Its roots contain a wide variety of chemical compounds including eurycomaside, tannins, high molecular weight polysaccharides, glycoproteins, mucopolysaccharides and alkaloids of the quassinoid group. The androgenic effect of increased serum testosterone is increased muscle mass resulting in increased potential for generating greater force in the muscles, evidenced by enhanced strength. Therefore, this study aimed at investigating TA as an ergogenic supplement for elderly people.

13 male and 12 female physically active seniors (57–72 years) were supplemented with 400 mg TA extract daily for five consecutive weeks. Treatment resulted in reported adverse side-effects.

After the treatment, hemoglobin, testosterone and dihydroepiandrosterone concentration, as well as the ratio of total testosterone/cortisol and muscle force remained significantly lower in the females than in the males. Hematocrit (46.2% vs. 47.8%) and erythrocyte count (4.7 $\times 10^6$/mL vs. 4.9 $\times 10^6$/mL) in males increased slightly, but were significantly higher than in females. In both men and women, treatment resulted in significant increases in total (3.8 ng/mL vs 4.2 ng/mL; $p = 0.0090$; and 0.3 ng/mL vs 0.5 ng/mL; $p = 0.0098$, respectively) and free testosterone concentrations (5.2 pg/mL vs 8.4 pg/mL; $p = 0.0005$; and 0.5 pg/mL vs 1.1 pg/mL; $p = 0.0032$, respectively) and muscular force (46.0 kG vs 53.7 kG; $p = 0.0375$; and 29.6 kG vs 33.7 kG; $p = 0.0641$, respectively). Although significantly elevated after the treatment, the testosterone levels (total and free testosterone) in the female subjects were still well within normal physiological levels of 0.063–0.836 ng/ml and 1.0–8.5 pg/ml respectively. The increase in free testosterone in women is thought to be due to the significant (59.7 nmol/L vs 47.3 nmol/L; $p < 0.0001$) decline in sex hormone binding globulin concentrations.

The study affirms the ergogenic benefit of TA for seniors, through enhanced muscle strength.

P21

Eurycoma longifolia Extract at Therapeutical Concentrations does not Negatively Affect Human Sperm Functions in vitro

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Eurycoma longifolia (Tongkat Ali; TA) is a Malaysian shrub used to treat various illnesses including male infertility. Considering that TA is also used to improve male fertility and no report regarding its safety has been published, this study investigated the effects of a patented, aqueous TA extract on various sperm functions.

Semen samples of 27 patients and 13 fertile donors were divided into two groups, washed and swim-up prepared spermatozoa, and incubated with different concentrations of TA (1, 10, 20, 100, 2000 µg ml^{-1}) for 1 hr at 37°C. A sample without addition of TA served as control. After incubation with TA, the following parameters were evaluated: vitality (eosin test), total and progressive motility (CASA), acrosome reaction (triple stain technique), sperm production of reactive oxygen species (ROS; dihydroethidium test; DHE), and sperm DNA fragmentation (TUNEL assay).

For washed spermatozoa, significant dose-dependent trends were found for vitality, total motility, acrosome reaction and sperm ROS production. However, these trends were only significant if the highest concentrations were included in the calculation. For progressive motility of washed spermatozoa, a marked ($p = 0.0770$) drop could only be observed at the highest TA concentration. Contrary, the increase in the percentage of acrosome-reacted spermatozoa with increasing TA concentrations is highly significant ($p < 0.0001$), and a significant difference ($p = 0.0069$) to the control could even be recorded at 20 µg TA per ml. For swim-up spermatozoa, only ROS-positive swim-up spermatozoa showed a biphasic relationship with its lowest percentage at 10 µg ml^{-1} but yet no significance could be observed ($p = 0.9505$). For sperm DNA fragmentation, no influence of TA could be observed, nei-

ther for washed nor for swim-up prepared sperm.

Results indicate that the Tongkat Ali extract has no deleterious effects on sperm functions at therapeutically used concentrations (<2.5 µg ml^{-1}).

P22

Algorithm for the Surgical Management of Obstructive Azoospermia

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Question Evaluation of a protocol for the surgical management of obstructive azoospermia to help identify and resolve possible multiple site obstruction.

Methods 80 infertile males with obstructive azoospermia were enrolled in this study. Intra-operative vasography pointed the site (or sites) of obstruction. According to the algorithm, single or multiple-site obstruction were managed by various combinations of epididymovasostomy, vasovasostomy, transurethral resection of the ejaculatory ducts and pelvi-scrotal vasovasostomy.

Results 92.5% of cases (74 out of 80 patients) showed appearance of spermatozoa in semen following surgery. The average count was 14 million/ml and the average motility was 40%. Simultaneous multiple-site obstruction was detected and managed in 32 out of 80 patients (40%)

Conclusion The humble success rate for surgical repair of seminal tract obstruction may be attributed to missing or failure to manage multiple-site obstruction, which appears to be common. The proposed algorithm guides the surgeon to full restoration of patency of the seminal tract along its whole length based on proper diagnostic and corrective measures.

P23

Common and Hormone-Specific Inhibitory Effects of Sex Steroids on Testes Development in the Rat

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Introduction Testosterone and estradiol may positively affect different aspects of testis development, but inhibition of follicle stimulating hormone (FSH) secretion by these hormones has negative impact on the testis. In rats administration of testosterone propionate (TP) or estradiol benzoate (EB) since the day of birth inhibits FSH secretion and testis growth on postnatal day (pnd) 6. However, when TP was administered later during first spermatogonial development (between 5 and 15 pnds) neither secretion of FSH nor testes growth were affected. In turn, EB may inhibit testis growth with no influence on FSH in this period.

The aim of this study was to compare the influences of TP and EB on testes development and FSH level depending on whether sex hormones were administered transiently during the initial 5 pnds, or continuously until pnd 15.

Material & Methods Male pups were daily injected with either 12.5 µg of EB or 2.5 mg of TP transiently from the day of birth until pnd 5, followed by daily injections of solvent until pnd 15 (tEB and tTP, respectively). Other pups were treated continuously from the day of birth until pnd 15 (cEB and cTP, respectively). Control group received solvent. At autopsy, on pnd 16, blood was taken for hormone measurements and testis for investigations of seminiferous tubule morphometry, cell number, proliferation and apoptosis.

Results Testis weight, tubule length, Sertoli and germ cell numbers were reduced after all treatments together with increased seminiferous epithelium cell apoptosis. In addition, c-EB inhibited premeiotic germ cell development together with further augmentation of cell apoptosis despite increased FSH secretion and proliferation of spermatogonia. In turn, c-TP reduced meiotic spermatocyte number with no influence on FSH, but together with inhibition of seminiferous tubule lumen formation, indicative of Sertoli cell adluminal secretion.

Conclusion

1. First 5 pnds in rats, the period corresponding to spermatogenic onset, appear to be susceptible to the inhibitory effects of sex steroids on testis growth via an increase of seminiferous epithelium cell apoptosis.

2. Hormone-specific inhibitions occurred after exposure to sex steroids during spermatogonial development. Estradiol inhibits germ cell compartment via further augmentation of cell apoptosis, whereas testosterone reduced meiotic cell number via inhibition of Sertoli cell function.

P24

FGFR3 as a Potential Marker for Human Spermatogonial Stem Cells

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Introduction Human type A spermatogonia (SPG) contain a stem cell population (SSCs). Knowledge concerning their molecular identity and the regulatory mechanisms involved in the transition from quiescent to proliferative state is still limited. Using microarray analysis, we previously listed more than 200 potential candidates for human SPG-specific markers, i.e. the Fibroblast Growth Factor Receptor 3 (FGFR3). We and others have shown that the FGFR3 protein in humans is expressed on the cell surface of SPG located along the basal lamina of the seminiferous tubules. FGFR3 as a candidate human spermatogonial stem cell marker protein may facilitate isolation and enrichment of human stem and/or progenitor SPG and could enable clinical application of these cells in the future. This work was performed to characterize the FGFR3-positive SPG on the protein level and to test isolation possibilities.

Material & Methods 3 FGFR3 antibodies directed against different epitopes of FGFR3 were utilized for immunofluorescence multiple staining on cross sections and whole mount preparations of human seminiferous tubules to characterize FGFR3-positive SPG and to estimate the sizes of the FGFR3-positive spermatogonial cell clones. For the isolation of the FGFR3-positive SPG, Magnetic-Activated Cell Sorting (MACS) via an antibody against the extracellular epitope of the protein was employed.

Results Tested FGFR3 antibodies produced largely superimposed staining patterns within cytoplasmic vesicles and on the cell surface of SPG. Using the nucleolus marker C23 (Nucleolin) in a co-staining, FGFR3 was primarily detected in A type SPG comprising the classical described A dark and A pale spermatogonial subtypes. Additionally, FGFR3 was found as co-expressed with the pluripotency marker Undifferentiated embryonic cell Transcription Factor 1 (UTF1) in a subpopulation of the UTF1-positive SPG. Proliferating (KI-67+) and differentiating (DMRT1+) SPG were FGFR3-negative. Whole mount preparations revealed FGFR3 expression predominantly in pairs or quadruplets of spermatogonial cells. FGFR3-MACS selected cells could be positively stained for the UTF1 protein.

Conclusion FGFR3 expression in non-proliferating and non-differentiating type A SPG including the A dark subtype, co-expression with UTF1 and the organization of FGFR3-positive SPG in small cell clusters could be indicative for an "early state" of these SPG and hence their stem cell potential. Optimization of the isolation protocol could form the basis for cultivation, xenotransplantation and for prospective medical application of this cell type.

P25

Effects of Flutamide on the Sperm Membrane Integrity, Mitochondrial Diaphorase Activity, and Sperm Morphology in Adult Boars: in vivo and in vitro Approach

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Introduction Androgens are crucial for mammalian sperm differentiation however their role in biology of male gamete is not still defined. Recently, we have reported morpho-functional alterations in the boar epididymis

as a consequence of flutamide exposure [1]. The aim of the study was to test whether flutamide or its metabolite through the blockage of androgen receptor could influence reproductive capability of the boar sperm.

Material & Methods In vivo effects of flutamide (50 mg/kg b. w.) on sperm morphology were investigated by electron microscopic observations [2]. In vitro effects of 5, 50, 100 µg of OH-flutamide (2 h and 24 h incubation time) on sperm membrane integrity were measured by LIVE/DEAD Sperm Vitality kit, while those on sperm membrane stability and mitochondrial diaphorase activity were examined using Merocyanine540 and NADH tests, respectively [3].

Results The incidence of abnormal spermatozoa increased significantly in flutamide-treated boars compared to controls. In an in vitro approach, low dose of OH-flutamide for 2 h appeared less effective in altering the sperm membrane integrity and its stability than two higher doses used. No further decrease in sperm membrane integrity was found when the effect of anti-androgen lasted for 24 h. On the other hand, sperm membrane destabilization and mitochondrial diaphorase activity were strengthened after 24 h.

Conclusion The alterations in functional parameters of the sperm correlating with the sperm morphology provide novel data on the sperm sensitivity to anti-androgen action.

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P26

Prenatal and Neonatal Exposure to Flutamide Alters Androgen Receptor Expression in the Prostate of Adult Boars

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Introduction It is well established that androgens play a central role in normal prostatic growth and the maintenance of tissue homeostasis, but they are also responsible for development of prostate disease [1]. Complete interruption of androgen signalling in utero causes prostate abnormalities or agenesis [2]. However, effects of reduced availability of androgen receptor during different stages of reproductive system development on prostatic cells function in adult males are not fully characterized. Thus, in the present study we examined prostate morphology and androgen receptor (AR) expression in adult boars exposed prenatally or neonatally to an antiandrogen flutamide.

Material & Methods Flutamide was administered to pregnant gilts at 50 mg/kg/day during the period of sex differentiation: gestational days 20–28 (GD20) or 80–88 (GD80) and to male offspring from postnatal day 2–10 (PD2) and from day 90–98 (PD90). Prostates were collected from 270 day-old animals. Routine histology was performed using hematoxylin-eosin staining. The expression and localization of AR were analyzed using qRT-PCR, Western blotting and immunohistochemistry, respectively.

Results Morphological examination demonstrated that flutamide exposure at GD20 and PD2 resulted in decreased prostatic alveoli size and stromal volume, as well as reduction of secretions in the lumen. Alveoli were often lined with cuboidal rather than columnar epithelium.

In the prostates of flutamide-exposed boars, expression of AR was decreased at mRNA and protein level when compared to the control group. Decrease was most significant in GD20 and PD2 groups. Immunostaining for AR was localized to both epithelial and stromal cells of control and flutamide-exposed pigs. In the epithelial compartment, the nuclei of secretory cells were strongly stained, whereas the nuclei of basal cells exhibited weaker staining. In the stroma, immunostaining of variable intensity was detected. The number of AR positive stromal cells was reduced following flutamide treatment, especially in GD20 and PD2 pigs.

Conclusion Our findings indicate that high level of androgen action and availability of androgen receptor during early gestational and neonatal periods is essential for the maintenance of normal prostate phenotype in adult boars. Moreover, decreased AR expression in the stromal compartment suggest that flutamide-induced alterations in adult prostate may result directly from disruption of androgen-dependent functions of stromal cells.

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P27

Effects of a Murine Germ Cell-Specific Knockout of Connexin 43 on Connexin Expression in Testis and Fertility

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Introduction Connexins (Cx) are gap junctional proteins facilitating direct intercellular communication. In rodent testis, Sertoli cells (SC) and germ cells (GC) synthesize amongst others Cx43 connecting by the way SC with SC and SC with different generations of adjacent GC. The SC-specific knockout (KO) of Cx43 (= SCCx43KO) was shown to prevent initiation of spermatogenesis, and male mice were infertile. In the present transgenic mouse model, we intend to investigate functions of Cx43 especially in GC using the Cre/loxP-recombination system by elucidating the consequences of its KO on testicular Cx26, 33, 43 and 45 expression and fertility.

Material & Methods Establishing a conditional GCCx43KO mouse model: Transgenic mice containing the Cx43 gene surrounded by loxP sites were mated with transgenic mice expressing the Cre gene under the control of the GC-specific Tissue Non-Specific Alkaline Phosphatase (TNAP) promoter. In ovaries and testes, the Cx43 gene was specifically deleted in GC. Genotypes were determined by Cre- and TNAP-PCR, homozygous GCCx43KO mice were mated with wildtype (WT) and homozygous GCCx43KO mice to test their fertility. Immunohistochemical analysis, RT-qPCR and Western blots of different aged KO and WT mouse testes homogenates were performed to investigate the distribution of Cx mRNA and protein.

Results A total of three male homozygous and 3 male WT animals could be evaluated, aged 19 days (pubertal), 131 days (adult) and 232 days (adult). Three female GCCx43KO$^{-/-}$ were only used for fertility survey. The adult male and all female KO mice have been mated and proved their fertility. Litter sizes were normal. Immunohistochemical analysis of testes of different aged homozygous mice revealed normal spermatogenesis and a reduced Cx43 immunoreaction. RT-qPCR and Western blots showed a downregulation of Cx43 mRNA and protein in KO mutants, a nearly unchanged expression of Cx26, Cx33 and Cx45 mRNA in pubertal and adult KO mice but an increase of Cx45 protein in adult GCCx43KO$^{-/-}$ mice.

Conclusion Our data suggest that persistent spermatogenesis and fertility in GCCx43KO$^{-/-}$ mice does not rely on GC-specific Cx43 expression. A selective loss of Cx43 in GC may be compensated by the persistence of other tubular Cx like Cx26 or Cx45 and a possible interaction between (1) Cx45 in both GC and SC, (2) Cx45 in GC and Cx43 in SC or (3) Cx26 expressed both in GC and SC. The precise cellular localization of these Cx as well as the demonstration of functional gap junctional intercellular communication between GC and SC remains to be investigated.

P28

The Effect of Treatment with Antioxidants/Vitamins/Aminoacids on Semen Quality in Idiopathic Infertile Males

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Question Is the treatment with antioxidants/vitamins/aminoacids able to improve semen quality in idiopathic infertile males; and which treatment regimen is the most effective?

Methods 109 males from infertile couples with decreased semen quality (oligo- and/or astheno- and/or teratozoospermia) with excluded evident causes for infertility (normal testicular volume, normal FSH and testosterone levels, no varicocele, excluded urogenital infections) were included into the study. They were randomly assigned into 2 treatment groups: Group I (n = 52) received commonly used vitamin/antioxidant combination with a daily dose of 1000 mg vitamin C, 800 mg vitamin E, 200 µg selenium and 1000 mg L-arginine for 2 months. Group II (n = 57) received more complex treatment including a daily dose of 1000 mg vitamin C, 800 mg vitamin E, 200 µg selenium, 3.5 mg vitamin B_6, 9 µg vitamin B_{12}, 800 µg folic acid, 40 mg zinc, 1,000 µg copper, 1000 mg L-arginine, 80 mg N-acetylcysteine, 440 mg L-carnitine, 170 mg omega-3-fatty acids, and 15 mg coenzyme Q10 for 2 months. Semen analyses were performed according to WHO guidelines (1999) before and after 2 months treatment by the same technician. Sperm concentration, progressive motility and normal morphology before and after the treatment were compared using the univariate linear regression analysis, adjusted for the abstinence time.

Results In Group I mean numbers (± SD) of sperm concentration ($\times 10^6$/ml), progressive motility (%) and normal morphology (%) before and after the treatment were 54.6 (± 31), 46.9 (± 14), 6.7 (± 6), and 73.1 (± 55), 49.1 (± 16), 7.4 (± 6), respectively, reaching statistically significant improvement only in sperm concentration ($p = 0.04$). In Group II all parameters showed significant improvement after the treatment: sperm concentration from 40.7 (± 29) to 66 (± 56), $p = 0.003$; motility from 41.8 (± 18) to 48.6 (± 18), $p = 0.048$; normal morphology from 3.9 (± 3) to 6.8 (± 4), $p < 0.01$.

Conclusion Our study shows that only complex treatment with the variety of vitamins, antioxidants, trace elements, aminoacids and other micronutrients that are essential for the spermatogenesis can provide improvement in semen parameters in idiopathic male infertility. Extended placebo controlled studies are necessary to confirm these results.

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P29

Androgen Signalling Disruption during Prenatal and Neonatal Period affects Leydig cell Function in Adult Boar

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Introduction Fetal and neonatal periods are crucial for fertility in adult life, as reproductive system development and programming of the hypothalamus-pituitary-testicular axis occur during these periods [1]. It has been reported that fetal hormonal disruption induced by anti-androgens gives rise to reproductive function impairment in adulthood [2]. However, the precise mechanisms of these alterations are still unclear.

In order to determine the role of androgen receptor signalling during fetal and neonatal period in porcine Leydig cell function, we focused on steroid hormones production and expression of aromatase and luteinizing hormone receptor (LHR) in testes of flutamide-exposed pigs. In addition, we measured plasma concentrations of gonadotropins in adult boars.

Material & Methods Flutamide, an androgen receptor antagonist, was administered (50 mg/kg/day) into pregnant gilts during gestational days 20–28 (GD20) and 80–88 (GD80), and into male piglets on postnatal days 2–10 (PD2). Flutamide- and vehicle-treated (control) boars were maintained under identical conditions until 270 days of age when they were slaughtered. Testes and blood samples were collected and used for qRT-PCR, Western blotting, immunohistochemical and radioimmunological analyses.

Results In flutamide-exposed boars, especially those of PD2 males, testosterone concentrations were reduced significantly in comparison to those of control animals. Reduced testosterone production in response to flutamide exposure appeared to be related to changes in testosterone metabolism, as demonstrated by increased aromatase mRNA, protein expression and elevated estradiol concentrations. Moreover, impaired Leydig cell responsiveness to luteinizing hormone (LH) was indicated by the decreased expression of LHR. No significant effect of flutamide was found on LH and follicle-stimulating hormone (FSH) concentrations.

Conclusion Taken together, our data indicate that flutamide when administered during prenatal or neonatal period have long-term effect on Leydig cell function, leading to androgen-estrogen imbalance. Leydig cell failure was most evident in adult boars neonatally exposed to flutamide suggesting that androgen action during neonatal development is of pivotal importance for the differentiation and function of porcine adult Leydig cell population.

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P30

Aromatase, Androgen and Estrogen Receptors in Bank Vole Spermatozoa

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Introduction Spermatoza represent a highly specialized cell type that transport a single-copy haploid genome to the site of fertilisation. In order to condense DNA, many of the proteins of the conventional somatic chromatin are removed. Changes in the sperm membrane and cytoplasm take place during transit through epididymis and female genital tract by the modifications in the composition and cellular localization of proteins [1]. However, the function of most of the proteins that are present in mature spermatozoa is not fully understood.

It is widely accepted that sex hormones play a crucial role in proper germ cell differentiation although implication of androgen/estrogen signalling in the biology of mature male gametes is still to be defined. Till now, only the presence of estrogen receptor β (ERβ) has been demonstrated in elongating spermatids and spermatozoa of mice and rats [2, 3].

In light of these data, it seems interesting to investigate whether androgen receptor (AR), estrogen receptor α (ERα) and ERβ, as well as aromatase, are present in spermatozoa of seasonally breeding rodent, the bank vole.

Materials & Methods Spermatozoa were isolated as described in detail [4]. Immuno fluorescent assay and Western blot were performed using primary antibodies against: (i) human AR (Santa Cruz Biotechnology, USA); (ii) human placental P450 aromatase (a gift from Dr. Y. Osawa, Hauptman-Woodward Medical Research Institute, USA); (iii) human ERα (Dako, Denmark); (iv) human ERβ (Serotec, UK). Immunohistochemical results were analyzed quantitatively [5].

Results Our study provides evidence that aromatase, AR, as well as the ERα and ERβ, are present in bank vole spermatozoa. We demonstrated the region-specific localization of these proteins using confocal microscopy. Immunoreactive aromatase was observed in the proximal head region and in both the proximal and distal tail regions, whereas steroid hormone receptors were found only in the proximal region of the sperm head. Western blot analysis confirmed the presence of these proteins in sperm lysates.

Conclusion In the present study we demonstrated for the first time that bank vole spermatozoa are both a source of estrogens and a target for steroid hormone action. Moreover, the presence of aromatase and steroid hormone receptors in the bank vole spermatozoa clearly indicate a potential role of these proteins during capacitation and/or the acrosome reaction.

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P31

Aromatase and Estrogen Receptors Expression at Complete Spermatogenesis and in Sertoli cell only Syndrome

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Estrogens are produced locally within testes in high amounts with an involvement of an enzyme aromatase (CYP19), that converts androgen into estrogen. Estrogens signalling in the cell is mediated by estrogen receptors α and β (ERα and ERβ). Wide distribution of CYP19 and ERs immunohistochemical staining within human testes has been reported. Uncertain knowledge concerns Sertoli cell only syndrome (SCO), where unchanged ERα immunostaining has been reported, not confirmed by Western-blot analysis.

The aim of this study is to investigate the expression of CYP19 and ERs in human testicular biopsies revealing complete spermatogenesis (CS) and SCO.

Material & Methods The archival human testicular biopsies taken from men diagnosed because of infertility were investigated. Cases with CS (n = 21) and SCO (n = 30) were selected. Immunohistochemistry against CYP19 and ERα, ERβ were performed to visualize the site of antigen in testis. The expression of CYP19 and ERs genes were performed from the same archival samples using real-time quantitative PCR. Relative copy number values (number of copies of CYP19 or ERs mRNA per 1,000 copies of GAPDH mRNA) were calculated.

Results In CS cell nuclei immunohistochemical localisation of CYP19, ERα and ERβ were found in spermatocytes, round spermatids, Sertoli and Leydig cells, whereas spermatogonia revealed only ERβ (lack of CYP19 and ERα). Cytoplasmic localisation of ERα and ERβ was present in both Sertoli and Leydig cells, but only Leydig cells revealed expression of CYP19.

In SCO Sertoli cell nuclei the labeling of CYP19 and week staining of ERβ were present, whereas cytoplasm was reach of ERα. Leydig cell number was increased in SCO in association with pronounced expression of CYP19 and ERα in both nuclei and cytoplasm.

Using real-time quantitative PCR, the expression of CYP19 did not differ between groups, but amounts of ERα and ERβ was significantly higher in CS compared to SCO **(Fig. 12)**. Moreover a significant negative correlation between the amount of CYP19 and ERβ was observed in SCO (–0.38).

Conclusion Immunocytochemistry revealed wide distribution of CYP19 among Sertoli and Leydig cells, as well as in germ cells except for spermatogonia.

Equal quantities of CYP19 in CS and SCO may be due to increased Leydig cell number in SCO.

Spermatocytes and round spermatids are reach of ERs and hence reduced expression of ERs in SCO might be due to lack of germ cells.

Spermatogonia express ERβ exclusively what suggest that ERβ-depended pathway plays critical role in the regulation of premeiotic steps of spermatogenesis by estrogen.

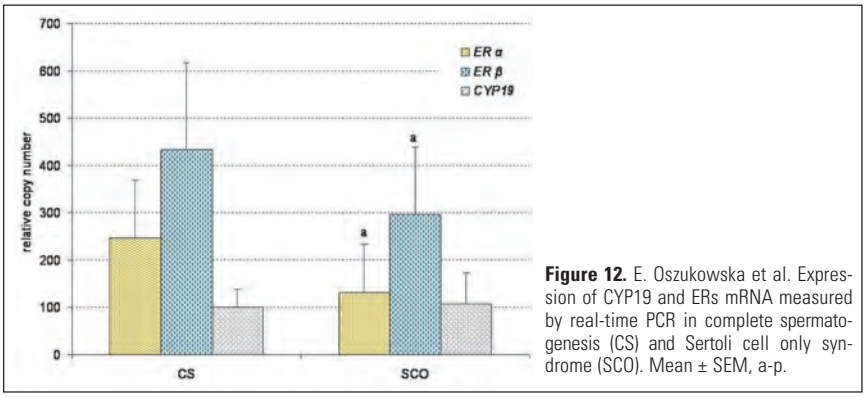

Figure 12. E. Oszukowska et al. Expression of CYP19 and ERs mRNA measured by real-time PCR in complete spermatogenesis (CS) and Sertoli cell only syndrome (SCO). Mean ± SEM, a-p.

P32

Transient Pubertal Administration of FSH Together with Triiodothyronine Produces Quantitative Reduction of Spermatogenesis and Endocrine Function of the Testes in Adulthood

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Introduction Follicle-stimulating hormone (FSH) and triiodothyronine (T3) are known regulatory factors of testicular development. We have recently shown that administration of FSH together with T3 evoked regressive changes in the seminiferous epithelium in pubertal rats.

Question The aim of the study was to evaluate the effects of pubertal administration of FSH and T3 on spermatogenesis and endocrine function of the testes 35 days later in adult rats.

Methods Newborn, male Wistar rats were divided randomly into experimental groups receiving daily subcutaneous injections with: FSH 7.5 IU/animal (FSH group), or 100 mg T3/kg body weight (T3 group), or both substances (FSH+T3 group), or vehicles in the same volume (control group-C). A part of animals in each group was eutanised in the postnatal day (pnd) 16 of life (progression of first meiosis), while the rest were eutanised in the pnd 50 (appearance of first spermatozoa). Each of 8 groups contained 10–17 males. Testes were removed and immunohistochemical reactions were performed using monoclonal antibody against Vimentin, used as a Sertoli cell marker. The ratio of Sertoli cells (vimentine positive) number to germ cells (vimentine negative) number in 20 subsequent seminiferous tubules cross-sections was and used to estimate the quantitative efficiency of spermatogenesis.

Results On pnd 16, total Sertoli cell number was increased to 156% of C after FSH given alone and was reduced to 66% of C after T3 alone. On pnd 50 Sertoli cell number changed to 128% and 63% of C after FSH and T3 respectively. Treatment with FSH+T3 did not change Sertoli cell number in pubertal animals, but increased it to 238% of C in adult rats.

On pnd 16 serum level of FSH and T3 increased in animals receiving the hormones. In adult animals significant changes in hormonal pattern were observed only after FSH+T3: blood level of FSH increased to 18.1 ± 5.9 ng/ml vs 8 ± 6.4 in C and testosterone declined to 1.5 ± 1 vs 3 ± 1.9 nmol/l in C, suggesting impairment of gonadal function. Hormone levels were not affected after separate treatments in adult rats.

An increase of the efficiency of spermatogenesis was observed only in pubertal rats treated with T3 alone (1:0.88 vs 1:0.67 in C). Qualitatively spermatogenesis advance expressed as a presence of spermatozoa, was the same in all adult animals. However, after FSH+T3 a significant reduction in the number of germ cells per Sertoli cell was present in both young (1:0.32 vs 1:0.67 in C) and adult animals (1:2.7 vs 1:4.5 in C). No difference was observed in other groups.

Conclusion Combined administration of FSH and T3 during prepuberty decreased ratio: Sertoli/germ cells, what was maintained until adulthood and produced impaired endocrine function of the testes.

P33

Three Dimensional Culture of Rat Testicular Cells in Collagen Sponges

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Introduction Spermatogenic differentiation requires support of somatic cell types, presence of extracellular matrix components, endocrine signalling, as well as a defined spatial arrangement of the different cell types. Previous studies on in vitro differentiation of male murine germ cells in three dimensional culture approaches attempted to resemble these requirements and could demonstrate differentiation of germ cells into morphologically mature spermatozoa. Here, the colonization of testicular cells of rats in a three dimensional artificial collagen sponge scaffold was analyzed focusing on survival and reassembly of testicular cells in vitro.

Methods Testicular cells obtained from Sprague Dawley and eGFP (enhanced green fluorescent protein) transgenic rats (10 dpp) were isolated enzymatically and the single cell suspension was transferred on collagen sponges (Matricel, 5 × 1.5 mm). Colonization was observed using the life cell imaging system (Pecon, Zeiss). 1.5 $\times 10^6$ cells per 40 µl were added with a drop-on seeding method to the scaffolds and the effect of gonadotropines (hCG and r-hFSH per 5 IU/l) was tested. The sponges were cultured in DMEM high Glucose + Glutamax (Gibco-Invitrogen) at 35°C and 5% CO_2 in air. Colonized scaffolds were analyzed after defined periods of culture by scanning electron microscopy (SEM), conventional histology and immunohistochemistry. Additionally, testosterone and cAMP levels were determined in the supernatants to check the functions of Sertoli- and Leydig cells.

Results The drop-on seeding method enabled a subsequent colonization of testicular cells across the entire scaffold. After two days of culture reaggregation in terms of cluster formation was observed. Size and surface structure of cells indicate aggregation of different cell types. These small clusters indicate first signs of initiating tubulogenesis and were attached to the collagen fibres. They were found to survive for at least nine weeks in vitro. Immunohistochemical stainings indicate that those clusters contain somatic and germ cells (germ cell marker: VASA, spermatogonial marker: Lin28, peritubular cell marker: αSMA, Leydig cell marker: 3βHSD). Measurements of cAMP and testosterone indicate vital Sertoli cells and steroidogenically active Leydig cells responding to gonadotropin stimulation.

Conclusions Testicular cells from rats obtained at 10 dpp are able to colonize artificial collagen scaffolds and can be cultured for several weeks, during which they reassemble. In further experiments we aim at systematic optimization of the seeding concentrations and culture conditions and detailed analysis of the reorganized cellular structures.

P34

Reduced Numbers of Sertoli, Germ, and Spermatogonial Stem Cells in Impaired Spermatogenesis

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Introduction Investigation of male infertility relies on the appropriate classification of impaired spermatogenesis. Besides mitosis, meiosis, and differentiation of germ cells also the functions of Sertoli cells are essential for male reproduction. In this study, we precisely quantified Sertoli and distinct germ cell types in azoospermic patients with defined spermatogenic defects. Our study provides a new method of categorizing spermatogenic defects.

Materials & Methods Testicular biopsies (n = 110) were obtained from normogonadotropic obstructive or non-obstructive azoospermia and showed histologically normal spermatogenesis (n = 33), hypospermatogenesis (n = 44) and maturation arrest at the level of spermatocytes (n = 33). To precisely identify the cell types, we detected the following marker proteins by specific antibodies: androgen receptor for Sertoli cells, UTF1 (undifferentiated embryonic cell transcription factor) for a subset of spermatogonia including stem cells, Smad3 for spermatocytes, histone H3 phosphorylated at serine 10 for meiotic divisions and CREM (cAMP response element modulator) for round spermatids. At least 10 cross-sections per patient were analyzed. Patients were classified into three groups: mainly meiotic deficiencies (reduced numbers of spermatocytes, meiotic divisions, and spermatids), mainly „founder pool"-related deficiencies (reduced numbers of Sertoli cells, spermatogonia, and stem cells) or both.

Results Remarkably, the numbers of Sertoli cells, spermatogonia and a subset of spermatogonia including stem cells are significantly reduced in patients with spermatocytic maturation arrest, however, the strongest reduction was found in spermatid numbers. Patients with hypospermatogenesis showed a significant reduction of spermatogonia but only modestly reduced numbers of spermatocytes and spermatids. No correlation was found with age or obstruction. Interestingly, patients with maturation arrest showed meiotic deficiencies (36%), while the majority exhibited deficiencies in the founder pool (58%). In contrast, patients with normal spermatogenesis most often had no deficiencies at all (45%) or founder pool-related deficiencies (33%) but an apparently normal meiosis.

Conclusions In conclusion, by antibody-based quantification of Sertoli and germ cells, we found that patients with spermatocytic maturation arrest showed besides mitotic and meiotic defects also additionally a significant reduction in Sertoli, spermatogonial, and stem cell numbers. Thus, analysis of meiotic defects should consider also deficiencies in the founder pool. Our findings pave the way to novel routes of investigation into the role of Sertoli cells and spermatogonial stem cells in male infertility.

P35

Sdf-1 Mediated Response of Germ Cell Loss in Male Mouse Testis After Cytotoxic Treatment

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Introduction The chemokine Sdf-1 (Stromal cell-derived factor-1α) and its receptor Cxcr4 (CXC chemokine receptor 4) are constitutively expressed by most organs. Furthermore, it is known that an up-regulation of Sdf-1 expression occurs after injury and stem cell loss. This up-regulation of Sdf-1 facilitates the recruitment of Cxcr4+ adult stem cells to the injured site and thereby aids organ regeneration. Apart from that, it has been shown, that the Sdf-1/Cxcr4 interaction is required for the colonization of the gonads by primordial germ cells in the mouse fetus. In the adult mouse testis Sdf-1 has been shown to be expressed in the somatic Sertoli and Leydig cells whereas Cxcr4 is expressed in germ cells. However, it is hitherto unknown whether an up-regulation of Sdf-1 can be detected after the loss of germ cells which would indicate a role of Sdf-1 and Cxcr4 in the regeneration process. The aim of this study was therefore to investigate the expression patterns of Sdf-1 and Cxcr4 following germ cell loss in adult mouse testes.

Material & Methods Adult NMRI mice were either given a single injection (i. p.) of busulfan (38 mg/kg) or dimethylsulfoxid (DMSO, control treatment). On days 1, 3, 7, 21 and 28 after treatment, 10 animals per time point and per treatment were sacrificed. Determination of testis weights was the first endpoint measurement. Furthermore, real-time PCR was used to perform a relative quantification of Sdf-1 and Cxcr4 in the testicular tissues. In addition, the expression of the germ cell marker genes Ddx4 and Lin28a and the somatic marker gene Erm was determined.

Results Testis weight decreased following busulfan treatment (average weight: 1d [93 mg], 3d [85 mg], 7d [90 mg], 21d [62 mg] and 28d [42 mg]). In addition, qPCR analyses revealed decreasing transcript levels of the germ cell marker genes Lin28a & Ddx4 (2-fold). Interestingly, while constant expression levels were detected for the somatic marker gene Erm, the expression levels of the chemokine Sdf-1 and its receptor Cxcr4 increased significantly (4- and 1-fold) within 28 days after treatment. DMSO treatment showed no effect on transcript levels of all investigated marker genes.

Conclusion In agreement with previous reports, our results show that busulfan treatment leads to a reduced testis weight in adult mice which is due to the loss of germ cells.

Apart from that, we could show for the first time that the transcript levels of Sdf-1 and Cxcr4 increased significantly following testicular injury and germ cell loss whereas the expression of other somatic marker genes remained constant. The next step will be to localize these proteins in the testes using immunohistochemistry. In summary, these data indicate that the Sdf-1/Cxcr4 interaction contribute to the recovery of spermatogenesis after cytotoxic treatment.

P36

Human Sertoli cells Produce High Amounts of Acetate that is Under Strict Hormonal Control

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Introduction Several important functions for a successful spermatogenesis are dependent on Sertoli cells (SCs). Besides their unique characteristics as support cells, they produce essential cofactors and metabolites, and are responsible for nurturing the developing germ cells. The continuous production of lipids, phospholipids and proteins by germ cells must require high amounts of metabolic precursors. Thus, we hypothesized that hSCs could produce acetate in a hormonally-regulated manner.

Material & Methods hSC-enriched primary cultures were maintained in the absence of insulin or in the presence of 17β-estradiol (E2) or 5α-dihydrotestosterone (DHT). Acetate production was determined by 1H-NMR. mRNA gene expression levels of Acetyl CoA hydrolase (ACoA Hyd) and Acetyl CoA synthase (ACoA Synt) were determined by RT-PCR.

Results hSCs produced high amounts of acetate suggesting that this compound should play a key role on the progression of spermatogenesis, namely as a metabolic precursor for the synthesis of cellular constituents. In addition, acetate metabolism proved to be under strict hormonal regulation. In the presence of E2 or DHT, hSCs produced different amounts of acetate. While E2 treatment increased acetate production, increasing ACoA Hyd gene transcript levels, DHT-treated cells showed decreased acetate production, differently modulating the ratio ACoA Hyd/ACoA Synt. Surprisingly, insulin-deprivation completely suppressed acetate production/export and significantly decreased the ACoA Hyd gene transcript levels.

Conclusions Taken together, these results suggest that, although hSCs are primarily described as lactate producers, the elevated production of acetate deserves special attention, in order to clarify the mechanisms behind its hormonal regulation and its role on a successful spermatogenesis.

P37

Visualization of Sildenafil Effects in Testis

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Introduction In the human testis, myofibroblasts are the main cellular components of the lamina propria of seminiferous tubules. These cells are crucial for the transport of immature sperm towards the epididymis. In many cases of disturbed spermatogenesis, the peritubular lamina propria is considerably thickened, with an increase of extracellular components, resembling fibrotic changes in other organs. The second messenger cGMP (cyclic guanosine monophosphate) contributes to the regulation of contractile cell function in the testis. Sildenafil (Viagra®), an inhibitor of cGMP-hydrolyzing PDE5, is used in an increasing number of young patients to treat pulmonary hypertension. However, there is only scarce knowledge on PDEs (phosphodiesterases) in the testis and on possible effects of PDE inhibition in male reproductive organs.

Material & Methods The expression pattern of PDEs within the lamina propria was characterized using laser capture microdissection (LCM) followed by RT-PCR analyses and immunohistochemistry. Sildenafil effects were analyzed by collagen gel assays in combination with video microscopy and in a rat model after long-term treatment with sildenafil.

Relative amounts of PDE isoforms in human isolated peritubular cells were investigated by Real Time PCR.

Results PDE1A, PDE1B, PDE3B, PDE5A, PDE9A and PDE10A isoforms were found in the regular and thickened (fibrotic) lamina propria. Use of additional primer pairs for smooth muscle cells (α smooth muscle actin), Sertoli cells (anti-Müller hormone) and germ cells (CatSperI) unequivocally showed PDE5 expression also in germ cells. The dual substrate PDEs PDE2A, PDE3A and PDE11A were only detected in intratubular cells of seminiferous tubules, but were absent from the isolated lamina propria. Different to the PDEs mentioned above PDE1C was lacking in thickened lamina propria, but could be found in the regular LP.

Involvement of PDE5A, the target of sildenafil, in contractility of seminiferous tubules was revealed by newly developed collagen gel-based assays in combination with video microscopy both in rat and men. Sildenafil induces a decrease contractile frequency.

Investigation of testis section from rats after long-term treatment with sildenafil provided no evidence for a pathological accumulation of sperm in the lumen of seminiferous tubules and in the rete testis.

Conclusion Different PDEs including PDE5 are expressed in regular and fibrotic lamina propria of human testis.

The PDE5 inhibitor sildenafil induces acute effects on contractility of seminiferous tubules. After long-term treatment with sildenafil no indication for pathological sperm retention was detected.

P38

Visualization of Sildenafil Effects in the Epididymis

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Introduction The transport of immotile and immature sperm from the testis towards the epididymis requires a well orchestrated action of smooth muscle cells of the epididymal duct. The drug sildenafil (Viagra®) elicits smooth muscle cell relaxation by inhibiting PDE5 which hydrolyses the intracellular second messenger cGMP (cyclic guanosine monophosphate). Since the drugs' therapeutic use extends from the treatment of erectile dysfunction to pulmonary hypertension, more young patients are exposed sildenafil. Therefore, it is of particular interest to investigate possible effects or side effects of sildenafil on epididymal smooth muscle function.

Materials & Methods Human and rat epididymal tissue (including a model of long-term sildenafil exposure) was investigated by Western blot, immunohistochemistry and RT-PCR of laser-microdissected tissue samples, protein preparations served to assess PDE activity. Rat epididymal duct segments were used to assess contractile function using time lapse videomicroscopy and organ bath experiments.

Results PDE5, the target of sildenafil, is expressed in human and rat epididymis and localizes to the smooth muscle cell layer of the epididymal duct in the caput, corpus and cauda region and vascular smooth muscle cells. RT-PCR of laser-microdissected epididymal smooth muscle or epithelial cells confirmed the exclusive localization of PDE5 to the smooth muscle layer of the epididymal duct.

As demonstrated in a PDE activity assay, sildenafil interferes with the hydrolysis of cGMP in a dose-dependent manner.

Organ bath studies with contraction force recordings reveal spontaneous and regular contractions of mid-cauda segments of the epididymis. Sildenafil decreases the contractile frequency similarly to ANP (atrial natriuretic peptide) or SNP (sodium nitroprusside) which are known to increase intracellular cGMP levels.

Time lapse video microscopy shows the same contractility pattern in the more proximal epididymal duct segments which are not suitable for organ bath studies.

In a rat model of long-term exposure to sildenafil, PDE5 expression remained unaltered and acute effects of sildenafil in organ bath studies were conserved.

Conclusion Visualization by video microscopy is a promising tool in investigating the contractile function of the epididymal duct.

PDE5, the target of sildenafil, is exclusively localized to contractile cells within the epididymal duct and sildenafil decreases epididymal contractile frequency. Long-term exposure to sildenafil neither changed PDE5 expression nor contractility of the epididymal duct and acute sildenafil effects were conserved.

P39

Spermatogonial Stem Cells in Marmoset Monkeys: Isolation and Characterization in vitro

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Introduction The marmoset monkey is widely used as a non-human primate model in reproductive medicine. During its testicular development, germline cells differentiate from primordial germ cells via gonocytes to spermatogonial stem cells (SSCs). In the newborn marmoset testis, gonocytes, which express pluripotency markers at the protein level, are still present. In contrast to that, SSCs are the most undifferentiated germ cells in the adult marmoset testis and it has been shown that these cells do no longer express pluripotency markers. Thus we hypothesize that pluripotent stem cells can be derived from newborn, but not from adult marmoset testes. In this study we aimed to establish germ cell cultures from newborn and adult marmoset testes and to determine their differentiation status in vitro.

Materials & Methods Testes obtained from newborn (n = 2) and adult (n = 3) marmosets were enzymatically digested and plated onto culture dishes. After 6 and 11 days the attached cells (AT) and the cells from the supernatant (SN) were separately analyzed by quantitative real time PCR (qPCR) analyses using marker genes for pluripotency (LIN28, OCT3/4), germ cells (VASA) and somatic cells (VIM, αSMA). In addition, cultures were fixed after 6 and 11 days and analyzed using immunohistochemical stainings. Both methods were used to detect the proportion of different cell types in the SN and the AT fraction of the cell cultures after 6 and 11 days.

Results After 6 days of culture, some cells were attached to the culture plastic, whereas about 40% (newborn)/90% (adult) of testicular cells remained in the SN. Interestingly, from 6–11 days the number of cells in the SN increased from 120,000–275,000 in the newborn cultures, whereas it decreased from 3.1–1.8 million in the adult cultures. Preliminary qPCR analyses show an increased expression of the pluripotency marker gene LIN28 and the germ cell marker gene VASA in the SN compared to the AT fraction of newborn and adult cultures. In contrast to that, an increased expression of the somatic marker genes VIM and αSMA was detected in the AT compared to the SN cell fraction (newborn + adult, each n = 1). These results indicate that germ cells remain in the SN, whereas somatic cells mainly attach to the culture plastic. Furthermore, our results suggest that the proportion of germline stem cells within the germ cell fraction is higher in the newborn, than in the adult cultures, but further analyses will have to confirm this at the RNA and the protein level.

Conclusion We showed that germ cells from adult and newborn marmosets are enriched in the SN. However, it is obvious that the putative germ cells from newborn marmosets exhibit a significantly higher proliferative activity, which is probably due to their more undifferentiated status compared to adult germ cells. In further experiments we will aim to improve the culture conditions for the maintenance of germline stem cells.

P40

Morphological, Immunohistochemical and Histochemical Study of Rat Prostate under Immunosuppression

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Introduction The usage of immunosuppressants in patients following the organ transplantation increases survival rate. The treatment with these agents reduces the risk of rejection of transplanted organ. On the other hand such an approach is related to numerous adverse effects. Immunosuppressants may lead to generation of reactive oxygen species (ROS). The consequence is oxidative stress that can cause either apoptosis or necrosis in the cells. The previous studies describe the effects of particular immunosuppressive agents to transplanted tissues and organs. It is also well known that when used in combined therapy they can change their metabolism to each other. The aim of the study is to evaluate the effect of immunosuppressants on morphology, cytoskeleton proteins and intensity of apoptosis in cells of three lobes of rat prostate.

Material & Methods 48 adult, not operated Wistar rats were divided into seven experimental groups and control group. In experimental groups the animals were treated with immunosuppressants including rapamycin, mycophenolate mofetil, cyclosporine, tacrolimus and encorton in different protocols for 6 months. Afterword the rats were killed and three lobes of prostate glands (dorsal, lateral and ventral) were obtained. The examination under light microscopy (H&E, PAS) and immunochemical reaction of cytokeratin, desmin and vimentin were carried out. TUNEL was applied to detect apoptosis.

Results The experiment showed the effects of combined immunosuppression in number of protocols on rat prostate cells. Immunosuppressive treatment caused different degree of glandular epithelium hyperplasia, varying changes in expression of cytoskeleton proteins and intensity of apoptosis depending on protocol of treatment.

Conclusions Different immunosuppressants induced focal epithelial hyperplasia of different degree.

In groups were TUNEL positive cells were numerous the hyperplasia was less prominent.

Different immunoreactivity and immunolocalization of proteins were determined depending on particular protocols.

P41

Hormonal Regulation of Na+-H+ Exchanger 3 (NHE-3) in Sertoli cells: A Possible Role for Estrogens in Spermatogenesis

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Introduction The secretion of the seminiferous tubular fluid (STF), as well as the control of the pH of this fluid is crucial for male fertility. Sertoli cells (SCs) express various types of ion membrane transporters that are directly involved on the movement of basic and acidic particles across the membrane. Among them is Na^+-H^+ Exchanger 3 (NHE3), which belongs to the NHE family, one of the most relevant epithelial ion transporter families, that catalyzes the electroneural transport of extracellular Na+ for intracellular H+. The NHE family plays a major role in intracellular pH regulation, transcellular absorption of Na^+, cell volume and possibly in cell proliferation.

It has been reported that 17β-estradiol (E2) can regulate the expression of NHE-3 and the rate of Na^+ transport in the male reproductive tract. Recently, we identified several membrane H^+ transporters in SCs, among which NHE3. Although NHE3-knockout mice are infertile, the role of NHE3 in providing the milieu for spermatozoa development remains unclear.

Material & Methods Primary rat Sertoli cell cultures were maintained in the absence or in the presence of 17β-estradiol (E2). The mRNA and protein expression levels of NHE3 were determined by qPCR and Western-blot, respectively.

Results In our study, we identified the expression of NHE3 mRNA and protein in cultured rat SCs. Quantification of the expression of NHE3 mRNA in SCs of the different experimental groups was performed. Assessment of the alteration of the NHE3 protein levels in SCs from the different experimental groups was also done. The possible role of E2 in NHE3 gene and protein expression will be disclosed.

Conclusion As far as we know, this will be the first report with the regulation of NHE3 by E2. Deepening the knowledge on the mechanisms involved in the secretion, composition and regulation of STF is essential and will be a major step in understanding the possible

mechanisms of infertility associated with some hyperestrogenic pathological conditions.

05

Results of a Prospective Analysis Indicating an Autoantibody Prevalence in Patients with Peyronie's Disease

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Purpose The pathogenesis of Peyronie's disease is still not resolved. An autoimmunological background is still under debate. We have performed an initial prospective analysis for the prevalence of typical autoantibodies in patients with Peyronie's disease.

Methods 50 patients with Peyronie's disease (mean age: 54.1 y) were analyzed for human antinuclear antibodies (ANA), anti-ENA Pool, anti-dsDNA antibodies, cANCA, pANCA, β2-glycoproteine antibodies (IgM and IgG), anti-cardiolipine antibodies (IgM and IgG) and anti-glomerular basement membrane (GMB) antibodies. ANA's were analyzed by immunofluorescence and ELISA technique in serum.

Results 5/50 patients with Peyronie's disease (10%) were positive for anti-β2-glycoproteine antibodies (IgM). In contrast, the prevalence of IgM anti-phospholipidantibodies in 206 healthy blood donors was reported to be 1%. Other autoantibodies were not detectable in any patient with Peyronie's disease.

Conclusion This study describes a significant association (p = 0.0037) of Peyronie's disease with anti-β2-glycoprotein IgM antibodies compared to a "normal" human population. Normally, IgM anti-β2-glycoprotein is an indicator for increased risk for thrombovascular complications on an autoimmunological basis. Our preliminary findings may implicate a possible autoimmune background in about 10% of patients with Peyronie's disease, which may contribute to the postoperative results.

P11

From Priapus to Priapism

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Introduction & Objectives According to Greek mythology, Priapus was a rustic fertility god, protector of livestock, fruit plants, gardens and male genitalia. Priapus was noteworthy for his oversized, permanent erection, which gave rise to the medical term priapism. Priapism nowadays is a well know treatable clinical entity which has been thoroughly examined and documented with over 2000 published articles covering this condition. The objective of this study is to examine the root between Priapus and priapism.

Materials & Methods A detailed search on the history of Priapus and priapism was undertaken on PubMed, Medline and Google.

Results Priapus was described as the son of one of the phallic gods, either Hermes or Dionysos depending on the source. His mother was Aphrodite. According to the legend Hera was informed that Aphrodite was unfaithful to Dionysus with Adonis. Dissatisfied with her conduct, she caused her to give birth to a child of extreme ugliness and large genitals. Other sources believe that Hera's action was due to her jealousy towards Aphrodite, for Paris of having judged Aphrodite more beautiful than Hera. According to myth, Priapus later on permanent erection was a result of his attempt to rape the nymph Lotis. He pursued Lotis until the gods took pity on her and turned her into a lotus plant and gave him a permanent erection.

Historically, the clinical condition of an unwilling permanent erection had been described as a pathological condition initially through ancient Greek doctors Galen and Soranus of Ephesus and later on from doctors of the Byzantine Empire Oribasius and Sicamus Aetius who eventually named this condition priapism. In there writings they distinguish hypersexuality from priapismus and tried several therapeutic modalities of that age. The contemporary literature of priapismus begines in the twentieth century with the article of Frank Hinman "Priapism: Report of cases and a clinical study of the literature with reference to its pathogenesis and surgical treatment" in which the treatment described was still inadequate. After the second half of the 20th century the condition was further understood and better treated due to the increasing knowledge of the anatomical structures of the penis and physiology of the erection. Nevertheless, the pathophysiological differentiation of high and low flow priapism was not developed until 1983.

Conclusions The clinical entity of priapism has been named after a God of Greek mythology Priapus and his permanent erection. Although priapism has been a known and documented for over 2000 years, only the last 30 years this condition has been deeply understood and adequately treated.

Postersession 3: Sperm Quality and Selection for ART

P42

Sperm DNA Integrity Defect of Infertile Male Patients at IIUM Fertility Centre

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Introduction Male factor is responsible for approximately 30% of the infertility cases. Sperm quality shown to decline worldwide including in Malaysia for almost all the infertility cases related to male factor. Recently, reports indicate [http://eca2012.abstract-management.de/index.phpe] that sperm DNA fragmentation process is one of the major factor contribute to the reduce of sperm quality. Most of the studies have shown that sperm DNA fragmentation are higher among oligozoospermia compared to normozoospermia patients. Although conventional semen analysis could determine the quality of the sperm, these parameters are unable to reveal the sperm DNA defects. The aim of this study is to investigate the sperm DNA fragmentation among infertile normozoospermia and oligozoospermia male patients. This study will also evaluate the relationship between the sperm DNA integrity and the sperm parameters.

Materials & Methods A cohort study was conducted on 41 infertile male patients (23 normozoospermia and 18 oligozoospermia) who seek fertility treatment at IIUM Fertility Centre from October 2011 until March 2012. Conventional semen analysis was performed on these patients according to World Health Organization (WHO), 1999 to determine the sperm parameters. Then, followed by the Acridine orange test (AOT) which was done on the same sample in order to analyze the sperm DNA integrity.

Results The DNA fragmentation Index (DFI) above 30% were 13 (56.5%) and 10 (62.5%) for normozoospermia and oligozoospermia patients respectively (p = 0.5). The mean DFI for oligozoospermia was higher; 50.8 ± 34.1% compared to normozoospermia which was 37.9 ± 27.3%. However, there were no significant differences in DNA fragmentation between both infertile groups. The DFI of the normozoospermia men (n = 23) was ranged from 0.9–85.3%. Smoking habit may effect the DNA fragmentation which contribute 30.4% (n = 7/23) in the normozoospermia and 55.6% (n = 10/18) in the oligozoospermia patients. In the sperm parameters, DFI above 30% were higher in the low motility group; 81.2% (n = 13/16) compared to normal motility groups which had 48% (n = 12/25). There were significant differences between both of the groups (p = 0.034).

Conclusions Our results have shown that DNA fragmentation not only effected the oligozoospermia but also for normozoospermia male patients. As for the sperm parameters, the DNA integrity defects have impaired the motility of the sperm.

P43

Body Mass Index has no Impact on Sperm Quality but on Reproductive Hormones

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Question Does body mass index (BMI) impact on sperm quality and reproductive hormones? To date, results on this issue are par-

Table 6. A. Aziz et al. Descriptive Statistics

n	16	1082	821	191	p
BMI	< 18.50	18.5–24.9	25–29.9	> 30	
Mean pathological spermatozoa morphology (%)	63.63	62.30	63.33	63.64	0.488
Mean sperm concentration (%)	30.70	63.09	66.39	62.06	0.122
Mean spermatozoa motility (%)	20.94	24.59	24.76	24.53	0.359
FSH	4.78	5.51	5.19	5.20	0.310
LH	3.62	3.92	3.63	3.43	0.006
T	5.43	5.48	4.61	3.87	< 0.001
PRL	13.20	12.79	11.61	12.66	0.135

Table 7. A. Aziz et al. Multivariable Analysis of Sperm Quality Regarding BMI (normal: 18.50–24.99 vs pathologic > 24.99)

Sperm quality	BMI	Median	n	p
Mean morphological pathologic spermatozoa in %	Normal	62.22	1098	
	Pathologic	63.39	1012	0.131
	Overall	62.78	2110	
Mean sperm concentration in %	Normal	62.63	1098	
	Pathologic	65.58	1012	0.293
	Overall	64.04	2110	
Mean spermatozoa motility in %	Normal	24.54	1098	
	Pathologic	24.72	1012	0.631
	Overall	24.63	2110	

tially contradictory and deserve further evaluation.

Methods Semen samples and serum levels of FSH, LH, T, and PRL of a total of 2110 men attending our andrology unit from 1994–2010 due to infertility work-up were analyzed. Patients were stratified according to their BMI in 4 groups. Main outcome measures were sperm motility, morphology and concentration and serum levels FSH, LH, T and PRL.

Results No statistically significant difference was found for sperm quality and BMI between patients categorized according the 4 BMI levels. T ($p < 0.001$) and LH ($p = 0.006$) significantly differed between the 4 groups. In multivariable analysis, BMI did not have significantly independent influence on all assessed sperm quality parameters, whereas BMI significantly influenced hormone values for LH ($p = 0.001$), T ($p = <0.001$), and PRL ($p = 0.044$).

Conclusions BMI had no significant impact on sperm quality parameters. However, serum levels of LH, T, and PRL were significantly influenced by BMI **(Tab. 6, 7)**.

P44

Zech-Selector: The Common Method for Assisted Reproduction Technology?

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Background In this study the Zech-selector, a new sperm preparation method that was published to completely eliminate spermatozoa with DNA-strand breaks was compared with a double density-gradient centrifugation and a direct swim-up method without centrifugation. DNA-fragmentation, motility and morphology of sperm were analyzed. It is a fact, that spermatozoa with DNA-damage have a bad impact on the whole assisted reproduction technique, thus several studies analyzed the effect of gradient centrifugation and swim-up techniques on the elimination of DNA-strand breaks, but their results are controversial.

Methods Semen samples from different men with low and high DNA-fragmentation (< 5 up to 40 %) rates and a minimum volume of 3 ml were analyzed. Before and after semen preparation DNA-fragmentation rates were analyzed with the HaloSperm Kit. Motility and morphology were analyzed manually using the WHO criteria 2010.

Results The results of the Zech-selector were convincing and will be discussed.

Conclusion According to these data, the Zech-selector could become the common method for assisted reproductive medicine.

P45

Nemaspermic DNA Fragmentation in Non-leukocytospermic Ejaculates is Related with the Formation of 8-Hydroxy-2'-deoxyguanosine and it seems to be Related to an Endogenous Sperm Defect

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Introduction Activated seminal macrophages (MΦ) are present in non-leukocytospermic subfertile ejaculates [Pelliccione et al. Int J Androl, 2009] and are associated with altered sperm features and sperm DNA damage [Pelliccione et al. Fertil Steril, 2011]. Oxidative stress is often involved in the aetiology of sperm DNA damage and it may result from reactive species of oxygen released by mononuclear cells (MNCs) such as MÖ, or the result of aberrant metabolic processes during the early phases of germinal development. 8-hydroxy-2'-deoxyguanosine (8-OHdG) is a sensitive biomarker for oxidative stress-related damage of sperm DNA. Here we analyzed by flow cytometry, the presence of 8-OHdG and of DNA fragmentation in human sperm and its association with immunophenotipic characterization of non-germinal MNCs and standard seminal parameters in non-leukocytospermic sub fertile ejaculates (WBCs < 1×10^6/ml).

Materials & Methods 60 non-leukocytospermic ejaculates from subfertile couples were first evaluated for the routine semen analysis. Flow cytometry in spermatozoa was used to detect DNA fragmentation assessed by TUNEL assay and 8-OHdG using a monoclonal antibody anti-8-OHdG. Non germinal MNCs where evaluated by flow cytometry, using monoclonal antibodies anti-CD45, a general marker of all leukocytes, anti-CD14 and anti HLA-DR as markers respectively of non-activated and activated MΦ.

Results Percentages of sperm with DNA fragmentation and with 8-OHdG were higher in oligozoospermic ejaculates (< 39×10^6 total sperm count) and the 2 parameters were significantly correlated ($r = 0.36$; $p = 0.005$). Percentage of sperm with DNA fragmentation was negatively correlated with progressive motility of spermatozoa ($r = –0.40$; $p = 0.001$). No correlation was found between the percentage of sperm with 8-OHdG and the number of CD45+, of CD14+ and of HLA-DR+ MNCs.

Conclusions The correlation between the presence of 8-OHdG and DNA fragmentation suggests an oxidative origin of sperm DNA damage. The lack of correlation between the occurrence of sperm 8-OHdG and the number of ejaculated MΦ suggests that the oxidative damage of sperm DNA is related to and endogenous sperm defect in non-leukocytospermic subfertile ejaculates. The determination of the oxidative adducts

of nemaspermic DNA offers a new tool in the evaluation of male subfertility and can be used to select and monitor subjects to antioxidant therapy before PMA procedures.

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P46

Intra-Acrosomal Protein Indicates Disturbed Acrosome Activity and Correlates with IgA Load on Human Spermatozoa

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Introduction Disturbed acrosome reaction is known to be a frequent cause of male fertility disorders and is usually surveyed by gelatinolysis and acrosin activity. Yet these tests cannot disclose why capacitation ability is reduced. Besides low vitality and already undergone acrosome reaction disturbance of acrosome integrity can be considered a cause. Thus the aim of this study was to show that a disturbed acrosome structure of different origin is related to low gelatinolysis ability.

Material & Methods In sperm samples of 30 patients percentage of disturbed acrosome integrity was detected by flow cytometry using the antibody IAP/FITC against intra-acrosomal protein (IAP) which can be detected after chemically induced acrosome reaction as well as in damaged spermatozoa. Furthermore acrosin activity was screened using the gelatinolysis assay and spermiograms were made according to WHO guidelines. Percentage of IAP was then compared with outcome of gelatinolysis, vitality, motility, morphology and signs of inflammation.

Results We could show that a disturbed acrosome integrity is significantly correlated with reduced gelatinolysis as well as with low vitality and motility. Furthermore we demonstrated that there is no relation to morphological alteration such as acrosome disturbance or elongation of spermatozoa. Most interestingly, percentage of detected IAP correlates with load of IgA on spermatozoa in mixed antiglobulin reaction test (MAR) and thus with auto-immunological events. Yet it could not be linked to signs of acute inflammation such as IL-6 and peroxidase in seminal plasma.

Conclusion When given high percentage of detected IAP in sperm samples a reduced acrosin activity can be assumed. Disturbance of acrosome integrity reflected by IAP cannot be detected using the light microscope and is likely not due to abnormal development of spermatozoa but a result of biochemical processes afterwards presumably undergone acrosome reaction, apoptosis and auto-immunological events namely IgA load. Albeit it is clear that acrosome reaction itself changes the acrosome structure it is likely that alteration of acrosome as detected by IAP/FITC can induce early acrosome reaction vice versa or inhibit it.

Table 8. N. Gatimel et al. Vacuole criteria under high Magnification with Nomarki contrast before and after Freezing-thawing.

	Before freezing	After freezing/thawing	p
Total vacuole area (µm²)	0.75 ± 0.20	0.73 ± 0.20	n.s.
Anterior vacuole area (µm²)	0.24 ± 0.13	0.21 ± 0.13	n.s.
Median vacuole area (µm²)	0.48 ± 0.15	0.49 ± 0.14	n.s.
Basal vacuole area (µm²)	0.04 ± 0.02	0.04 ± 0.03	n.s.
Relative vacuole area (RVA) %	6.2 ± 1.8	6.1 ± 1.7	n.s.
% of sperm with RVA ≤ 6,5 %	63.5 ± 16.1	63.4 ± 13.5	n.s.
% of sperm with RVA > 13 %	9.2 ± 7.2	8.8 ± 6.8	n.s.

P47

Sperm Vacuoles are not Modified by Freezing-Thawing Procedures

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Introduction Since the development of a new concept called Motile Sperm Organellar Morphology Examination (MSOME) in 2001 for observing the cephalic vacuoles under high magnification (×6000), no study to date has assessed the effect of cryopreservation on these vacuoles. Despite the success of sperm cryopreservation in assisted reproductive technology (ART), the process of freezing and thawing is undoubtedly associated with impaired sperm quality. Evaluation of the vacuoles under high magnification before and after freezing/thawing could allow us to determine whether it is possible to use for IMSI with frozen-thawed sperm the same criteria as those established from fresh semen.

Material & Methods Conventional semen analysis (motility, vitality, morphology) and assessment of cephalic vacuoles at high magnification using a digital imaging software (Leica Application Suite v 3.6) allowing accurate and objective measurements of vacuoles area were performed for 27 fertile men before and after sperm freezing-thawing. The main outcome measures are relative vacuole area, vacuole area (in µm²) in the different positions (anterior, median and posterior), and the percentage of sperm with a relative vacuole area less than 6.5% and more than 13.5%.

Results While we find a significant decrease after freezing-thawing in motility (49 ± 15 to 25 ± 12 %; $p < 0.0001$), vitality (77 ± 9 to 46 ± 9 %; $p < 0.0001$) and normal forms (17.8 ± 6.5 to 14.9 ± 7 %; $p < 0.05$), there is no difference in the different vacuole criteria assessed at high magnification with Nomarsky contrast **(Tab. 8)**.

Conclusion This study demonstrate that freeze-thawing procedures has no effect on human sperm vacuoles.

P48

Epididymis Response Partly Compensates for Spermatozoa Oxidative Defects in snGPx4 and GPx5 Double Mutant Mice

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Introduction Oxidative stress has been shown in male infertility to promote loss of sperm functions. Oxidative stress occurs when reactive oxygen species (ROS) generation is not sufficiently controlled by antioxidant players such as for example the glutathione peroxidase enzymes (GPx). To investigate the role of the sperm nucleus GPx4 (snGPx4) and GPx5 in epididymal sperm maturation, we generated double knockout sngpx4;gpx5$^{-/-}$ mice (DKO).

Material & Methods Sperm DNA analysis was performed by staining, immunofluorescence and flow cytometry. Expression of ROS-recycling enzymes and disulfide bridging enzymes was quantified by qRT-PCR. Antioxidant activity was measured by enzymatic assay.

Results Spermatozoa of sngpx4;gpx5$^{-/-}$ mice display sperm nucleus structural abnormalities including delayed and defective nuclear compaction associated with DNA damage. We showed that to counteract GPx activity losses, the epididymis of the DKO animals mounted an antioxydant response resulting in a strong increase in its global H_2O_2-scavenger activity especially in the cauda territory. Quantitative RT-PCR data show that together with the up-regulation of epididymal scavengers (of the thioredoxin/peroxiredoxin system as well as glutathione-S-transferases) the epididymis of double mutant animals increased the expression of several disulfide isomerases in an attempt to recover normal disulfide bridging activity. Despite these compensatory mechanisms, cauda-stored spermatozoa of DKO animals show high levels of DNA oxidation, increased fragmentation and greater susceptibility to nuclear decondensation. Nevertheless, the enzymatic epididymal salvage response is sufficient to maintain full fertility of DKO

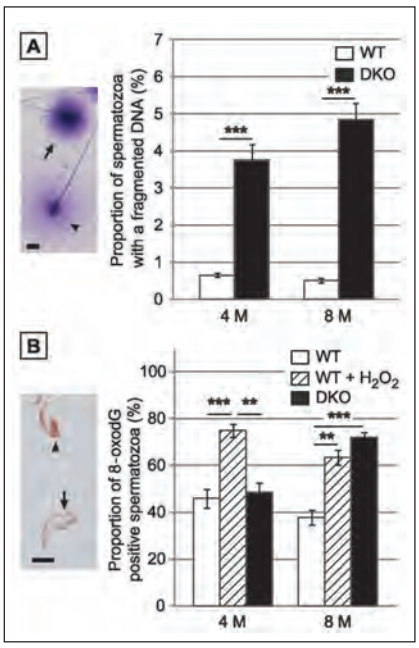

Figure 13. A. Noblanc et al. Cauda-retrieved spermatozoa of DKO animals suffer oxidative damage. **a: Left panel:** Typical picture of fragmented (arrowhead) or non-fragmented (arrow) sperm nucleus as shown by the modified Sperm Chromatin Dispersion Assay. **Right panel:** Histograms show the proportion of WT and DKO cauda collected spermatozoa with a fragmented DNA. **b: Left panel:** Typical immunodetection of the nuclear adduct 8-oxodG in cauda epididymidis-retrieved spermatozoa preparations from DKO male mice. **Right panel:** Histograms show the percentage of 8-oxodG positive spermatozoa in cauda epididymidis-retrieved spermatozoa preparations, respectively from WT, positive control (WT spermatozoa treated with H_2O_2) and DKO male mice. (Mean ± SEM; n = 5; *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$). Scale bar = 5 µm.

males whatever their age, when crossed with young WT female mice.

Conclusion Our data emphasize the ability of the epididymis to counteract excessive ROS and that normal sperm nucleus condensation should not be considered as an absolute indicator of full nuclear integrity **(Fig. 13)**.

P49

Seminal Plasma microRNAs are Differentially Expressed in Normozoospermia, Oligozoospermia and Azoospermia

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Introduction microRNAs are small, non-coding RNAs of endogenous origin, which function intracellularly as translational repressors of gene expression. Furthermore, microRNAs can be detected in many extracellular fluids and they can be applied as biomarkers for several diseases including tumors, sepsis or pre-eclampsia. The expression of microRNAs in seminal fluid was recently described in several forensical approaches covering the discrimination of different fluidal traces. However, regarding male infertility data about the relevance of microRNAs in seminal fluid and sperm cells are rare.

Material & Methods microRNA expression was studied using MicroRNA Arrays facilitating the Northern Blot principle (Human MicroRNA Array I–IV, Signosis Inc., Carlsbad, USA). 352 microRNAs was analyzed in three independent patient samples (normozoospermic, oligozoospermic, azoospermic men). Candidate microRNAs significantly different expressed in fertile and non-fertile patients can be analyzed in a vaster cohort by quantitative real-time PCR. Statistical analyses were carried out by SPSS 18.0 software (IBM Inc., Ehningen, Germany).

Results Total expression of 193 microRNAs was detectable in patients' samples by the MicroRNA Arrays. Of these, especially microRNA miR-19a and -b, miR-22, miR-122a, mir-196a, miR-199a and -b, miR-224, and miR-375 exhibited an elevated expression compared to other microRNA entities. 193 microRNAs were expressed in the seminal plasma of the normozoospermic proband, 190 in the oligozoospermic and 166 in the azoospermic patients. 16 microRNAs differed significantly in expression between normozoospermic and azoospermic samples, 19 microRNAs between the normozoospermic and azoospermic samples and 5 microRNAs between oligozoospermic and azoospermic samples. MicroRNAs miR-9, miR-93, miR-125a, miR-155, miR-204, miR-368 and miR-373 were markedly differentially expressed and may be interesting for further analysis on their relevance in male infertility.

Conclusion We were able to detect several microRNAs in the seminal plasma serving as candidates for the diagnosis of fertility disturbances. Due to the relatively easy accessibility of seminal plasma and the unbiased detection of the microRNA fingerprint, detection of a selected number of infertility-associated microRNAs may be a new and promising addition to classical routine diagnostic methods.

P50

Piwi Gene Expression is Associated with Ejaculate Parameters and Male Infertility

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Introduction The Piwi gene family (P-elementinducedwimpytestis) is a subgroup of evolutionarily highly conserved genes coding for Argonaute proteins that play an essential role in RNAi and posttranscriptional gene regulation. While members of AGO subclass are ubiquitous present in diverse tissues, Piwi genes are only expressed in the germline. It is known that the Piwi genes, which are expressed in pre-pachytene and pachytene stages of spermatogenesis, are associated with germ cell development and silencing of retrotransponsons to maintain genomic integrity in mice while no data have been described so far in humans. In Europe 10% of male population is affected by infertility caused by defects in spermatozoa maturation or transport. The aim of this study was to investigate the relevance of the mRNA expression of human Piwi genes (Piwi like 1–4) for evaluation of male infertility.

Material & Methods The study was performed in 31 infertile patients and 43 healthy volunteers after approval of the ethics committee of the university and informed written consent of all study volunteers. RNA was extracted from spermatozoa, cDNA synthesis was conducted and expression of Piwi like 1, 2 and 4 was measured by real-time-PCR. The expression was correlated with various clinical parameters (fertility status, sperm motility, sperm concentration, occurrence of morphological defects of spermatozoa). Bivariate correlation analysis according to Spearman-Rho and Chi-square test were applied.

Results The appearance of spermatozoa with head defects was positively correlated with expression of Piwi like 2 mRNA ($p = 0.015$; $r = 0.42$). Likewise, a significant positive correlation was detected between Piwi like 2 mRNA expression and percentage of immotile sperm ($p = 0.049$; $r = 0.449$) and the diagnosis of infertility ($p = 0.001$; $r = 0.384$), respectively. Furthermore, mRNA expression of Piwi-like 2 was negatively correlated to sperm concentration ($p = 0.002$; $r = –0.352$). Elevated Piwi like 1 mRNA expression is negatively correlated with mid-piece defective spermatozoa ($r = –0.356$; $p = 0.042$). There is a significant association between low Piwi like 2 mRNA expression and the presence of a normozoospermia ($p = 0.026$, Chi-square test).

Conclusion Elevated expression of Piwi like 2 is associated with defects in spermatogenesis and associated infertility. Piwi like 1 expression has opposite effects. Further studies have been initiated to clarify the diagnostic relevance and the mechanistic background of both Piwi genes for male infertility.

P51

Lysophosphatidylcholine Content in Human Spermatozoa Depends on BMI?

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Introduction The lipid composition of the human spermatozoa membrane is complex. The most abundant glycerophospholipid is phosphatidylcholine (PC) (16:0/22:6). The

fatty acyl composition of sperm is unique and comprises large amounts of highly unsaturated fatty acyl residues, in particular docosahexaenoic acid (22:6) which are crucial for the fluidity of the membrane and, therefore, the successful fertilisation process. However, as a consequence of this significant amount of unsaturated fatty acyl residues, the spermatozoa are very sensitive to reactive oxygen species (ROS). It has been already shown that PC is converted into lysophosphatidylcholine (LPC) under conditions of oxidative stress, and, thus, the PC/LPC ratio may be used as a measure of sperm quality. Oxidative stress is massively involved in the pathology of many diseases. It has already been reported that obesity evokes a state of systemic oxidative stress and an elevated body mass index (BMI) negatively affect the male reproductive potential. Surprisingly, however, ROS-induced changes of the sperm lipids have been scarcely investigated.

Material & Methods Semen samples from 58 donors were used after signing informed consent. The men were grouped according to their BMI: 21 normal weight men (20.00–24.99 kg/m²), 20 overweight men (25.00–29.99 kg/m²), 17 obese men which were subdivided into subcategory named obesity I (30.00–34.99 kg/m², n = 9) and obesity II (≥ 35.00 kg/m², n = 8). The lipid composition of spermatozoa was determined by matrix-assisted laser desorption and ionization time-of-flight mass spectrometry because this technique offers some important advantages: the required sample preparation steps are simple and can be quickly performed, derivatization of the samples is not required and considerable concentrations of impurities such as buffer compounds, salts or detergents are tolerated.

Results The lipid composition is significantly different if the spermatozoa from extremely obese men (BMI ≥ 35.00 kg/m²) are considered in comparison to normal weight volunteers. The PC/LPC ratio clearly reflects the changes of the lipid compositions (normal weight men: PC/LPC: 23 [7–60], obese men subgroup II: PC/LPC: 4 [1–7], data expressed as median [interquartile range]). This clear effect is not observed if men with a slightly elevated BMI were included.

Conclusions The significant reduction of the PC content accompanied by an increased LPC could be a sign for a restricted fertilisation competence of those ejaculates. The large variability of the PC/LPC ratios may indicate highly diverse capabilities of the individuals to manage oxidative stress. Therefore, it is important to clarify whether either ROS or PLA_2 activity represent the prime effectors of the observed effects.

P52

A Cross-Platform Cross-Laboratory Microarray Study as a Powerful Tool to Reveal Gene Expression Signatures of Male Infertility

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Introduction The molecular basis of idiopathic male infertility is largely unknown. Gene expression profiling of normal and pathological human ejaculates/spermatozoa is a tool to identify causes on a molecular level. We present a cross-laboratory cross-platform microarray study with gene expression profiles of 127 human ejaculates/spermatozoa. Involved are donors/patients belonging to different groups in respect to fertility status and spermiogram parameters (according to WHO guidelines, 2010).

Material & Methods 25 ejaculates with different outcomes of IVF treatment (fertilisation rates, pregnancy rates) were collected at the Fertility Centre Hamburg. RNA was isolated and whole genome microarrays (Codelink, 55k) were hybridized. For cross-platform analysis, seven sets of raw data were downloaded from GEO (NCBI) additionally. All data were background corrected. Log(2) data were quantile normalized using the "Affy" package (Bioconductor). Datasets were merged by EntrezID common to all platforms. In case of multiple probes targeting one EntrezID the one with highest MAD (Median absolute deviation) was chosen. Batch effects were eliminated using different R packages.

Result In a first attempt we investigated mRNA expression patterns of 25 normozoospermic donors with different outcomes in the IVF program. The degree of individual heterogeneity of the resulting gene expression patterns was very high and disturbed the detection of relevant genes. We extended the study to data downloaded from GEO database. A total of 127 gene expression profiles of human spermatozoa from 6 different laboratories and 5 different microarray platforms were analyzed in silico.

When merging datasets from different laboratories and platforms the main challenge is to overcome non-biological technical bias while keeping an optimum of biological information at the same time. Applying the "ComBat" package (Bioconductor) we were able to cluster samples according to their biological class and no longer to platform/laboratory. As expected, the transcripts for protamine 2 (PRM2) and transition protein 1 (TNP1) showed the highest average expression in all samples. Furthermore functional annotation analysis of the 200 transcripts with highest expression detected overrepresentation of GOterms "spermatogenesis" and "translation" (DAVID6.7, NIAID/NIH).

Conclusion Successful merging of different datasets opens new possibilities for understanding effects on male infertility by analyzing gene expression patterns in different combinations.

P53

Sperm DNA Fragmentation as Assessed by Tunel/Mean Values in Fertile Men and Intra Individual Variability

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Introduction Sperm DNA Fragmentation (SDF) is a chromatin anomaly frequently found in sub and infertile patients and negatively impacting on human reproduction. Given that, many authors propose that tests detecting SDF should be included in the clinical management of male infertility. TUNEL/PI [Muratori et al. Hum Reprod, 2008] is an innovative version of the TUNEL assay, one of the most popular methods to reveal SDF by flow cytometry. The new technique couples the nuclear staining of sperm with the detection of DNA breaks and thus excludes M540 bodies from the sperm analysis and greatly ameliorates the accuracy of the measures of SDF. In previous studies [Muratori et al. JAndrol, 2010] we further demonstrated that when TUNEL/PI is executed by a standardised procedure, it presents very low (< 5%) intra-assay coefficients of variation (CVs), thus resulting a quite precise test. In the present study, we faced two further aspects important for using TUNEL/PI in the clinical practice: the development of reference values and the determination of the intra-individual variability.

Material & Methods For the first aim, 53 proven fertile men (i.e. natural pregnancy within one year from the day in which TUNEL/PI was executed) were recruited and SDF was measured by TUNEL/PI. For the second aim, within patients afferent to our clinics, we retrospectively selected those who performed TUNEL/PI at least twice. The variability between the two measures was calculated as CV.

Results In the collected fertile men, the average age was 37.2 ± 4.8 y, (average female age = 34.5 ± 3.9 yrs) and semen parameters were as following: sperm count (millions): = 189.7 ± 159.4; concentration (millions/ml) = $71.0 \times 10^6 \pm 90.8 \times 10^6$; morphology = 9.4 ± 6.1%; progressive motility = 45.8 ± 17.5%; total motility = 55.0 ± 17.6%. In this group of fertile men, we found an average value of SDF of 36.4 ± 14.8% and a range of 12,0–65,56 %. Regarding the intra individual variability of SDF, we observed that SDF remains quite stable within 3 months. Indeed the average intra-individual CV was 8.9 ± 5.8% (range: 1.5–16.4%, n = 11) resulting smaller than CVs for sperm count (36.7 ± 33.2%), concentration (29.0 ± 23.5%), progressive motility (14.5 ± 12.3%), total motility (19.4 ± 20.2%) and morphology (30.0 ± 22.5%). When the length between the 2 determinations of SDF was greater than 3 months, the intra-individual CV for SDF increased to 17.1 ± 15.0% (range: 0.8–66.0%, n = 42).

Conclusion In this study we began to collect fertile men to the final aim of building the reference value for SDF as assessed by TUNEL/PI.. In addition, we reported that within 3 months, SDF is the most stable intra-individual semen parameter.

P54

Characterization of Sumoylated Proteins in Human Sperm

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Introduction SUMOylation is a post-translational protein modification involved in the regulation of essential cell functions. Recently our group investigated the expression of SUMOylated proteins in human ejaculated spermatozoa [Marchiani et al., 2010]. We found several SUMO-1 and SUMO-2/3ylated proteins in spermatozoa in a molecular weight range of 25-85 kDa. Moreover we showed that SUMO-1 is mainly present in live spermatozoa and the percentage of SUMOylated spermatozoa was inversely correlated with total and progressive motility. Such correlations become stricter when only asthenospermic subjects were included in the analysis. By immunoconfocal fluorescence analysis and electron microscopy, we demonstrated that SUMOylated proteins are mainly located in the nucleus and in the midpiece. To better understand the role of this protein modification in sperm we aimed to characterize possible target proteins of SUMO-1. In particular, we evaluated RanGap-1 (Ran GTPase-activating protein 1) one of the main target of SUMO in somatic cells, and DRP1 (Dynamin-related protein 1), whose SUMOylation in somatic cells provokes alterations of mitochondrial function.

Material & Methods By using immunoprecipitation/western blot analysis, immunofluorescence and immunoconfocal fluorescence we determined the occurrence of SUMOylation of RanGap-1 and DRP1 and, subsequently, their relative localization in sperm.

Results By immunoprecipitation both with anti-SUMO-1 and with anti-RanGap-1 antibodies, we demonstrated that RanGap-1 is SUMO-1ylated in human spermatozoa. With same strategy, we demonstrated that DRP-1 is SUMO-1ylated and that this protein modification is found at higher levels in sperm pools from asthenozoospermic men respect to normozoospermic. By confocal microscopy we observed that RanGap-1 is located both in the neck area and at acrosomal level of sperm, whereas co-localization between SUMO-1 and RanGap-1 is mainly found in the neck area.

Conclusion We identified RanGap-1 and DRP-1 as two targets of SUMOylation in human spermatozoa. Our preliminary data suggest that SUMOylation of DRP1 may be related to sperm motility. Taken together, our data [Marchiani et al, 2010 and present study] suggest that SUMOylation could play different roles in human sperm functions and the characterization of target proteins is fundamental to understand such roles.

P55

Small Human Sperm Vacuoles Observed under High-Magnification (×10000): Small Nuclear Concavities Linked to Failure of Chromatin Condensation

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Introduction Intracytoplasmic morphologically selected sperm injection (IMSI), using high-magnification microscopy with differential interference contrast, could allow to improve embryos' implantation by selection of a normal spermatozoon with a vacuole-free head. If the nature of large vacuoles was recently well described, the nature of smaller ones remained unclear. The present study set out to determine whether multiple small vacuoles were of nuclear or acrosomal origin.

Material & Methods For 15 infertile men with various sperm profiles, high-magnification was used to select and assess (1) 450 normal spermatozoa with a vacuole-free head and (2) 450 spermatozoa with multiple small vacuoles (> 2 vacuoles occupying each less than 4% of the head's cross-sectional area). Sperm acrosomal status (reacted or not using Pisum sativum lectin staining) and their degree of chromatin condensation (aniline blue staining) were subsequently analyzed on the same spermatozoa using three-dimensional deconvolution microscopy.

Results The mean proportion of sperm with a non-condensed chromatin was significantly higher for spermatozoa with multiple small vacuoles than for normal ones (18.9 ± 1.9% vs 6.7 ± 1.5% in average respectively; $p < 0.001$). Spermatozoa with small vacuoles tended to be more often acrosome reacted than normal ones (6.0 ± 1.6% acrosome reacted spermatozoa among spermatozoa with vacuoles vs 2.9 ± 1.0% among normal spermatozoa; $p = 0.06$), even if this difference did not reach significativity. No association was observed between chromatin condensation and acrosomal status. In all the 450 spermatozoa observed, multiple small vacuoles were identified as small abnormal nuclear concavities.

Conclusions Multiple small vacuoles are small nuclear concavities linked to failure of chromatin condensation.

P56

Cryopreservation of Human Spermatozoa Decreases the Number of Motile Normal Spermatozoa, induces Nuclear Vacuolisation and Chromatin Decondensation: Interests of High-Magnification for Frozen/Thawed Sperm

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Introduction Even though cryopreservation of human spermatozoa is known to alter sperm motility and viability, it may also induce nuclear damages. The present study set out to determine whether or not cryopreservation alters motile sperm morphology under high-magnification and/or is associated with chromatin decondensation.

Material & Methods For 25 infertile men, we used high-magnification microscopy to determine the proportions of various types of motile spermatozoa before and after freezing-thawing: morphometrically normal spermatozoa with no vacuole (grade I), < 2 small vacuoles (grade II), at least one large vacuole or > 2 small vacuoles (grade III) and morphometrically abnormal spermatozoa (grade IV). The spermatozoa's chromatin condensation and their viability were also assessed before and after freezing-thawing.

Results Cryopreservation induced sperm nuclear vacuolization. It decreased the proportion of grade I + II spermatozoa ($p < 0.001$). It induced a decrease in the sperm viability rate ($p < 0.001$) and increased the proportion of sperm with non-condensed chromatin ($p < 0.001$). The latter parameter was strongly correlated with sperm viability ($r = 0.71$; $p < 0.001$). However, even motile sperm presented a failure of chromatin condensation after freezing/thawing because the proportion of sperm with non-condensed chromatin was correlated with high-magnification morphology ($r = –0.49$ and $+0.49$ for the proportions of grade I + II and grades III+IV, respectively; $p < 0.001$).

Conclusions Cryopreservation alters the organelle morphology of motile human spermatozoa and induces sperm chromatin decondensation. High-magnification microscopy may be useful for evaluating frozen-thawed spermatozoa before use in assisted reproductive technology procedures (such as intrauterine insemination, in vitro fertilisation and intracytoplasmic sperm injection) and for performing research on cryopreservation methods. If frozen-thawed sperm is to be used for intracytoplasmic sperm injection, morphological selection under high magnification may be of particular value.

P57

Cryptozoospermia Negatively Affects Pre-Implanation Embryo Development: Results from a Study on 798 ICSI Cycles with Fresh Ejaculated Sperm

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Introduction Semen quality may affect the early embryonic development in humans, that seems to be strongly dependent also on male factors. Thus, we evaluated the effect of semen quality on the main ICSI outcomes in cycles where fresh ejaculated sperm were used.

Material & Methods In this retrospective study, we considered 1,100 couples submitted to ICSI with fresh ejaculated sperm. To limit the confounding effects of female factors, we excluded all cycles where the female partner was > 40 years or where < 3 oocytes were retrieved. According to these exclusion criteria, 798 cycles were analyzed and divided in four groups based on male partner sperm concentration: cryptozoospermic ($\leq 1 \times 10^6$/ml; n = 182), severely oligozoospermic (1–5 $\times 10^6$/ml; n = 156), oligozoospermic (> 5 and < 20 $\times 10^6$/ml; n = 201) and with a normal sperm concentration ($\geq 20 \times 10^6$/ml; n = 259). The differences among the number of fertilized oocytes, total embryos, good quality embryos, and the fertilisation rate, the pregnancy rate per embryo-transfer (ET) and the implantation rate were evaluated in the 4 groups.

Results Only in cycles with cryptozoospermic semen we observed significantly lower fertilisation rates ($p < 0.0001$), number of fertilized oocytes ($p < 0.01$), number of total embryos ($p < 0.01$) and number of good quality embryos ($p < 0.001$) compared to those cycles in which male partner semen parameters were better; whereas, no differences among the same ICSI outcomes were found in cycles in which the male partner was severe oligozoospermic or oligozoospermic or with a normal sperm concentration. Finally, no differences were found among the four groups regarding the pregnancy rate per ET and the implantation rate.

Conclusions Cryptozoospermia negatively affects the early human embryogenesis in ICSI cycles in which fresh ejaculated sperm were used, corroborating the hypothesis of an important paternal impact on the pre-implantation phase of embryo development.

P58

Reliability of Flow Cytometry in Quantifying Antibody-Molecules on Sperm Surface

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Introduction Antibody load on sperm surface when all ejaculated spermatozoa are antiboby coated at screening tests (IBT or MAR test) can represent a main determinant of fertility impairment. The reliability of its quantification by flow-cytometry (FCM) has been questioned [Nikolaevaet al. Hum Reprod, 2000]. In this study we re-assessed the reliability of indirect flow-cytometry, which allows the manipulation of controlled variables.

Materials & Methods Motile sperm suspensions from 3 different donors were incubated with serial dilutions (1:8 to 1:128) of 2 sera with high titre of sperm-agglutinating activity (SAA) from patients with > 90% positive direct IgG-MAR test and, after 2 washing, with FITC-F(ab') antibody anti-human IgG. Propidium iodide was used to exclude from the analysis non-vital sperms. The median fluorescence intensity (in arbitrary units) was assessed by FCM and converted in number of antibody-molecules using Quantum FITC MESF Kit®, including several populations of microspheres associated to a known and increasing number of fluorescein molecules. Moreover, sera from 12 patients with direct IgG-MAR test positive >90% were tested at a fixed dilution 1:16 with the same procedure.

Results Serial dilutions of the 2 immune sera produced regression curves with a decay of fluorescence intensity, reproducible with different donors. The interassay coefficient of variation ranged from 2.2% to 15%. When converted in number of antibody-molecules, median values ranged from a maximum of 81,000 ± 1,700 at 1:8 dilution to a minimum of 6,700 ± 214 at 1:128 dilution. Below 10,000 antibody molecules the percentage of positive spermatozoa began to decline from > 90%. When sera from 12 patients with direct IgG-MAR test positive > 90% were tested at a fixed dilution 1:16, the median number of antibody-molecules/spermatozoon ranged from 81,000 to 12,000. More than 95% of live spermatozoa were positive at FCM when the median number of antibody-molecules/spermatozoon was > 20,000; ≥ 85% for values > 10,000; < 85% in the only case with < 10,000 antibody-molecules/spermatozoon. The median number of antibody-molecules/spermatozoon was correlated with the titre of sperm agglutinating activity detected in the patients' sera ($r = 0.83$; $p = 0.0008$).

Conclusions FCM is a reliable method to quantify the antibody load on sperm surface, which can be easily converted in median number of antibody-molecules/spermatozoon. More complicated methods have been proposed in the past. This could contribute to shed light on the variable interference of antisperm antibodies with fertility. In clinical setting, direct FCM can have a role in quantifying antibody load when screening tests are strongly positive (> 90%), with potential clinical relevance in the choice of treatment (IUI or ICSI).

P59

MMP-12 Expression is Detectable in Human Seminal Plasma and Represents a Predictor for Inflammatory Processes in Semen Analysis

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Introduction Macrophage metalloelastase-12 (MMP-12), a protein of the matrix metalloproteinase family is involved in the breakdown of extracellular matrix in normal physiological processes as well as in disease processes. MMP-12 is almost exclusively produced by macrophages and is associated with inflammatory processes. Giving the fact, that inflammatory processes do negatively influence ejaculate parameters, we investigated a possible presence and relevance of MMP-12 in seminal plasma especially in ejaculates with peroxidase positive granulocytes.

Material & Methods 33 patients who presented for semen analysis were assigned to four groups depending on the result of semen analysis according to the WHO guidelines 2010: normozoospermia (n = 10), OAT-syndrome (n = 6), azoospermia (n = 8) and leukocytospermic samples (> 1 mio peroxidase positive cells/ml) (n = 10). MMP-12 was detected in seminal plasma by ELISA. The statistical analyses were performed with GraphPad Prism.

Results MMP-12 was measurable in all seminal plasmas. Generally, MMP-12 concentrations in seminal plasma were significantly higher in patients with leukocytospermia than in patients with less than 1 mio. peroxidase positive leucocytes ($p < 0,001$). Leukocytospermic samples displayed significantly higher levels of MMP-12 in seminal plasma than patients with normozoospermia, OAT-syndrom and azoospermia.

Conclusion MMP-12 is present in seminal plasma and is correlated with inflammatory processes in human semen and therefore may serve as predictor of ongoing inflammatory processes.

P60

Success Predictors in Homologous Intrauterine Insemination

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Objective To identify factors influencing the outcome of infertility treatment using homologous intrauterine insemination (H-IUI).

Design Retrospective study of H-IUI cycles performed at the Reproductive unit, Department of Obstetrics and Gynecology, University Medical Centre Ljubljana from 2002 to 2011.

Setting University-affiliated infertility clinic.

Patients & Ovarian Stimulation 919 couples undergoing 2247 H-IUI treatment cycles, stimulated by clomiphene citrate (CC), letrozole (L) and gonadotropins: Pergonal, Gonal-F (G), Puregon and Menopur (M),

Methods Female and male infertility factors diagnosis, duration of infertility, age, type of hormonal treatment, follicles diameter, endometrial thickness, and semen quality 3 months before and at H-IUI, related to the occurrence of a pregnancy. First, bivariate analysis (Mann-Whitney U and $\chi 2$ tests) of first attempt H-IUI cycles, secondly logistic regression of the overall cycles.

Results Throughout the 10 year period the overall clinical pregnancy rate per H-IUI cycle was 11.7% and per couple 28,6% after a mean of 2.0 treatment cycles. The pregnancy rate was identical (mean of 11.6% per cycle) in the 4 first attempts. Pregnancy rate did not differ according the type of female factors of infertility: endometriosis (11.6%), ovulation abnormalities (12.9%), tubal pathology (12.0%), uterine pathology (11.6), unexplained infertility (12.4%) and cervical pathology (15.4%). Pregnancy rate was lower in cases of asthenozoospermia (7.1%) and teratozoospermia (2.3%). At the contrary, H-IUI success was not influenced by isolated leukocytospermia (19.5%). No influence of the following parameters on pregnancy rate was observed: type and duration of infertility (respectively, $p = 0.478$ and $p = 0.192$), female and partner's age (respectively, $p = 0.753$ and $p = 0.493$), follicles diameter ($p = 0.371$), endometrial thickness ($p = 0.634$). Stimulation by CC and L was less successful than that with gonadotropins G and M (respectively, 9.4% and 9.5% vs. 19.6% and 16.7%).

Conclusion H-IUI is a simple and inexpensive treatment giving good pregnancy rates at least up to 4 treatment cycles. Success is dependent on the type of ovarian stimulation used and on the concentration of motile sperm at IUI.

P61

Human Sperm Likes Sugars: The Role of the Glycolytic Pathway in Male Gamete Functionality

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The sperm cell can survive for days in the female reproductive tract requiring energy for several purposes, such as progressive motility, that can theoretically be obtained by glycolysis and oxidative phosphorylation (OXPHOS). The relative significance and differential input of these metabolic pathways remains controversial.

In this work human sperm samples ($n = 6$) were maintained for 48 h at 37°C (normoxia, 5 % CO_2) in PBS medium containing (1) no exogenous substrates; (2) glucose, pyruvate and lactate (glycolysis and OXPHOS substrates). Additionally, the effects of potassium cyanide (an OXPHOS inhibitor, acting at the complex IV of the mitochondrial electron transfer chain) and iodoacetic acid (a glycolysis inhibitor acting at the glyceraldehyde 3-phosphate dehydrogenase level) in sperm viability, motility, ATP content and energy charge were tested.

Interestingly, human sperm maintained some functionality after 24 h at 37°C, even in the absence of any exogenous substrates. However and as expected, the percentage of motile sperm was higher in the presence of substrates (76.7 ± 4.3 compared to 55.7 ± 9.3; $p < 0.05$). Likewise, the sperm energy charge was better in the supplemented medium (0.74 ± 0.03 vs 0.59 ± 0.06; $p < 0.05$). The inhibition of glycolysis clearly affected sperm motility, ATP content and energy charge, lowering all parameters even in the presence of exogenous substrates. On the other hand, the mitochondrial poison potassium cyanide did not seem to affect sperm functionality.

These data clearly points to the importance of glycolysis in human sperm homeostasis.

03

Asthenozoospermia in Spinal Cord-Injured Men: a Model for Shedding Light Upon the Contribution of Glycolysis and Mitochondrion in Supporting Sperm Motility

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Introduction The multi-factorial etiology of severe asthenozoospermia, usually occurring in men with spinal cord injury (SCI) includes an adverse impact of seminal plasma (SP) on sperm motility. In this study we tested the hypothesis of a double energetic blockage (glycolysis and mitochondrial respiration) as a metabolic determinant of the adverse effect exerted by SP from men with SCI on sperm motility.

Material & Methods 22 seminal plasmas were recovered by centrifugation of ejaculates obtained from SCI men by penile vibratory stimulation. Seminal fructose levels were determined by spectrophotometry. Each SP sample from SCI men as well as from healthy donors was tested on donor motile sperm suspensions for its effect on sperm motility, vitality, mitochondrial membrane potential ($\Delta\Psi m$) and caspase activation. Sperm motility was evaluated with CASA and spermatozoa exhibiting an average pathway velocity > 5µm/sec were categorized by the software as motile spermatozoa. Sperm vitality was evaluated under light microscope with the eosin exclusion staining. Sperm $\Delta\Psi m$ was assessed at flow cytometry with JC-1, which emits red or green fluorescence in the presence of high or low $\Delta\Psi m$, respectively. Caspase activation was evaluated at flow cytometry using permeable FITC-conjugated peptides (LEHD-FMK and DEVD-FMK), which irreversibly bind to the activated caspase-9 and -3, respectively.

Results Only SP from asthenozoospermic samples, exhibiting both low fructose levels and inhibitory effect on $\Delta\Psi m$, affected donor sperm motility (motile sperms were 20.5 ± 12.7% in sperm samples exposed to SP from asthenozoospermic SCI ejaculates and 71.8 ± 13.3% in those exposed to donor SP, $p < 0.001$). No significant effect on sperm vitality was observed. The inhibitory effect on sperm motility was reverted by washing in medium containing glycolysable sugars (48.8 ± 8.5% vs 45.2 ± 6.5% observed in washed controls, $p = 0.1$), in spite of persistently depressed $\Delta\Psi m$, as indicated by the lower percentage of spermatozoa emitting red JC-1 fluorescence (38.9 ± 5.0%) with respect to washed controls (79.4 ± 7.2%, $p < 0.001$). Activation of caspase-9 (mitochondrial caspase) and caspase-3 (executioner caspase) accompanied the loss of $\Delta\Psi m$.

Conclusions A double energetic blockage (glycolysis and mitochondrial respiration) represents a metabolic determinant of the adverse effect exerted by SP from men with SCI on sperm motility. Mitochondrial dysfunction-related apoptotic/oxidative mechanisms might account for later consequences on sperm motility/vitality.

P154

Pregnancy Rate, Semen Analysis, Varicocele Profile in Surabaya 2007–2010

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Introduction Varicoceles have classically been described to induce a stress pattern semen analysis. Improvements in semen pa-

rameters after varicocelectomy had been the main outcome of most studies, but the results were different.

Objective To assess varicocele information from Soetomo and Ramelan Navy Hospital including patient's detail, semen analysis before and after operation, and pregnancy rate. We reviewed medical records of patients retrospectively who underwent Varicocelectomy 2007–2010 in Soetomo and Ramelan Navy Hospital. The data collected and classified based on patient's age, chief complaint, type of varicocele, severity, semen analysis and pregnancy rate.

Results There were 151 patients in both hospital, but only 19 patients had information about pregnancy and 24 patients had information about semen analysis after operation.In four years there were 151 patients with varicocele who underwent varicocelectomy. Most of them were 20–30 years old (61%), complained testicular pain (88.7%), unilateral (84.1%), grade II (55%), had stress pattern of semen analysis before operation (95.4%) and 4.6% were azoospermia. Overall Pregnancy Rate was 42.1%. Post operative semen analysis improved in 12/24 (50%) patients and normospermia in 4/24 (16.7%) patients **(Fig. 14, 15)**.

Postersession 4: Determinants of Male Reproductive Health

P62

Morphometric and Immunohistochemical Evaluation of the Testis of Sexually Mature Mice (*Mus domesticus*) intoxicated with Boron

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Introduction It has been reported that boron causes changes in various systems, including the male reproductive system. Residents in some towns in northernChile were consuming a few years ago in the drinking water 20 times more than the amount established as permissible limit by WHO. This study evaluates the effects of high intake of boron in the testis using an animal model. Boron was administered in the drinking water.

Material & Methods 20 male mice (*Mus domesticus*), sexually mature, divided into 2 groups: the experimental group was given Boron at a dose of 12 mg/L, and the control group 0.6 mg/L, for 42 days. Sections of testis were obtained for: HE staining (Morphometry and Histopathology), Immunohistochemistry (Cox-2), Mallory and Picrosirious stain (evaluation of tunica albuginea).

Results The results indicate that ingestion of a dose of 12 mg boron/L produces vacuolization, tubular epithelial desquamation and tamponade. Morphometry revealed decreased tubular diameter and epithelial height and lumen diameter and increased interstitial area in the exposed group. Immunodetection of Cox-2 was positive in high percentage of tubules in the group intoxicated. The tunica albuginea was thinner, with decreased percentage of type I collagen fibers and an increase in the percentage of type III collagen fibers in animals exposed to boron in contrast to the control group.

Exposure to critical levels of boron produces severe histopathological changes in the testis, altering morphometric parameters and causing overexpression of Cox-2. Finally, evaluation of collagen fibers suggests that Boron produced a degradation of the collagen of the tunica albuginea, causing a decrease in the thickness of it and altering the percentage ratio collagen I/collagen III, a process called collagenolysis.

Conclusion Boron compromises testicular function in mice, altering several reproductive testicular and sperm parameters, provokes collagenolisis and therefore altering testicular architecture.

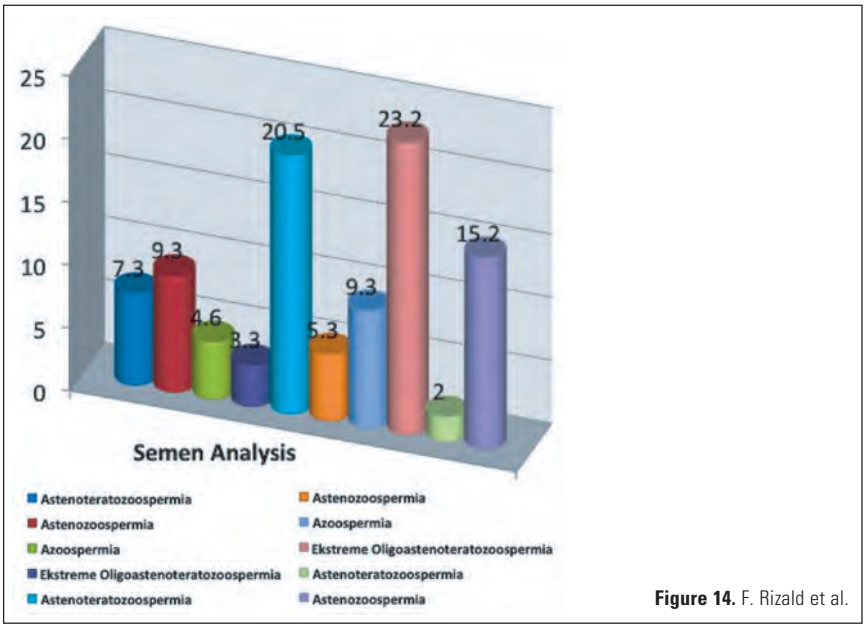

Figure 14. F. Rizald et al.

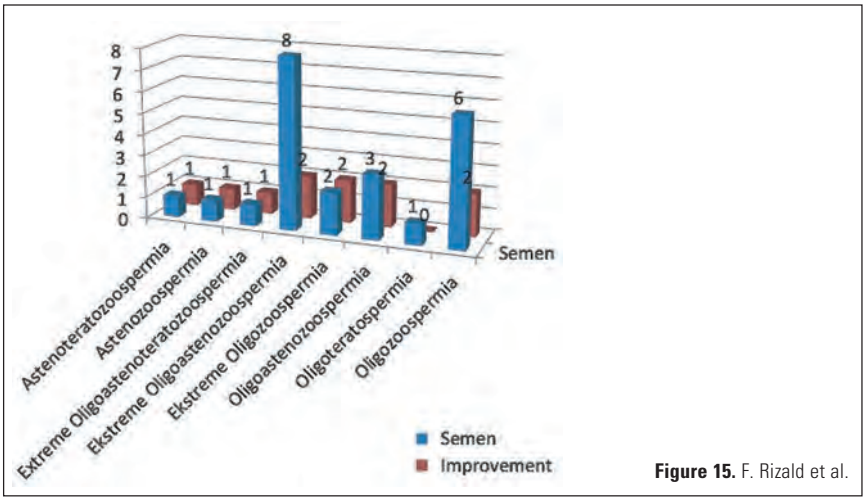

Figure 15. F. Rizald et al.

P63

The Impact of Vitamin D on Semen Quality

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Introduction Hypovitaminosis D is a significant public health issue in Northern Europe. A role for Vitamin D (VitD) in human fertility has been suggested as its receptors/metabolising enzymes are expressed in the male genital tract and VitD improves sperm motility. We investigated:
- The prevalence of VitD deficiency in men attending a fertility clinic.
- The impact of VitD supplementation on semen quality in men with VitD deficiency.
- The effect of the seasonal rise of serum (sVitD) levels on semen quality in non-sVitD deficient men.

Methods We recruited125 men attending our fertility unit into a prospective cohort

study. (Ethical approval obtained. Men on VitD therapy excluded). Participants completed a fertility lifestyle questionnaire, submitted blood and semen samples, and were invited to return 6 months later for further samples. All participants with sVitD deficiency (75: Optimal).

Results 124 results were analyzed. sVitD levels were deficient in 52 men (41.9%), insufficient in 51 (41.1%), adequate in 15 (12%) and optimal in 6 (4.8%). The prevalence of sVitD deficiency was significantly higher in non-Caucasians (80% vs 36.7%; p = 0.002).

87/125 (69.6%) of participants returned for follow-up. 37 had been sVitD deficient and 36 had received VitD supplementation. Their sVitD levels improved from 14 ± 3.8 to 86.5 ± 36.7 nmol/l (Mean ± SD).

50/125 participants who were not sVitD deficient returned for follow-up. There was a seasonal rise in sVitD levels from winter/spring to summer/autumn (from 46.1 ± 17.5 to 64.6 ± 19.5 nmol/l, Mean ± SD).

Conclusions This is the first longitudinal study to examine the impact of vitamin D elevation on semen quality. The prevalence of VitD deficiency was twice as high in recruits as in the general population. VitD deficiency was not associated with poor semen quality and supplementation did not improve semen quality. We identified a significant decline in semen parameters with increasing sVitD levels, but the reason for this is unclear and further studies are warranted.

P64

Increased Urinary Levels of Endocrine Disrupting Chemicals in Filaggrin Gene Null Mutation Carriers: A Cross-Sectional Population-based Study

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Background Phthalates can impair male reproductive development and function in animals, and studies also indicate a negative effect on human testicular function. Humans are exposed to phthalates through the diet, and from plastic products and personal care products. Filaggrin is an epidermal protein crucial for skin barrier function, and carriers of filaggrin gene (FLG) null mutations are at risk of having impaired skin barrier with facilitated transfer of allergens and chemicals across the epidermis. We investigated whether FLG null individuals have higher urinary levels of phthalate metabolites, and secondarily whether testicular function is associated with FLG genotype in a cross-sectional study of young men from the general Danish population.

Materials & Methods 868 men, median age 19 years, were genotyped for the R501X, 2282del4 and R2447XFLGnull mutations. 65 men had anFLGnull genotype. Urinary concentrations of 14 metabolites of short and long-chained phthalates were measured, as well as serum levels of reproductive hormones and semen quality.

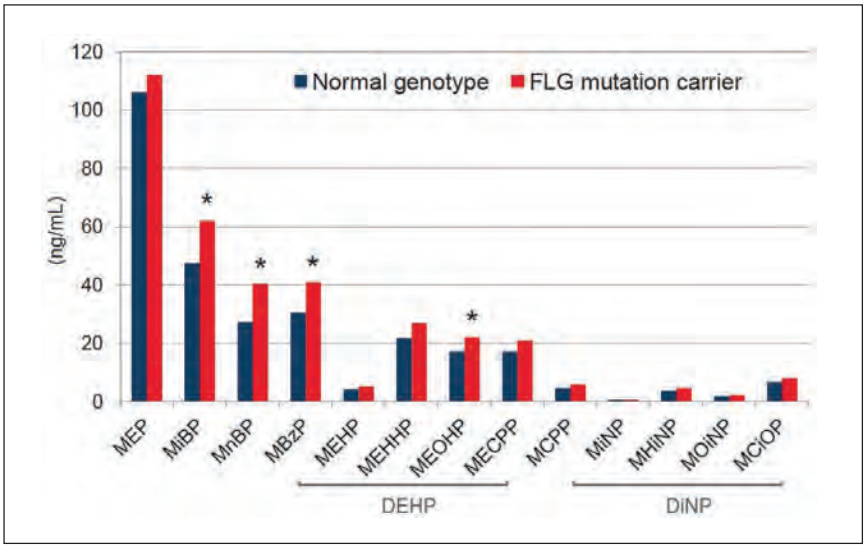

Figure 16. U. Joensen et al.

Results Men withFLGnull genotype had higher urinary excretion of most common phthalate metabolites, both short and long-chained, up to 39% (95%-CI: 17–61%) higher in the mutation carriers for MnBP.FLG mutation carriers had slightly lower BMI and slightly higher sex hormone-binding globulin (SHBG). We did not observe associations betweenFLGgenotype and other reproductive hormones or semen quality.

Conclusion The results are the first evidence thatFLGnull individuals are at risk of higher internal exposure to phthalates, as a result of a defective skin barrier. The same may apply to other chemicals with potential to disrupt the male endocrine system **(Fig. 16)**.

P65

Perfluorooctanesulfonate (PFOS) in Serum Negatively Associated with Testosterone Levels in Healthy Men

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Background In animals, some perfluorinated compounds (PFCs) have endocrine disrupting potential, but few studies have investigated PFC exposure in relation to testicular function in humans. In a previous, smaller study we found a significant negative association between serum PFC levels and number of normal sperm, but no significant association with reproductive hormone levels. We aimed to investigate associations between PFC exposure and reproductive hormone levels and semen quality in a larger group of healthy men.

Methods 247 men delivered serum and semen samples. Serum samples were analyzed for total testosterone (T), estradiol (E), sex hormone-binding globulin (SHBG), luteinizing hormone (LH), follicle-stimulating hormone (FSH) and inhibin-B, as well as content of 14 PFCs, including perfluorooctanesulfonate (PFOS). Semen samples were analyzed according to WHO criteria.

Results In multivariate linear regression models, PFOS levels were inversely associated with T, calculated free testosterone (FT), free androgen index (FAI), and ratios of T/LH, FAI/LH, and FT/LH **(Fig. 17)**. Other PFCs were detected at lower levels than PFOS and did not exhibit the same associations. PFC levels were not significantly associated with semen quality. PFOS levels in these samples collected in 2008–2009 were lower in than in our previous study of men participating in 2003 **(Tab. 9)**.

Conclusion The negative associations between serum PFOS and testosterone confirm trends observed in a smaller study, indicating that PFOS exposure may still contribute to health risks associated with decreased testosterone, in spite of decreasing serum levels. In contrast, we could not confirm our previous finding of decreased sperm mor-

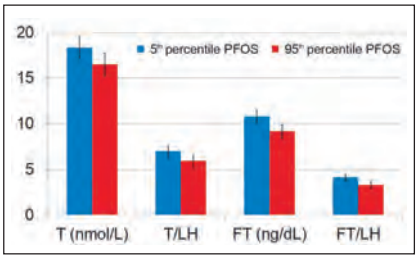

Figure 17. U. Joensen et al. Estimated levels of testosterone (T), free testosterone (FT) and related hormone ratios for 5th percentile (4.28 ng/ML) and 95th percentile (14.6 ng/ML) of PFOS exposure. Adj. to BMI = 23.0 kg/m² and no smoking.

Table 9. U. Joensen et al. PFOS levels in the current study (247 samples collected 2008–2009) were lower than in a previous study (105 men participating in 2003).

		Selected percentiles		
PFC ng/mL	**Mean ± SD**	**5th**	**50th**	**95th**
PFOS 2008–2009 raw	8.46 ± 3.74	4.28	7.79	14.59
PFOS 2008–2009 [a, b]	11.9 ± 5.26	6.02	11.0	20.5
PFOS 2003	25.3 ± 7.85	14.3	24.5	39.6
PFOA 2008–2009 raw	3.46 ± 1.99	1.83	3.02	6.15
PFOA 2008–2009 [a, b]	3.97 ± 2.28	2.09	3.47	7.05
PFOA 2003	4.80 ± 1.33	2.77	4.85	6.88
PFNA 2008–2009 raw	1.23 ± 0.63	0.64	1.07	2.41
PFNA 2008–2009 [a, b]	1.35 ± 0.70	0.70	1.17	2.66
PFNA 2003	0.89 ± 0.45	0.43	0.80	1.61

[a] Adjusted for inter-laboratory difference btw. previous and current study
[b] Statistically significant difference between 2003 levels and 2008–2009 levels. All $p < 0.002$ after adjustment for smoking, alcohol intake and age.

phology in the highest tertile of PFC exposure, possibly due to the lack of highly exposed individuals in this study.

P66

Psychological Impact of Fertility Treatment among Malaysian Men

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Introduction Mental stress has a reciprocal relationship to infertility and it constitutes many unknown reasons leading to the problems related to infertility. The impact of physical and emotional stress can be detrimental to infertile couples especially during the course of fertility treatment. Previous studies indicated that emotional stress has a negative impact on semen parameters. Our aim was to investigate the relationship between psychological stress and semen quality among Malaysian men during fertility treatment.

Materials & Methods This was a prospective study involving 122 male patients who attended a fertility clinic from January to June 2012. Patients were given questionnaires after seminal fluid analysis (SFA) test, intrauterine insemination (IUI) procedure and in vitro fertilisation (IVF) procedure. The questionnaires was constructed to gather data on patients' demographics, medical history and consumption of tobacco. It also included the Depression, Anxiety and Stress (DASS) test. Semen samples were collected by coitus interruptus or masturbation and were evaluated based on the World Health Organization (WHO), 1999 criteria. Analysis was conducted using SPSS version 16.0 with Chi-square statistical tests with p-value 0.05 was considered significant.

Results The sample included 68 (55.7%) patients who had undergone seminal fluid analysis (SFA), 35 (28.7%) intrauterine insemination (IUI) and 19 (15.6%) in vitro fertilisation (IVF). There were 88 (72.1%) normozoospermia, 31 (25.4%) oligozoospermia and 3 (2.5%) azoospermia. Psychological morbidities were mainly anxiety (33.3%) followed by stress 12.3% (n = 15/122) and depression 9.8% (n = 12/122). There were significant differences in the level of anxiety in patients with sperm volume ($p = 0.013$) and sperm motility ($p = 0.015$). All psychological morbidities had no effect on sperm count and sperm morphology. No statistical significant differences were seen in the fertility investigation or treatment (SFA/IUI/IVF) with all the sperm parameters. Patients with depression were associated with duration of infertility ($p = 0.022$). Anxiety patients were related to occupation ($p = 0.013$) and income ($p = 0.030$). Other factors such as age, type of infertility and level of education seem did not affect the psychological morbidities on the sperm parameters.

Conclusions This study has shown that psychological impact has affected the sperm quality of Malaysian men especially in sperm volume and motility.

P67

Clinical Evaluation of the Male Partners of Infertile Couples: A Waste of Time?

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Introduction The male factor has rapidly emerged as playing a major role in difficulties in conceiving, and is involved in 50% of cases. Although development of intracytoplasmic sperm injection treatment allows men to become fathers, management of male infertility is not limited to semen evaluation.

Material & Methods We described andrological examination and sperm results in a large retrospective cohort study including all 1672 couples consulting for infertility at the Toulouse Male Sterility Centre from 2000 through 2004. Andrological investigation included reproductive, surgical and medical histories, and clinical evaluation.

Results In 85% of the 1672 men, at least one andrological examination was abnormal: 21% had a history of infection, 13% a history of cryptorchidism, 9% a surgical history, 4% a history of testicular trauma, 23% an epididymal abnormality, 22% a varicocele, 20% a scrotal abnormality and 4% a vas deferens abnormality. Oligospermia was mainly associated with cryptorchidism or varicocele, whereas azoospermia was related only to epididymal abnormality. Abnormal testicular volume increased the risk of oligospermia or azoospermia.

Conclusion Properly standardized clinical evaluation is informative in diagnosing the causes of male infertility in complement to sperm analysis for clinicians who could better assist couples in management of male infertility, especially for treatment of particular clinical abnormalities, and in improving the relationship between clinicians and patients.

P68

Development of Semen Quality During Young Adulthood – Longitudinal Follow-up of 2 Finnish Cohorts

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Introduction Objective of our research was an assessment of trends in semen variables during young adulthood (19–29 years).

Material & Methods Study is based on the longitudinal follow-up of 2 cohorts of young adult Finnish men. The study was conducted in the University Andrology Research Unit (Turku). 2 cohorts of voluntary men were followed up from the age of 19 years till the age of 29 years. Semen analysis of the participating men was performed at 2 to 5 year intervals over a period of ten years (cohorts A and B). Physical examination was carried out at each visit to rule out any significant andrological abnormalities. Main outcome variables were semen volume, sperm concentration, total sperm count, motility and morphology. Statistical results were also adjusted for the time of abstinence.

Results 336 men in the cohort A participated in the first round of the study, and in the final 4th round 111 men were examined. 197 men attended the 1st visit in the cohort B, and in the final 3rd visit 90 men were evaluated. Sperm concentration (median 60 and 50 million/mL in A and B, respectively at the age of 19; 70 and 62 at the age of 29) did not change significantly during follow-up. How-

ever, the total sperm count in the cohort B increased significantly from 172 million at 19 to 225 million at 29. The percentage of motile sperm increased (66 and 76 % motile sperm in A and B at 19; 82 and 81 % at 29, respectively). Percentage of sperm with normal morphology also increased during the follow-up, as judged on the 61 men that participated in all 4 follow-up visits of the cohort A: 7.5 – 7.0 – 8.0 – 10.0 % at 19, 21, 24, and 29 years of age. Adjustment for the time of abstinence did not change outcome.

Conclusion(s) Full spermatogenic capacity is almost reached by the age of 19 years. Both sperm motility and the percentage of structurally normal sperm increase during young adulthood. Furthermore, sperm production capacity also shows slight improvement. Thus, semen quality seems to improve between 19 and 29 years. Current abstract complements our previous data presented in Boston(Massachusetts) at the Endocrine Society's Annual Meeting (ENDO) in 2011.

P69

Testis and Epididymis Morphology in Rats Treated with Soya Isoflavones From Prenatal Life until Sexual Maturity

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Introduction Xenobiotics with estrogen activity may have a significant influence on the function of the male reproductive system. Such xenobiotics are manufactured (e. g. pesticides, polyphenols, organochlorines) or are naturally present in the environment (e. g. phytoestrogens). Phytoestrogens are present especially in leguminous plants and are divided into three groups: isoflavones, lignans and coumestanes, of which isoflavones are the most common. Isoflavones (genistein and daidzein), after entering the body, may have estrogen-like action and can be expected to exert biological effects at the molecular, cellular or physiological level. They possess ability binding to estrogen receptors (ERs), with higher affinity for ERb, than for ERa. As natural selective estrogen receptors modulators (SERM) can affect estrogen-regulated gene products.

However, in certain periods of life, male exposure to xenoestrogens may have an adverse effect, for example on the male reproductive system. The periods of particular sensitivity are the fetal period and early infancy [9], when disruption of hormonal balance in favor of estrogens can lead to some abnormalities in adulthood.

Objective This study aimed to determine the influence of soy isoflavones (daidzein and genistein) administered from prenatal life to sexual maturity on testicular and epididymal morphology of rats.

Methods Pregnant Wistar rats received orally soy isoflavones, daidzein and genistein at a dose of 200 mg/kg/b.w./d. After separating sucklings from their mothers, male rats received the same dose of isoflavones until reaching the age of sexual maturity, i.e., for 3 mo.

Results The isoflavones induced changes in the morphology of the seminiferous epithelium of rat testes. There were numerous, strong PAS-positive, degenerated, without lumen, shrunken seminiferous tubules with destroyed arrangement of seminiferous epithelium, seminiferous tubules with sloughing of premature germ cells into the tubular lumen. Infoldings of the tubular basement membrane in some seminiferous tubules, and deep invaginations of the lamina propria with myoid cells were observed. However, there were no significant changes in the morphology of the epididymis. The levels of estradiol in serum and cauda epididymis homogenates of rats receiving phytoestrogens were significantly higher than in the control group. No differences were observed in testosterone concentrations in the serum of treated and control rats. The testosterone levels in the homogenates of the treated rat testes were significantly lower than in the control group.

Conclusion The exposure of rats to genistein and daidzein during intrauterine life until sexual maturity influenced the morphology of testes in greater degree than the morphology of epididymides.

P70

Perinatal Origins of Adult Leydig cells and Function: Role of Developmental Androgens

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Introduction Several lines of evidence show that human fetal events can adversely affect adult testosterone levels, but precisely how is unknown. Given the evidence that testosterone levels in men may be declining at the population level in a birth cohort-related fashion, this issue has added importance, which this study addresses using experimental rodent studies. Adult Leydig cells (ALC) are essential for normal spermatogenesis and overall male health, but their developmental origin is unclear. We identify a population of fetal interstitial cells expressing Chicken Ovalbumin Upstream Promoter-Transcription Factor II (COUP-TFII), essential postnatally for ALC development. We hypothesise these cells (3β-HSD$^-$/AR$^+$/COUP-TFII$^+$) are progenitor cells that differentiate into ALC (3β-HSD$^+$/AR$^+$/COUP-TFII$^+$) from puberty onwards. We investigated the fetal origins of these "progenitor ALC" and their potential regulation by fetal androgens so as to provide insight into how fetal events could determine testosterone levels in adult men.

Methods Three approaches were used: (1) complete androgen receptor (AR) knockout (ARKO) mice, (2) dibutyl phthalate (DBP; 500mg/kg/day; e13.5–e21.5) exposed pregnant rats, in which fetal intratesticular testosterone is substantially reduced, and (3) dihydrotestosterone (DHT; 10mg/ml/kg e15.5–e21.5) exposed pregnant rats, in which fetal intratesticular androgen exposure may be increased. In these groups, the numbers of progenitor ALC were quantified in fetal life through to adulthood, and related to ALC number and function in adulthood.

Results Presumptive progenitor ALC (COUP-TFII$^+$/AR$^+$) are abundant in the fetal testis in human, marmoset, rat and mouse. In ARKO mice, there was ~40% reduction in progenitor ALC number on pnd2 through to adulthood ($p < 0.05$). This was paralleled by a similar shortfall in ALC ($p < 0.05$) and there was also compensated ALC failure. In adulthood there was a significant correlation ($p < 0.0001$) between progenitor and ALC numbers. Similarly, fetal DBP exposure reduced progenitor ALC numbers by ~40% in fetal and postnatal life and induced compensated ALC failure, although final ALC number was unaffected. Effects of fetal dihydrotestosterone exposure are currently under investigation.

Conclusion This study suggests that COUP-TFII marks a population of cells in the fetal testis from which ALC develop from puberty onwards, and that the numbers of these cells is regulated by fetal androgens. Altered fetal androgen exposure also altered adult Leydig cell function and although the mechanism is unclear, we suggest this effect is mediated by altered fetal androgen action on the ALC progenitors. This adds a new dimension to growing evidence that fetal androgen exposure may determine functional competence of the adult testis and overall male reproductive health.

P71

Effects of Quercetin on mRNA Expression of Steroidogenesis Genes in Primary Cultures of Leydig cells Treated with Atrazine

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The effect of the phytoestrogen, quercetin (QT) on the reproductive toxicity of atrazine (ATZ) was explored in interstitial Leydig cells (ILCs). We measured the mRNA expressions of steroidogenesis genes in isolated ILCs by real-time-PCR after cultured cells were treated in vitro with ATZ (232 μM) and QT. The mRNA expression of tested genes increased with ATZ treatment and was normalized by quercetin except androgen re-

ceptor (AR) and estrogen receptor-alpha (ER-α) expression. Treatment of cells with QT alone (15–50 μM) caused a dose-dependent increase in AR and ER-á mRNA expression. The expressions of tested genes were unaffected by cyclic-AMP at 6 h when the stimulatory effects of ATZ on tested genes were sustained. When cyclic-AMP was absent in the culture medium at 1 h of incubation, QT (50 μM), did not stimulate the expression of AR and ER-α except cyclic-cAMP was added, and increasing the concentration of cyclic-cAMP resulted in dose-dependent expression of AR and ER-α. These findings suggest that ATZ may stimulate the expression of tested steroidogenesis genes via a mechanism independent of cyclic-AMP which was partially antagonize by QT **(Fig. 18, 19)**.

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P72

The Identification of Pre-diabetes Condition with ARIC Algorithm, Predicts Long-term CV Events in Patients with Erectile Dysfunction

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Question The Atherosclerosis Risk in Communities (ARIC) algorithm is one of the most efficient instruments for the prediction of incident type 2 diabetes (T2DM). Recently it has been shown to predict another relevant cardiovascular (CV) risk factor, such as chronic kidney disease. The aim of the present study is to verify whether, in patients with erectile dysfunction (ED), the use of ARIC diabetes risk score might improve the efficacy in predicting major CV events of other CV risk algorithms specifically developed for the assessment of CV risk.

Methods A consecutive series of 2,437 men (mean age 52.5 ± 12.9 years) attending our outpatient clinic for sexual dysfunction was retrospectively studied. A subset of this sample (n = 1687) was enrolled in a longitudinal study (mean follow-up of 4.3 ± 2.6 years). Several clinical, biochemical (including testosterone) and instrumental (penile color doppler ultrasound; PCDU) factors were evaluated. The assessment of metabolic risk was evaluated with the ARIC algorithm. The assessment of CV risk was evaluated using the Progetto Cuore risk engine.

Results In the cross sectional study ARIC score was inversely related with reduced testosterone levels, worse sexual functioning and reduced penile blood flow. When longitudinal sample was analyzed, higher baseline ARIC score significantly predicted MACE

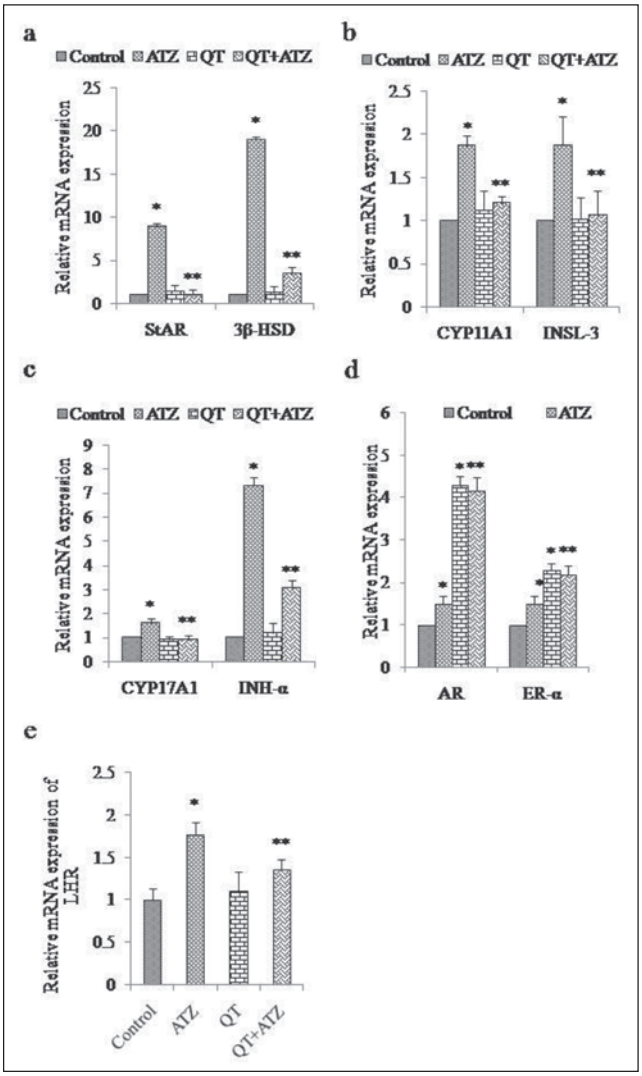

Figure 18. S. Abarikwu et al. Effects of quercetin (QT) and atrazine (ATZ) on the mRNA levels of steroidogenesis genes in cultured ILCs after a 6 h culture period.

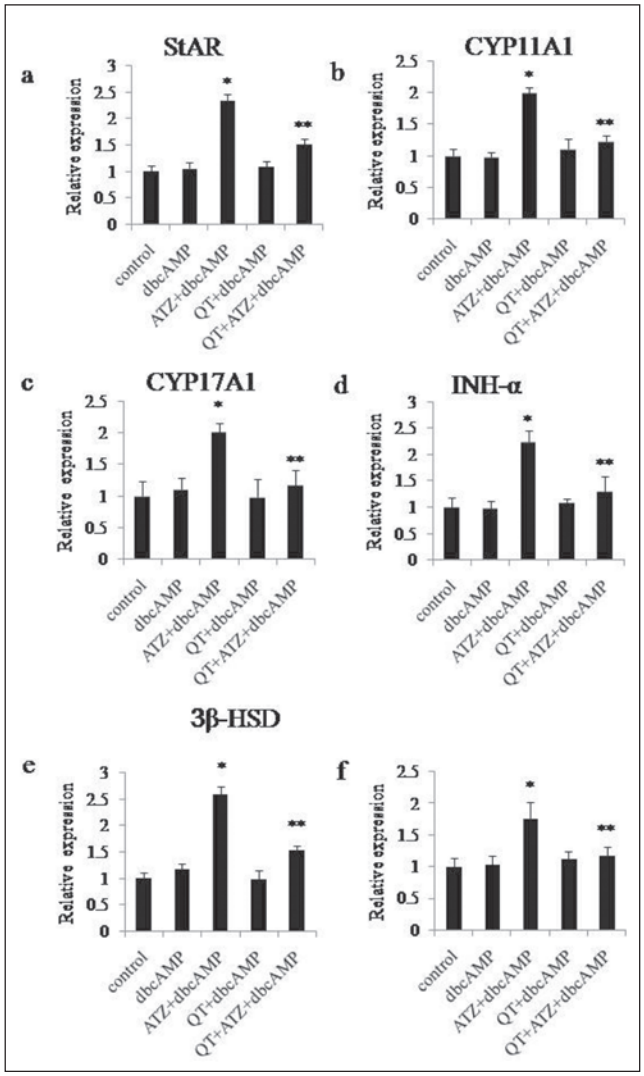

Figure 19. S. Abarikwu et al. Effects of quercetin (QT) and atrazine (ATZ) on the mRNA levels of steroidogenesis genes in cultured interstitial Leydig cells in the presence of dbcAMP after a 6 h culture period.

even when subjects with diabetes mellitus at baseline were excluded from the analysis (HR = ; 1.522 [1.086–2.135] p = 0.015 for trend). In addition, among subjects classified as "low-risk" (CV risk < 20% at 10 years corresponding to < 9% at 4.3 years) by Progetto Cuore, a ROC curve analysis for ARIC (vs. MACE) allowed the identification of a threshold of 0.22, which had a positive predictive value for 4.3-year MACE of 9%. Applying the ARIC score (with a threshold of 0.22) to Progetto Cuore "low risk" subjects, we could classify as "at high risk" 89.8% of subjects with incident MACE vs 79.6% with Progetto Cuore only.

Conclusions In patients with ED, identifying pre-diabetes, even with algorithms, predicts long-term CV events.

P73

Semen Parameters in Estonian Fertile Men

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Introduction Reduced semen quality is commonly accepted as a major cause of couple subfertility and infertility. The definition of male subfertility based on semen analysis has been extensively discussed over the last years. Recent (2010) WHO manual for semen analysis includes for the first time reference values for basic human semen parameters. As substantial regional differences are well-known in semen quality in both fertile and general populations studied so far, thus universal reference intervals should be used with caution.

Therefore we undertook a study of reproductive parameters in fertile Estonian men – partners of pregnant women – to analyze suitability of WHO reference ranges for our population and to compose a control group for future studies on male reproductive diseases.

Material & Methods Male partners of pregnant women were invited, during 2010 and 2011, to participate in this study. A total of 3175 pregnant women got invitation for their partners to participate in our study. 907 (28,6 %) men agreed to complete the questionnaire, modified version of European study (Jørgensen et al. 2001) and 284 from them (31,4%) also accepted to deliver semen sample, blood for tests of reproductive hormones and basic biochemical profile, passed STD tests, genital examination and analysis of body composition. Three persons were excluded from the study since the pregnancies were achieved after assisted reproduction. Therefore final number of study subjects was 281.

Semen analysis was performed according to WHO 2010 guidelines. Physical examination of the male participants was performed by two andrologists.

Results The mean age of the 281 men was 32 years. Basic clinical findings and genital and chronic diseases are shown in **Table 10**.

Table 10. K. Pomm et al. Clinical Findings of the Fertile Estonian Men.

Parameters	Mean ± SD	Median
Age (years)	32 (6.7)	31
Height (cm)	181 (6.2)	181
Weight (kg)	83.5 (13.2)	82
BMI	25.6 (3.8)	25
Testicular volume (ml)[a]	23.52 (4.8)	23.3
Genital and chronic diseases		
– Varicocele, (n%)	71 (25)	
– Cryptorchidism, n (%)	2 (0.7)	
– Cryptorchidism operated, n (%)	3 (1)	
– Testicular cancer operated, n (%)	2 (0.7)	
– *Chlamydia trachomatis*, n (%)	4 (1.4)	
– Ureaplasma urealyticum, n (%)	11 (3.9)	
– HIV, n (%)[b]	1 (0.4)	
– Diabetes, n (%)	1 (0.4)	
– Hypertension, n (%)	16 (5.7)	
– Hypothyreosis, n (%)	1 (0.4)	

[a] Mean of left and right testis, measured by use of Prader's orchidometer
[b] Patient is on the antiviral treatment

Mean values and 5th–95th percentile of basic semen parameters were as following: sperm concentration 78 mill/ml and 12–235 mill/ml; total sperm count 304 mill and 48–980 mill; progressive motility 52% and 28–70%; normal morphology 10% and 2–20%.

There were significant differences in sperm concentration, progressive motility and proportion of ideal spermatozoa and also FSH/LH ratio and testosterone level between groups of men categorized into TTP < 4 and TTP > 12 months.

Conclusion Preliminary analysis indicates that the results of basic semen parameters of Estonian fertile men are in substantial accordance with WHO 2010 reference values.

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P74

The Expression of Metalloproteinases 9 and 2, and their Tissue Inhibitors 1 and 2 as a Potential Markers of Asthenozoospermia

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Introduction 2 types of metalloproteinases: MMP-9 and MMP-2, and their tissue inhibitors TIMP-1 and TIMP-2, have been studied in human ejaculates [1, 2]. Our study was aimed to examine the expression of MMP-9, MMP-2, TIMP-1 and TIMP-2 in the different groups of subfertile men. These parameters may contribute to further understanding of the mechanisms of male infertility serve as additional diagnostic markers of semen quality and reproductive potential.

Material & Methods The ejaculates were collected from men living in childless couples, prepared for insemination. Seminal plasma was obtained as a spare fraction of the insemination procedure.

From 195 samples, 123 were normozoospermic, 15 oligozoospermic, 26 asthenozoospermic and 22 oligoasthenozoospermic, according to WHO criteria [3]. The reference group enrolled apparently fertile normozoospermic men (n = 9).

In all the samples MMP-9, MMP-2, TIMP-1 and TIMP-2 levels were determined using ELISA tests (R&D Systems), and the protein level with Bradford's method. The results have been presented as the content of MMP/TIMP [mg] per mg of the total seminal plasma protein.

Statistical analysis was done using STATISTICA 10.0 (StatSoft, Poland).

Results The expression of MMP-9 was elevated in all groups of childless men, when compared to the fertile subjects. The difference occurred relevant in asthenozoospermic, but not oligozoospermic subjects. No significant differences were found in MMP-2 expression among all analyzed groups.

Both TIMP-1 and TIMP-2 level were lowered in all the groups of childless men when compared to the physiological samples. In normozoospermic, asthenozoospermic and oligoasthenozoospermic groups the difference was significant for TIMP-1, and in normozoospermic and asthenozoospermic groups for TIMP-2. The latter was also significantly lower in asthenozoospermic vs. oligozoospermic group.

Conclusions Only MMP-2 expressed no variability among the studied groups. The

remaining three analyzed proteins expressed some relevant difference between the fertile and subfertile men, related rather to the reduced motility than the lowered count of spermatozoa. Thus metalloproteinase activity and its regulation through endogenous inhibitors may be involved in maintaining the proper motility of sperm cells.

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P75

The Impact of Paternal and Maternal Smoking on Reproductive Parameters of Adolescent Men

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Maternal smoking during pregnancy has been found to have negative impact on the sperm counts of sons. Sufficient data on the effect of paternal smoking are lacking. We wished to elucidate the impact of smoking (parental during pregnancy and own) on reproductive function of the male offspring. Semen parameters including sperm DNA integrity were analyzed in 310 adolescents from the general Swedish population. Serum was analyzed for reproductive hormones. Information on smoking was obtained from the Swedish Medical Birth Register and questionnaires. The impacts of maternal, paternal and own smoking were evaluated in a multivariate (I) model with all types of exposure tested at the same time and in a stratified (II) model with only those having one type of exposure. For both models, paternal smoking was associated with lower sperm numbers, sperm concentration being 44% lower (95%-CI: 16%, 62%) (model II) and total sperm count 32%/50% lower (model I/II) (95%-CI: [I] 6.3%, 51%, [II] 23%, 68 %). Maternal smoking was only significantly associated with sperm concentration in model II (36% lower [95%-CI: 3.0%, 58%]). We conclude that paternal smoking during pregnancy was negatively associated with sperm counts of the sons.

P76

Spermatogonial Expansion in Marmoset Testes

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Introduction Problems at all stages of spermatogenesis contribute to human infertility. We are aiming to elucidate whether male infertility can be related to dysfunction of germline stem cells. Using marmosets (*Callithrix jacchus*) as a primate animal model we want to explore the starting point and premeiotic expansion during spermatogenesis byin vivoadministration andin vitroexposure of BrdU and use of testicular organ culture.

Materials & Methods BrdU labeling: 6 adult male marmosets received injections of BrdU (IP, 100 mg/kg) either 2h (n = 3) or 11 days (n = 3) before tissue collection. The testes were dissected under sterile conditions and some tissue was fixed in Bouin's solution. The remaining tissue was transferred into ice cold Leibovitz medium for organ culture.

Organ Culture The testes are teased into tubule fragments which are placed onto membranes (pre size: 8 µm) in the interface of air and medium and cultured for 2 hours and 1, 3, 5, 9, and 11 days in low-glucose DMEM medium (supplemented with antibiotics and non-essential amino acids) at 5% CO_2, 35°C. rhFSH and/or hCG were added to defined wells. BrdU exposure (100 µM) occurred constantly or for the last 2 hours prior to fixation of the tissue.

Immunohistochemical staining of whole-mounts and paraffine sections: An antibody against BrdU was used to localize proliferating cells in cross sections (5 µm) by routine immunohistochemical staining (fluorescence or enzymatic). Whole mounts were subjected only to immunofluorescence.

Results Organ cultures with seminiferous tubules revealed a goodin vitrosurvival as the structure of tubules and the organization of the seminiferous epithelium remained almost normal until day 11. Germ cells remained viable and progressed normally. A subfraction of the Fractions of spermatogonial population and of the preleptotene spermatocytes are BrdU-positive and present as many small cohorts of proliferating cells in whole mounts. The analysis of sections and whole mounts allows description of the spatial organization, numerical identification and determination of labelling indices of spermatogonial subpopulations. Meiotic and postmeiotic germ cells were abundant while the number of premeiotic germ cells declined at later timepoints in organ cultures without hormones. BrdU labeled cells could be traced in organ cultures until postmeiotic stages.

Conclusion We were successful in maintaining functional tissue for long periods in organ culture. This provides us with an advanced approach and a new model to study the initial events during spermatogenesis.We evaluated that the clonal and spatial organization of proliferating cells in marmosets mimics the situation in man showing a high number of progenitor cells and no synchrony in the initial division of premeiotic germ cells.

P77

Oxidative Protein Damage and Oxidative Stress Markers in the Seminal Plasma of Subfertile Men

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Introduction Although low levels of reactive oxygen species (ROS) are essential for acrosome reaction and the other fertilisation events [1], the oxidative stress is indicated among the agents responsible for disturbed male fertility. It is defined as disturbed balance between ROS and antioxidants, leading to a damage of many macromolecules and disruption of their function [1, 2].

Our aim was to estimate the efficiency of an antioxidative system of seminal plasma: the ability to reduce excessive free radicals by endogenous antioxidants and the level of oxidative protein damage markers.

Materials & Methods Semen samples of normozoospermic (N; n = 123), oligozoospermic (O; n = 15), asthenozoospermic (A; n = 26) and oligoasthenozoospermic (OA; n = 22) men living in infertile couples were obtained from patients, attending the clinic for insemination procedure.

The spectrophotometric methods were applied to determine Ferric Reducing Antioxidative Power (FRAP) with ferric tripyridyltriazin complex [3], the level of Advanced Oxidation Protein Products (AOPPs) with potassium iodide [4], and the level of SH groups with Ellman's reagent [5].

Results The level of AOPPs was higher in N and O groups and significantly decreased in both A and OA seminal plasma. Moreover, the AOPPs concentration in OA group was also significantly lowered when compared to A group. Similar distribution of values was found for the other marker of the oxidative protein damage, the SH level.

The lowest antioxidative potential measured by means of FRAP level was observed in the A group. The value was slightly higher in the OA samples and significantly increased in N and O subjects. Decreased motility of sperm may be thus related to the reduced antioxidative power, although in these samples the proteins seem not to be so much affected with the oxidative damage.

Conclusions The analysis of oxidative stress and oxidative protein damage markers in seminal plasma, have shown significant differences in AOPPs levels between analyzed groups, thus indicates the potential useful-

ness of AOPPs in the identification of patients with different fertility problems and may serve as preliminary diagnostic marker.

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P78

Mixed Testicular Atrophy Related to Atherosclerosis: Further Lessons from the ApoE(–/–)/LDL receptor(–/–) Double Knockout Mouse Model

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Introduction Age-related testicular changes are associated with declining spermatogenesis and testosterone levels. A relationship to atherosclerosis has never been investigated systematically. The ApoE(–/–)/LDL receptor (–/–) double knockout (KO) mouse model, providing a remarkable homology to human atherosclerosis, is an ideal tool to investigate spermatogenetic alterations in this context.

Material & Methods Mouse tissue: Paraffin and EPON embedded as well as contrast agent-perfused testes from KO mice and wild-type mice at the age of 20, 40, 60 and 80 weeks were used. Contrast agent-treated ones were scanned with micro-CT to quantify whole testis and total vascular volume fraction. Subvolumes of the testes were also analyzed. Total length, volume and surface area of the capillaries were estimated in semithin sections of EPON-embedded tissue using computer program newCAST. Moreover, spermatogenesis was analyzed by spermatogenetic scores. Sperm counts were quantified in the epididymis. Testosterone levels were determined in serum.

Human tissue (clinical studies): In 30 patients undergoing testicular sperm extraction (TESE) because of azoospermia, peak systolic velocity (PSV) of the testicular artery (supratesticular) as well as PSV of intratesticular arteries was assessed by ultrasound.

Results KO mice exhibit diminished testis and total vascular volume fraction with respect to that of controls. Data also provide evidence for a change of total length, volume and surface area of capillaries. These findings were associated with a reduction of testosterone levels. Mixed atrophy was present in various seminiferous tubuli in KO mice at the age of 80weeks. Sperm counts from the epididymis demonstrated a significant decrease in KO mice.

In men PSV of the testicular artery was 8.0 ± 2.4 cm/sec, and 4.5 ± 1.3 cm/sec for intratesticular arteries.

Conclusions Mixed testicular atrophy in KO mice is linked to reduced testis volume, vascular volume fraction and low testosterone serum levels, suggesting a direct relation between atherosclerosis and disturbed spermatogenesis.

Further evaluation of testicular perfusion in human tissue is necessary to find possible associations with TESE outcomes.

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P79

Effects of In Utero Exposure to Environmental Agents on Human Fetal Testis Development and Function Using a Xenograft Approach

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Background Disruption of the human fetal testis by endocrine disruptors has been postulated as a cause of testicular dysgenesis syndrome (testicular germ cell tumors, cryptorchidism, hypospadias, low sperm counts). In-utero-exposure of rats to oestrogens, phthalates or paracetamol have demonstrated that all reduce fetal testis testosterone production. We have shown that xenografting of human fetal testis tissue results in normal testis development/function and that this represents a system in which to investigate the effects of proposed endocrine disruptors on testosterone production and testis development in the human fetus.

Objectives To determine the effect of exposure to diethylstilboestrol (DES), di-n-butyl phthalate (DBP) and paracetamol on testosterone production and germ cell development in the human fetal testis using the xenografting approach.

Methods We xenografted testis tissue from human fetuses (14–20 weeks gestation, n = 3–13) subcutaneously into castrate male nude mice. Host mice were treated with DES (0.1 mg/kg/day), DBP (500 mg/kg/day), paracetamol (350 mg/kg/day) or vehicle for 1–6 weeks. Testosterone production by the grafts was determined by measurement of host seminal vesicle (SV) weight and serum testosterone. Immunohistochemical analysis was performed to investigate steroidogenesis and germ cell effects of treatments.

Results Host SV weights were significantly reduced in paracetamol exposed grafts compared to vehicle exposed controls (9.87 vs 7.63 mg, p = 0.01), whereas exposure to DES or DBP had no effect on testosterone production as indicated by host animal SV weight, serum testosterone or 3b-HSD expression. Testosterone independent effects that altered germ cell numbers or aggregation were found in the DBP exposed xenografts, whilst the germ cell effects of exposure to DES and paracetamol are currently under investigation and will also be presented.

Conclusions These results suggest that testosterone production by the human fetal testis may be reduced by in-utero-exposure to paracetamol but not by exposure to DBP or DES; testosterone-independent germ cell effects can also occur. These results have important health and regulatory implications for determining the risk of in-utero exposure to these agents and also show that the rat may not always prove a reliable model for human fetal testis effects.

P80

Non-Linear Association between Androgen Receptor CAG Repeat Length and Reproductive Parameters in Fertile European and Inuit Men

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Introduction Male reproductive function is critically dependent on androgen action. It is generally assumed that there is an inverse linear association between parameters of male reproductive function and androgen receptor (AR) CAG /GGN repeat lengths. Recent experimental data as well as analyses of AR genotype in relation to fertility status, cryptorchidism and hypospadias risk have challenged this view. We have now aimed to analyse the pattern of association between a broader range of parameters of male reproductive function and AR CAG/GGN repeat length in fertile men.

Material & Methods Semen and blood were collected from 587 partners of pregnant women from Greenland, Warsaw (Poland), and Kharkiv (Ukraine). A total of 21 reproductive parameters including serum hormone levels, semen characteristics, and markers of accessory sex gland function were measured. CAG and GGN repeat lengths were determined by direct sequencing of leukocyte DNA. CAG and GGN lengths were categorised (CAG: ≤ 21, 22–24, ≥ 25; GGN: ≤ 22, 23, ≥ 24) and the associations between CAG/GGN category and reproductive markers were analyzed in a linear regression model, adjusted for country, age and abstinence period. Non-linear relationships were visually confirmed using a penalised spline regression model, which adjusts to the distribution of the data and not an á priori given model.

Results Following categorisation of the CAG and GGN lengths, in comparison to the reference CAG length (22–24), the short CAG (≤ 21) group had statistically significantly higher levels (mean difference (β) ± SD, 1.18 ± 1.07 mg/ejaculate; p = 0.021) of seminal prostate-specific antigen (PSA). The same was true for neutral α-glucosidase (NAG) (β ± SD, 1.17 ± 1.06 mU/ejaculate; p = 0.007), DNA fragmentation index (β ± SD, 1.19 ± 1.07%; p = 0.007) and expression of the apoptosis marker FAS (β ± SD, 6.72 ± 3.35% positive cells; p = 0.045). In the long CAG (≥ 25) group increased levels of estradiol (β ± SD, 1.11 ± 1.04 pmol/L; p = 0.011) and NAG (β ± SD, 1.29 ± 1.07 mU/ejaculate; p < 0.001) were seen. No significant associations were found between GGN and the markers when compared to the median length of 23. No interactions between CAG and GGN in relation to the reproductive markers were observed. A linear association was only found between inhibin and GGN length (β ± SD, -5.74 ± 2.23 ng/L; p = 0.020).

Conclusion These data confirm the hypothesis that, at least, for some markers of male reproductive function the association with CAG lengths is non-linear.

P81

Differentiation of Seminal Leukocyte Subpopulations by Flow Cytometry

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Introduction & Objective Infections and inflammation of the male genital tract are known to affect reproductive function. The presence of peroxidase-positive (pox+) leukocytes as well as polymorphonuclear (PMN) elastase in the ejaculate have been established as surrogate diagnostic markers. To date, however, systematic investigations concerning the prevalence and putative significance of different subpopulations of seminal immune cells are scarce. Therefore, we analyzed the distribution of leukocyte subpopulations in human semen measured by flow cytometry (FACS) in men with and without genital tract inflammation.

Methods In this study*, semen samples of 96 men from different clinical collectives were included: patients undergoing andrological work-up for infertility or various urogenital pathologies (n = 58); patients with HIV infection (n = 25), patients with metabolic syndrome (n = 6), and men before vasectomy (n = 7).

From each patient an aliquot of native ejaculate (500 µl) was prepared for FACS analysis. Cells were fixed with paraformaldehyde (in PBS) and stained with specific direct-fluorescent antibodies (CD45; CD14; CD24; CD3; CD181) in order to identify seminal leukocytes and their subpopulations. Cells were counted by BD FACSCanto™ Flow Cytometer and the obtained data were analyzed with FACSDiva 6.1.3.

Results CD45+ leukocytes were detected in a wide range of 0.5–90% of total cell counts (median 19); these results correlated with pox+ cell counts and seminal PMN elastase. Among CD45+ cells, the most frequent cell type were PMN (36%) followed by macrophages (13%), and lymphocytes (3%). This overall distribution pattern of seminal immune cells was found in all investigated groups including HIV-infected men, independent of the categorization of samples according to pox+ cell counts or elastase levels. However, the detection rate of leukocyte subpopulations showed a wide variation across the samples; in individual specimens, the proportion of macrophages exceeded or equalled that of PMN, whereas samples with significantly higher numbers lymphocytes are rare.

Conclusions Our results confirm previous reports, that PMN represent the dominant leukocyte subpopulation in the majority of human semen samples of different clinical origins. Other patterns are observed only in a minor subset of patients. Further FACS analyses including "functional" markers as well as correlation of the data with other parameters used to detect genital tract inflammation, i.e. cytokines, might allow to define profiles for specific urogenital tract disorders.

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P82

Comparative Analysis of Morphological Changes in Mice Testes Omparative Analysis of Morphological Changes in Mice Testes Following Nano- and Micro-Dispersed Manganese Oxide (III, IV) Effect

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Due to growing contacts of a human to nanomaterials including nanoparticles of heavy metals that are common in environmental objects there are arising highly topical problems of their effect on different organs and reproductive ones in particular.

The aim of work was the comparative analysis of testes alterations as a result of oral administration of nano- and micro-dispersed manganese oxide (III, IV).

Synthesis of nano-dispersed manganese oxide (III, IV) solution was carried out in the Laboratory of Multiphase Disperse Systems at the Institute of Technical Chemistry, UB RAS. Determination of particle concentration in a solution was made in the Analytical Laboratory for Nanomaterials and Physical Factors at the Department of Chemical-Analytical Methods of Investigations ("Federal Scientific Centre for Medical and Preventive Health Risk Management Technologies"). Aqueous micro-dispersed solution of manganese oxide (III, IV) was used for comparison.

Experiment used 30 wild-type male white mice with the mass of 27 ± 2 g (M ± m) were divided in 3 groups (each with 10 animals). Nano-dispersed manganese oxide (III, IV) was once orally introduced via probe to mice of the 1st group.

Mice from the 2nd group received micro-dispersed manganese oxide (III, IV) while complying with similar conditions. Both substances were used in a dose of 3500 mg/kg in the form of aqueous solution. Animals of the 3rd group (control) received water in the same way based on the same calculations. Period of observation was 14 days. Histological sections of mice gonads (from all of 3 groups) were stained with hematoxylin and eosin.

Following the application of micro-dispersed manganese oxide the spermatogenesis did not significantly differ from the standard values in most sections of convoluted seed tubules. The spermatozoon number was reduced in the minority of slices. Moderate expansion and plethora of large blood vessels was observed. Leydig cells were located as small clusters in interstitial connective tissue.

After the administration of nano-dispersed manganese oxide the number of mature gametes in convoluted seed tubules was found to be sharply lowered as compared with the previous group.

In addition, there were tubules with cell dissociation, sustentocyte detachment from basal membrane and spermatogonia number decrease. Marked widening and plethora of not only large but microcirculatory bed vessels was noticed. Leydig cells were less detected and were located in isolation. Therefore, single oral introduction of nano-dispersed manganese oxide (III, IV) to mice resulted in more pronounced changes in mice testes such as spermatogeny suppression and marked vascular disorders.

Postersession 5: Genetics and Epigenetics

P83

Mutation Studies in the CFTR Gene in Asian Indian Subjects with Congenital Bilateral Absence of Vas Deferens: Report of 2 Novel Mutations and 4 Novel Variants

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Background Congenital bilateral absence of vas deferens (CBAVD) is a form of male infertility in which mutations occur in the cystic fibrosis transmembrane conductance regulator (CFTR) gene. The molecular basis of CBAVD is not completely understood, especially in developing countries.

Methods We characterized the mutations/variants in the CFTRgene by single strand conformation polymorphism followed by sequencing in 35 CBAVD patients. None of the patients had systemic manifestations of cystic fibrosis. Fifty normal subjects were studied as controls.

Results Mutations/variants in CFTR gene were found in all CBAVD patients. 5 mutations and 10 variants were detected in 35 patients. The most frequent severe mutation was F508del (34.2%) and most common variant was IVS8-5T (54.2%). 2 novel severe mutations (p.E217Gfs*11 and p.A1285V) and 4 novel variants (pT438A, c.4095þ30insCT, c.-737G > A, and c.2909-92A > G) were detected.

Conclusion The protocol for identification of mutations in cases of CBAVD in developing countries would have to include a different set of mutations than those reported from western countries.

P84

Molecular Analysis of Sex Chromosomes in 47,XYY Men and Patients with Klinefelter's syndrome and/or Sex Chromosome Polysomy

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We evaluated 59 males, among them 50 Klinefelter's syndrome (KS) patients with following karyotypes: 47,XXY (n = 42), 47,XXY, t (3;8) (q23;p21) (n = 1), 47,XY+derX (n = 1), mos 46,XY/47,XXY (n = 3), 48,XXYY (n = 1), mos 47,XXY/48,XXXY/46,XY (n = 1), mos 46,XX/47,XXY (n = 1); 4 mosaics with disomy or polysomy Y (mos 45,X/46,XY/47,XYY (n = 2), mos 45,X/46,XY/47,XYY/48,XYYY, mos 45,X/46,XY/47,XYY/48,XYYY/49,XYYYY); and 5 47,XYY infertile men.

Chromosome analysis was performed using standart GTG-staining technique. Molecular study included the analysis of AZF microdeletions (PCR amplification of 19 STS loci of the Y chromosome) and CAG-repeat of exon 1 of the Androgen Receptor (AR) gene, and X chromosome inactivation (XCI) assay.

Y chromosome microdeletions were detected in 8 of 59 (13.6%) patients. Ñomplete AZFñ (b2/b4) deletion was found in 2 mosaics (mos 45,X/46,XY/47,XYY and mos 45,X/46,XY/47,XYY/48,XYYY). No "classic", complete Y chromosome microdeletions and incomplete (partial) AZFa and AZFb deletions were detected in KS patients. Partial AZFc deletions (b2/b3, n = 4; gr/gr, n = 1; delsY1197 and sY1206, n = 1) were found in 6 of 50 (12%) KS patients including 48,XXYY patient, also in 1 of 5 47,XYY infertile men and in the patient with mosaic tetrasomy Y. AZFc deletions were detected in 4 of 9 individuals who had cell line(s) with ≥ 2 Y chromosome in the karyotype.

AR CAG-repeats number varied from 16–30, but alleles with 26–30 (high) repeat number were detected in 16% KS patients. Homozygosity and heterozygosity for AR-allele were revealed in 16 and 27 of 43 individuals presented two and more X chromosomes in the karyotype, respectively. XCI analysis was done in 20 heterozygous KS patients. Skewed XCI with the rate 70–80% was found in 15% 47,XXY individuals. Furthermore 20% examined KS patients presented XCI rate that near to skewed (67-69%).

Klinefelter syndrome is not associated with "classic" AZF deletions. Relatively high prevalence of partial AZFc deletions in KS and di-/polysomy Y is requires further large cohort studies. No strong correlation was revealed between KS patients with or without Y chromosome microdeletions, normal or longer AR CAG-alleles, and random/skewed XCI. However less severe spermatogenesis defects (oligozoospermia) was detected only between KS patients with normal CAG-repeats, not-skewed XCI and with no AZF deletion. The patients with higher number of AR CAG repeat, skewed XCI or Y chromosome microdeletions presented secretory azoospermia.

P85

Human Catalase c-262t Polymorphism and Male Infertility

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Purpose Infertility is an inability to conceive after one year of unprotected intercourse. Male factor is solely responsible nearly 50%. It has been reported that reactive oxygen species contributed to pathogenesis of various disease. To inactivate ROS cells biosynthesize several antioxidant enzymes, One of them is Catalase which contributes H_2O_2 to H_2O and O_2. The aim of this study was to investigate the association of Catalase C-262 T polymorphism with male infertility.

Methods Inthis study we evaluated the distribution of a functional polymorphism in the gene for Catalase C-262T SNP in northern Iran. The study included 51 patients and 42 healthy volunteers. Genomic DNA was prepared from peripheral blood leukocytes. Genotypes and allele frequencies were determined in patients and controls using allele-specific PCR (AS-PCR).

Results Statistical analysis has not emerged significance differences from the comparison of either genotype (p > 0/05).

Conclusion We conclude that the catalase gene -262C >T polymorphism does not conter a protective effect with respect to male infertility.

P86

Molecular Markers of Male Infertility: Single Nucleotide Polymorphism of Protamine-1 and -2 Genes and Human Sperm Protamine Deficiency

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Introduction Male infertility can be associated with sperm chromatin abnormalities resulting from abnormal nucleotide sequence of protamine genes (PRM1, PRM2), expression of these genes and translation of the protamines. The defects lead to protamine deficiency and can trigger the decrease of fertilizing ability of sperm cells and impair development of embryo in natural and assisted procreation or cause spontaneous recurrent abortion. The purpose of this research was to determine the incidence of spermatozoa with abnormal protamination and to find single nucleotide polimorphisms (SNPs) in PRM1 and PRM2.

Material & Methods The study was performed on ejaculated spermatozoa of men with normal (n = 173) and abnormal (n = 189) standard sperm parameters (WHO 2010). The sperm protamine deficiency was studied by means of chromomycin A3 (CMA3) and was identified with a fluorescence microscope. DNA samples were extracted from semen samples. Sequencing of PCR products of the genes revealed 2 SNP in PRM1 (c.-190C > A, rs2301365, promoter region; c.139C > A, rs737008, exon 2, synonymous

polymorphism, p.Arg47Arg) and 1 SNP in PRM2 (c.*62G > C, rs79674436, 3'UTR).

Results The proportion of CMA3-positive sperm cells was significantly higher in men with abnormal sperm parameters as compared to subjects with normal sperm characteristics (23% vs 19%; Mann-Whitney test). A significant negative correlation (Spearman rank correlation) was found between the incidence of sperm showing deprotamination and sperm concentration, morphology and motility. In the case of c.-190C > A 41.49% and 44.79% were homozygous (CC), 38.30% and 43.75% were heterozygous (CA), 20.21% and 11.46% were heterozygous (AA) type, in the c.139C > A 4,44% and 6.67% were CC, 37.78% and 30.0% were CA, 57.78% and 63.33% were AA type and in the c.*62G > C 97.46% and 97.52% were GG and 2.54% and 1,24% were GC type in men with normal and abnormal sperm characteristics, respectively. In the case of c.-190C > A among man with 1–25% and with > 26% CMA3-positive sperm cells 38.89% and 30.43% of subjects were characterized by the CC genotype, 41.67% and 52.18% were CA, 19.44% and 17.39% were AA, in the case of c.139C > A 4.17% and 2.18% were CC, 34.72% and 23.91% were CA, 61.11% and 73.91% were AA, respectively. Moreover, in the case of c.*62G > C among man with 1–25% and with > 26% CMA3-positive sperm cells 98.61% and 93.48% of subjects were carriers of the GG genotype, 1.39% and 6.52% were GC, respectively.

Conclusions This preliminary report suggests that low sperm parameters seems to be accompanied by sperm chromatin defects expressed as abnormal DNA protamination. It should be emphasized that rare polymorphism c.*62G > C causing disturbances of protamine translation was identified mostly in man with > 26% CMA3-positive cells.

P87

From Sperm to Early Embryo Stages: Potential Functions of Acetylated-Histone-Bound Genes for Idiopathic Male Infertility (a Bovine Model)

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Introduction Microarray studies revealed that nucleohistones in spermatozoa associate with gene promoters relevant for early development. We suggest that epigenetic aberrations in sperm cause incorrect post-fertilisation gene activation and may explain the idiopathic male infertility. Here we aimed to identify relevant risk genes using a bovine model.

Material & Methods Immunohistochemistry (IHC) was applied to confirm histones in bovine sperm. Native chromatin immunoprecipitation (NChIP) and ChIP-Sequencing were performed to view the overall histone-bound gene status in bovine sperm. Histones and protamines in NChIP fractions were detected by western blot. Cross-linked ChIP (XChIP) against H3K9ac and ChIP-Seq were performed to preselect risk genes. mRNA expression and promoter methylation status of selected genes in bovine germ cells and early embryos were analyzed by qRT-PCR and combined bisulfite restriction analysis (COBRA). Single-nucleotide polymorphism (SNP) analyses were used to determine the paternal allele expression in zygote. Histone-bond relations and promoter methylation status of bovine imprinted genes were also analyzed.

Results Nucleohistones in bovine sperm were confirmed by IHC. Western blot also confirmed the presence of histones and protamines, respectively, in the appropriate NChIP-fractions. Preliminary NChIP result showed that 5–10% of DNA is related to histones in bovine sperm. Subsequent ChIP-Seq data reveal an enrichment (80%) of histone-associated DNA within intragenic regions (repetitive sequences, non-coding regions). 20% of mapped DNA show association to gene regions, thereof 5% to gene promoters. Analyzing DNA-enrichment on H3K9ac, we identified in bovine sperm first candidate risk genes for early embryo development including several Ca^{2+}- and apoptosis-regulators. 10/12 analyzed genes exhibited CpG-promoters and seven of them were unmethylated in bovine sperm. qRT-PCR showed that ten genes are expressed already in the zygote stage and SNP analyses confirmed the paternal allele expression. Methylation analyses on bovine imprinted genes with CpG- promoters showed that maternal imprinted H19 and MEG3 have highly methylated, MEST and IGF2R unmethylated promoters in sperm. All analyzed paternal imprinted genes with CpG-promoters (PEG10, IGF2, NNAT, NAP1L5 and XIST) were unmethylated. Especially, IGF2 and IGF2R showed association of their promoters to histones.

Conclusions Our study confirms the hypothesis that sperm histones tag genes activated in early embryos and presents the first candidate risk genes for early development. These genes will be validated in further studies on human fertile versus infertile sperms.

P88

Mutations in the NR5A1 Gene Encoding Steroidogenic Factor 1 Cause Spermatogenic Failure

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Introduction The Steroidogenic Factor 1 protein (SF1), encoded by the NR5A1 gene, plays a central role in gonadal development and steroidogenesis. Male Nr5a1 null mice exhibit adrenal agenesis and testicular dysgenesis. Mutations in NR5A1 have first been described in patients with primary adrenal insufficiency and 46,XY disorders of sexual development (DSD) and recently also in men with hypospadias, bilateral anorchia and micropenis as well as women with primary ovarian insufficiency. In 2010, Bashamboo et al. found missense mutations in NR5A1 in 4% of infertile men with unexplained reduced sperm counts. Since all mutation carriers found were of non-European ancestry, we performed a comprehensive mutation analysis in a large group of infertile German men.

Subjects & Methods NR5A1 was sequenced in 488 patients who attended the Department of Clinical Andrology, Centre of Reproductive Medicine and Andrology, University Clinic Muenster, a tertiary-referral centre for infertility. Only patients with severe oligozoospermia (sperm concentration < 5 Mill./ml, n = 218) or azoospermia (n = 270) were included. Major causes for male infertility (karyotype anomalies, Y-chromosomal AZF deletions, oncologic disease and/or chemo-/radiotherapy) were excluded. To explore an association of NR5A1 mutations and disturbances of testicular decent, patients with history of maldescended testes were included (n = 186).

Results 5 previously undescribed NR5A1 mutations were found in the 488 patients (1%). All were missense mutations leading to amino acid substitutions and predicted to be damaging to SF1 protein function. 3 were found in men with severe oligozoospermia (3/218, 1.4%) and 2 in those with azoospermia (2/270, 0.7%). One mutation (c.493G > C, p.Gly165Arg) was found in a man with azoospermia caused by complete bilateral meiotic arrest seen in his testicular biopsy. One patient carrying a different mutation (c.968T > C, p.Ile323Thr) had severe oligozoospermia with a sperm concentration of 0.2 Mill./ml. Interestingly, one mutation – c.769G > A, p.Asp257Asn – was found recurringly in 3 patients. While 2 of these men had severe oligozoospermia and 1 had azoospermia, all three had cryptorchidism (2 bilateral, 1 unilateral) as children. No mutations in NR5A1 were found in 237 normozoospermic controls.

Conclusions Missense mutations in NR5A1 can be considered a novel cause for spermatogenic failure with the phenotypic spectrum encompassing cryptorchidism, severe oligozoospermia and azoospermia.

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P89

Y chromosome-linked CNVs in the Spanish Population

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Introduction The Y chromosome is singular for the accumulation of a high proportion of segmental duplications which provides the structural basis for the generation of CNVs. AZF deletions are the most common molecular genetic cause of male infertility and it became a routine diagnostic test in severe oligozoospermia/azoospermia. The AZFc region is particularly prone to rearrangements affecting gene dosage inside this region.gr/grdeletion (a type of partial AZFc deletion) has been proposed as genetic risk factor for impaired spermatogenesis mainly in Caucasian populations. The role of partial AZFc duplications remains unexplored since data are restricted to the Italian and Chinese populations. Here we present a comprehensive analysis of Y-linked CNVs in Spain.

Materials & Methods 754 patients were screened for Y microdeletions (according to the EAA Guidelines). 288 idiopathic infertile and 241 normozoospermic controls were tested for partialAZFc deletions/duplications. The screening for partial deletions/duplications was performed through STS ± analysis followed by quantitative/qualitative analysis of DAZ and CDY1 copies. Y haplogroup definition was performed by analysing 6 binary markers.

Results Y microdeletions were found in 3.3% of the patients. The highest frequency, 8.5%, was found in idiopathic azoospermic men.gr/grdeletions were significantly more frequent in patients versus controls (4.2% and 1.5% respectively; $p < 0.05$) while b2/b3 deletions were detected only in patients. DAZ/CDY1gene dosage and RFLP analysis allowed to determine: (1) a significantly higher frequency of subjects with 2 DAZ copies in the infertile group ($p < 0.05$); (2) the lack of association of specific DAZ/CDY1copy combinations with semen phenotype. Although the frequency of partial duplications was higher in patients (5.3%) than in controls (3%), the difference was not significant. A Y haplogroups distribution was similar between cases and controls allowing to rule out population stratification bias.

Conclusions This is the largest screening for Yq microdeletions performed in a study population attending an infertility clinic in Spain providing conclusive data on the prevalence of this genetic anomaly. gr/gr deletion resulted a significant risk factor for infertility also in Spain. In addition, the AZFc gene dosage allowed us to establish that: (1) the reduction ofAZFc gene copy number independently from the type of partial deletion has a significant impact on spermatogenesis; (2) increased gene dosage is more frequent in infertile men, supporting the hypothesis about the importance of "optimal" gene dosage. Further enlargement of the study population is ongoing in order to obtain conclusive data also on the role of AZFc partial duplications in spermatogenesis.

P90

The PARP2 Gene is Associated with Male Subfertility and Prostate Cancer Protection

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Introduction Prostate cancer (PCa) is a one of the most common malignancies in men. Previous studies have indicated an association between male subfertility and a reduced risk of prostate cancer, giving the hypothesis that testicular dysfunction might alter the androgenic stimulation of the prostate tissue. The aim of this study was to investigate the genetic background of this relationship.

Material & Methods Candidate genes were selected based on previous GWAS studies reporting associations to prostate cancer or male subfertility. Thirty men categorized as subfertile, 403 fertile men with PCa and 394 fertile men without PCa were identified using data from the population based Malmö Cancer and Diet Study. Genotyping was performed using the Sequenom iPLEX Gold assay and MassARRAY MALDI-TOF platform (Sequenom Inc., San Diego, CA). Odds ratios (OR) and 95% confidence intervals (CI) for the associations were calculated using logistic regression models.

Results For polymorphisms in the genes PARP2 and RPPH1, the results showed a negative association to prostate cancer and a positive association to male subfertility (OR 2.83, 95%-CI: 1.04–7.67 for PARP2 and OR 3.44, 95%-CI: 1.63–7.23 for RPPH1), as well as an overall association to male subfertility (OR 3.07, 95%-CI: 1.12–8.43 for PARP2 and OR 2.80, 95%-CI: 1.34–5.84 for RPPH1).

Conclusion Polymorphisms in the PARP2 gene and its neighbour gene RPPH1 were found to be associated with male subfertility as well as decreased PCa risk. PARP2 is believed to be activated by DNA strand breaks and is important in normal spermatogenesis, whereas RPPH1 encodes RNA that makes up the RNA unit in RNase P, an endoribonuclease involved in the maturation of tRNAs. These genetic mechanisms might, at least partly, explain the inverse association between male subfertility and PCa risk.

P91

Polymorphisms in the Follicle-Stimulating Hormone Receptor Affect Receptor Activity In Vitro

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Introduction Follicle-stimulating hormone (FSH) regulates gametogenesis through binding to its receptor (FSHR). Although the Thr307Ala and Asn680Ser polymorphisms in the FSHR gene affect reproductive function in both men and women in vivo, so far in vitro studies have failed to show differences in FSHR activity between the polymorphic receptor variants. The aim of the present study was, using a broader FSH concentration range than previously tested, to examine whether the Thr307Ala and Asn680Ser polymorphisms affect the FSHR activity in vitro in COS-1 cells.

Material & Methods Variants of the FSHR were cloned and transfected into COS-1 cells. Cells were stimulated with 5, 10, 20 and 50 mIU/mL rFSH (Gonal-F) and cAMP concentration in the culture medium was measured and adjusted for total protein concentration.

Results There were no differences in cAMP concentration between the Thr307/Asn680 receptor variant and the Ala307/Ser680 receptor variant, when cells were stimulated with 5, 10 and 20 mIU/mL rFSH. However, in response to 50 mIU/mL rFSH, the Thr307/Asn680 receptor variant displayed a two times higher cAMP response compared with the Ala307/Ser680 receptor variant.

Conclusion In accordance with in vivo findings, we found that the Ala307/Ser680 receptor variant exhibits notably lower activity in vitro when cells were stimulated with doses of rFSH outside the physiological range. This may, at least partly, explain the need for higher FSH doses needed for ovulation stimulation prior to in vitro fertilisation, and also higher FSH levels and marginally lower sperm counts reported in subjects with this FSHR variant.

P92

Sperm Y:X Chromosome Ratio and Exposure to Persistent Organic Pollutants in Young Men From the Faroe Islands

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Introduction Decline in birth sex ratio has been observed during recent decades in several countries and it has been suggested that

exposure to endocrine disruptors (EDs) might be the underlying cause. The present study sought to investigate whether exposure to dichlorodiphenyl dichloroethene (p,p´DDE), dichlorodiphenyl trichloroethane (p,p´DDT) or polychlorinated biphenyls (PCBs) had an effect on the distribution of sex chromosomes in sperm cells.

Methods The sperm Y:X chromosome ratio was established by 2 color fluorescent in situ hybridization (FISH). p,p´DDT and a mixture of known PCBs were measured using a gas chromatographic system in n = 241 Faroe men, 24–27 years of age. In n = 27 cases not enough sperm for the FISH analysis was available. Linear regression as well pairwise comparisons of exposure levels stratified into 25th percentiles were performed. All analyzes were adjusted for age, abstinence time and smoking.

Results The efficiency of the FISH method was 98.1% and the Y:X chromosome ratio of the Faroe population was 0.501 (0.498–0.504, 95%-CI). There was a positive linear association between Y:X chromosome ratio and log transformed p,p´DDT (p = 0.030), but not for PCB. When exposure data was stratified into quartiles, no differences in Y:X chromosome ratio were found regarding p,p´DDT, while the 4th PCB exposure quartile had a significantly lower X:Y ratio (0.488; 0.478–0.498, 95%-CI) than both the 2nd (0.502; 0.493–0.510, 95%-CI; p = 0.047) and the 3rd (0.504; 0.492–0.516 95%-CI; p = 0.038).

Conclusion This study indicates that exposure to p,p´DDT leads to an increase in the sperm Y:X chromosome ratio whereas exposure to PCBs lead to a decrease in sperm cells from young Faroe men. Thus, changes in sperm Y:X chromosome ratio may be linked to ED exposure and subsequent alterations in birth sex ratio.

P93

Decreased Transactivating Capability due to a Deletion in the Transactivating Domain of the Androgen Receptor Gene is Associated with Testicular Cancer and Male Infertility

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Background A deletion of the amino acid leucine at codon 57 (Del L57) in the androgen receptor (AR) has been reported in a man with testicular cancer. Our objective was to investigate the impact of this deletion on AR activity in vitro and its prevalence in patients with testicular cancer or male infertility, both conditions are supposed to be related to subnormal androgen activity.

Methods Leukocyte DNA was extracted and the AR gene directly sequenced in n = 476 patients with testicular cancer and n = 406 patients with infertility problems and n = 322 samples from the general population. Hormone concentrations (Testosterone, FSH, LH, InhibinB, SHBG, estradiol) and sperm parameters were measured. The mutation was cloned into pCMV4 and transiently co-transfected into COS1-cells together with a luciferase reporter gene driven by the PSA-promoter, which is an AR target gene, in the absence or presence of 10 nM of the synthetic testosterone R1881. A mock transfected AR served as control.

Results The L57 deletion was detected in 2 infertile men (0.5%), 2 men with testicular cancer (0.4%) and in 1 control (0.3%). Hormonal levels and sperm parameters in these individuals were within the ranges found in the general population. The transactivating capability of the Del-L57 was 67% of the wild-type AR.

Discussion Despite lack of any obvious signs of androgen insensitivity among the subjects included in current study, this deletion hampers AR function in vitro. Our findings support the hypothesis that decreased androgenic activity in males during critical time-windows in gestation may be associated with risk of testicular cancer and reproductive function in adulthood.

P94

Array-CGH Studies In Male Infertility: Deletion Burden on the X-Chromosome and Sex Chromosomes-linked CNVs

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Introduction The etiology of male infertility can be related to genetic factors in about 15% of men with spermatogenic disturbances, but still a consistent portion of so-called "idiopathic cases", plausibly concealing a genetic origin, remains unexplained. Copy Number Variations (CNVs) have progressively drawn the attention in relation to various complex human diseases, thus raising the question whether such structural variants play a role in male infertility as well. In order to better understand the contribution of CNVs to male infertility, we used a high resolution array-CGH approach to fulfil two purposes: (1) to identify novel X-linked variants potentially linked to male infertility; (2) to determine whether men carrying Y chromosome microdeletions display structural defects in the PARs, as well.

Materials & Methods For the first aim, 96 infertile men with different grade of spermatogenic impairment (49 azoospermic, 25 cryptozoospermic and 22 oligozoospermic men) and 103 normozoospermic men were analyzed with a high resolution array-CGH platform customized for the X chromosome. Selected losses were validated by PCR (±) or TaqMan Assays and then screened in a case/control setting (359 infertile vs 370 normozoospermic men). For the second aim, we examined 22 infertile men with different types of Y-chromosome microdeletions and 12 men with an intact Y chromosome referred to as controls. CNVs identification was performed by the abovementioned array-CGH platform and then carried on by quantitative PCR of selected PAR genes (SHOX, PLCXD1).

Results Regarding the first aim, we found 73 CNVs, of which 55 are novel, providing the largest collection of X-linked CNVs in association with semen phenotype. Twelve deletions were patient-specific, thus suggesting their potential relevance from a clinical standpoint. The salient finding of our study is a significantly higher global "deletion burden" in patients compared to controls due to an excessive rate of deletions/person (0.57 vs 0.21, respectively; $p = 8.785 \times 10^{-6}$) and to a higher mean sequence loss/person (11.79 Kb and 8.13 Kb, respectively; $p = 3.435 \times 10^{-4}$). As for the second aim, we assessed the presence of PAR defects only in those Y microdeletion carriers presenting abnormal karyotype (i. e. mosaicism), whereas no PAR aberrations were found in carriers with a normal karyotype and in the controls.

Conclusions We propose the observed "deletion burden" as a new genetic factor that may mirror a vaster genomic instability, with potential implications to not only reproductive but also general health. Our preliminary data on PAR defects are discordant from a previous finding of PAR CNVs in men with Y microdeletions, indicating that Yq deletion carriers are unlikely to be at risk for developing PAR-related pathologies.

P95

Is Abnormal Karyotype the Essential Reason to Cancel Azoospermic Men From Testicular Biopsy?

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Background Infertility is described as the inability of a couple to conceive after 1 year of unprotected intercourse. The present is1 in6 couples. Male factor is implicated near 50% and genetic factors – chromosomal abnormalities – are recognized in 5.8% (Guidelines EAU, 2012).

Material & Methods During 2008–2011 a group of 338 azoospermic men (mean age 32,5 yrs) had regular andrological evaluation by WHO/EAU guidelines. The needle percutaneous testicular biopsies from both gonads (adapted method by [Hendricks, Fertil Steril 1969]) were performed under short iv anesthesia. All specimens were analyzed by pathologist using simplified classification for

IVF-ICSI procedure: Present Sperm (PS), Maturation Arrest (MA), Sertoli Cell Only syndrome (SCOs) and Tubular/Peritubular Fibrosis (TPF). Genetic test – karyogram of human male using Giemsa staining – had 138 (41%) patients.

Results Proper male karyotype – 46,XY – had 123 men (89.1%). Pathologist evaluations of testicular specimens were as follow: PS-73 (59.3%), MA-17 (13,8%), SCOs-30 (24.4%) and TPF-3 (2.4%). Non-homogenous abnormal karyotypes were recognized in 15 (10,9%) patients and pathologist results were: PS-7 (46.7%), MA-3 (20%), SCOs-5 (33.3%).

Conclusions

1. Chromosomal abnormalities were recognized in 11% in our group of azoospermic men.

2. Proper male karyotype is connected with present sperm in testicular biopsies near 60%.

3. Abnormal male karyotype gives the probability to find sperm in gonadal specimen near 47% then final exclusion from testicular biopsy procedure should take into consideration completely andrological evaluation of azoospermic men.

P96

Lack of Evidence for a direct Association of Y chromosome Microdeletions in Children with Testicular Maldescent

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Objective Testicular maldescent (TMD) represents the most common congenital abnormality in humans. Furthermore, normal control of spermatogenesis is regulated by genes located at the euchromatic region of the Y chromosome long arm (Yq11). It is currently clear that Yq11 microdeletions (Yq11-md) represent the most frequent genetic aetiology of severe testiculopathy among infertile patients. The association of Yq11-md with TMD and related infertility has been specifically evaluated in a limited number of studies with inconclusive results. The aim of the study was to investigate the hypothesis that Yq11-md are directly implicated in testicular maldescent.

Materials & Methods Genomic DNA was extracted from the peripheral blood of 292 subjects. This population consisted of (1) 180 children with all phenotypes of isolated (non-syndromic) testicular maldescent from 174 index families, (2) affected adult relatives available (n = 12), and (3) 100 unrelated children with normal external genitalia (controls). The sequence tagged site primer set and the conditions of conventional polymerase chain reaction amplification were based on the current laboratory guidelines for molecular diagnosis of Y chromosome microdeletions recommended by the European Academy of Andrology and the European Molecular Genetics Quality Network. 2 multiplex reactions were designed to screen AZFa, AZFb, and AZFc regions. Each multiplex reaction included adequate internal and external amplification controls. Amplification products were submitted to electrophoresis on 2% agarose gel impregnated with ethidium bromide dye solution for 80Vhrs and visualized under ultraviolet light.

Results No microdeletions were detected in any subject.

Conclusions These results indicate that Y chromosome microdeletions are not directly implicated in the pathogenesis of testicular maldescent. Other factors should be investigated to potentially explain the genetic predisposition that seems to exist in at least a subgroup of these patients.

P97

Influence of Androgens on the Differentiation of Human regulatory T cells

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Introduction Regulatory T cells (Tregs) are able to inhibit proliferation and cytokine production in the effector T cells and play a major role in immune responses and prevention of development of autoimmune diseases. The experimental autoimmune orchitis (EAO) is a model of chronic testicular inflammation. Our previous results have shown supplementation of EAO rats as well as rat splenic T cells in vitro with testosterone causes differentiation of Tregs characterized by increased expression of the transcription factor Foxp3. Foxp3 expression is under epigenetic control and it is known that common transcription factors are involved in Foxp3 regulation as well as in the methylation status by binding theFoxp3locus. In the present study, we aim to investigate the effect of androgens on the expression, regulation and methylation status of human Foxp3 and putative interaction with androgen receptor (AR).

Material & Methods Human T cells were isolated from fresh blood of patients with decreased testosterone concentration by magnetic beads and cultivated in vitro in the presence of testosterone or DHT. The Foxp3 staining was analyzed by flow cytometry. The detection of the AR expression in Treg cells was performed by immunohistochemistry and RT-PCR. The non-classical pathway of the AR signal transduction was analyzed by Western Blot, using antibodies against MAP kinases. Genomic DNA was used as a template to amplify Foxp3 promoter fragments by PCR. The luciferase reporter gene assay was performed to analyze a putative binding site of the AR to the Foxp3locus.

Results After the treatment of female T cells with DHT we detected significant increase in the Foxp3 expression T cells from male patients with a metabolic syndrome showing low testosterone levels, displayed an elevated Foxp3 expression after DHT treatment. No activation of the Foxp3 expression in T cells from healthy male donors could be observed. Human Treg express the AR and 5-α reductase, which is important to convert testosterone into DHT. The AR acts probably through the classical and not the non-classical pathway to activate the Foxp3 gene. After induction of Foxp3 by testosterone or DHT no changes in the phosphorylation of the classical MAP kinases could be observed. Furthermore, a potential direct binding of the AR to the Foxp3 locus with the luciferase reporter gene assay in human HEK 293 cells will be investigated. Our preliminary results show that the AR is able to bind and activate the Foxp3 promoter.

Conclusions Our preliminary results show that androgens are able to activate the Foxp3 expression not only in the rodent but also in human Treg cells which could to exert an immunomodulatory effect on the immune homeostasis of the testis. The activation of the Foxp3 protein can probably be affected by binding of the AR to the Foxp3 gene. This binding could possibly induce a change in the methylation status of the Foxp3 gene. To determine precisely the binding region and changes in the methylation status of the Foxp3 gene, further experiments need to be performed.

P98

Recurrent X Chromosome-linked Deletions: Discovery of New Genetic Factors in Male Infertility

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Introduction Both sex chromosomes are enriched with genes prevalently or exclusively expressed in the testis. Nevertheless, only Y-linked CNVs have been demonstrated to contribute to spermatogenic impairment in humans, whereas whether the X chromosome contains AZF-like regions remains unknown. In fact, up to now only a few "rare" X-linked deletions/duplications have been reported in the literature and given their rarity only exceptionally large study populations could confirm their contribution to oligo/azoospermia. Through our first X-chromosome specific array-CGH screening of 199 subjects, we identified 3 recurrent deletions (frequency > 1%). Here we perform a case-control association study in order to explore the role of such deletions in oligo/azoospermia.

Material & Methods The 3 deletions of interest, named CNVX1, CNVX2, and CNVX3, all mapping to the Xq, were studied by a multi-step PCR plus/minus method includ-

ing the main screening and confirmatory steps. Idiopathic infertile men (excluded all known causes) were strictly selected and compared to normozoospermic controls. The study populations included Italian (60%) and Spanish (40%) subjects and for each CNV we analyzed: (1) 494 cases and 491 controls (CNVX1); (2) 591 cases and 590 controls (CNVX2); (3) 359 cases and 390 controls (CNVX3).

Results All 3 deletions were found with a frequency significantly higher in patients than controls. CNVX1 resulted the most recurrent in both the patient (6.7%) and the control group (3.8%) ($p < 0.05$; OR: 1,726. 95%- CI: 0.996–2.993) followed by CNVX3 detected in 4.2% of patients and 1.5% of controls ($p < 0.05$; OR: 2.716; 95%-CI: 1.065–6.924) and CNVX2 which was exclusively found in patients (1.2%). The analysis of flanking regions does not reveal Segmental Duplications, therefore NHEJ is the most likely mechanism for the formation of these deletions. The breakpoint's definition is ongoing and will help define the recombination substrate. The deletions do not remove directly coding genes, with the exception of CNVX2, which could affect the expression of one gene belonging to the cancer testis antigen family. CNVX1 and CNVX3 may also affect the activity of a number of genes expressed in the testis localized at < 0.5 Mb from the deletion.

Conclusions In this study, based on two independent study populations of different geographic origin, we provide strong clues supporting the involvement of two deletions CNVX2, and CNVX3 in the etiopathogenesis of spermatogenic impairment. Our most relevant data, based on 1182 subjects, is the discovery of an AZF-like region on the X-chromosome which is specific to men with impaired sperm production. The associated phenotype ranges from azoospermia to severe oligozoosperrmia and similarly to Y-linked deletions it may become a new diagnostic test.

P99

The Molecular Role of Tcd1b in Transmission Ratio Distortion

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Males heterozygous for the t-haplotype (t/+), a variant form of the mouse chromosome 17, transmit the t-haplotype at a high ratio to their offspring (99%). This phenomenon, termed Transmission Ratio Distortion (TRD), is caused by the cooperative effect of several t-complex distorters (Tcds 1–4) and the single t-complex responder (Tcr) on sperm motility. Previously, we have identified Tcr, as a catalytically compromised protein kinase (Smok) and the distorters Tcd1a, Tcd2a and Tcd2b which encode Rho protein regulators.

Here we show that Tcd1b, encoding a Rho guanine nucleotide exchange factor (GEF), is differentially expressed in t-haplotype testis. Overexpression of its wild-type and long form reduces the transmission rate of the th49 haplotype. A loss-of-function allele also affects the transmission rate of the partial tw18 haplotype. The combined data clearly demonstrate that Tcd1b acts as a distorter.

P100

Treatment of Patients with a Late Diagnosis of Klinefelter's Syndrome with Testosterone undecanoate for a Duration of up to 5 years

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Introduction Males suffering from Klinefelter's Syndrome (KS) experience an increased hospitalization rate from a variety of disorders, some caused by hypogonadism, and some linked to the syndrome per se, others not readily explained, maybe socioeconomic. The degree of hypogonadism is variable with the vast majority below the normal range. Mortality in KS is significantly increased, with excess deaths due to diabetes, cardiovascular, respiratory, and gastrointestinal diseases. KS is markedly underdiagnosed with less than 10% of cases identified by puberty and less than 20% ever diagnosed during life.

In the present study we describe a cohort of men suffering from KS, diagnosed at advanced age. The majority of the patients had been referred by an orthopedic surgeon after a diagnosis of osteoporosis to be checked for hypogonadism.

Methods Open-label, single-centre, cumulative, prospective registry study of 22 middle-aged men with testosterone levels between 2.1 and 3.5 ng/mL (mean: 3.08 ± 0.46). 21 men were studied for at least 2 years, 18 for 3 years, 11 for 4 and 8 for at least 5 years. They received parenteral testosterone undecanoate 1000 mg/12 weeks after an initial interval of 6 weeks.

Results After 5 years the following changes were observed: Testosterone (ng/mL) increased from 3.08 ± 0.46 to 4.86 ± 0.47 ng/mL ($p < 0.0001$). Body weight (kg) decreased from 105.05 ± 11.18 (minimum: 70, maximum: 139) to 90.88 ± 9.76 ($p < 0.0001$ vs baseline). Waist circumference (cm) declined from 104.32 ± 6.39 to 96.50 ± 6.63 ($p < 0.0001$ vs baseline). The mean T-score increased from –2.91 ± 0.31 (min –3.90, max –2.60) to –1.50 ± 0.28 ($p < 0.0001$ vs baseline). Glucose levels remained unchanged with mean levels below 100 mg/dL at all time-points. Total cholesterol decreased from 240.95 ± 18.89 to 177.75 ± 16.69 ($p < 0.0001$), LDL from 124.09 ± 46.4 to 64.13 ± 18.13 ($p < 0.0001$) and triglycerides from 235.09 ± 22.07 to 180.75 ± 7.59 ($p < 0.0001$). HDL did not change significantly.

Conclusions Raising serum testosterone to normal in patients with a late diagnosis of Klinefelter's syndrome improved body composition including bone mineral density. Other features of the metabolic syndrome were also improved.

P101

The Muenster EXAKT Project: Cardiovascular Risk Factors in Klinefelter Patients and Healthy Controls

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Background & Aim Klinefelter syndrome (47,XXY; KS) is a very common chromosomal disorder, affecting 1:600 men. Klinefelter men have been described to exhibit clinically relevant metabolic patterns related to a pro-inflammatory status, resulting in a high prevalence of insulin resistance and cardiovascular impairment. Testosterone deficiency in form of primary hypogonadism is a common feature in these men.

EXAKT (Epigenetics, X-Chromosomal features and clinical applications in Klinefelter's syndrome Trial) is a Muenster-based prospective project involving Klinefelter patients (n = 130), and their parents assessing a wide area of cardiovascular, inflammatory and metabolic factors as well as sex steroids and questionnaires in comparison to age-matched healthy male and female controls (2 × n = 50). A broad range of genetic and epigenetic investigations completes the approach.

Here, we present first and novel clinical data comparing Klinefelter patients to healthy male controls with regard to cardiovascular and metabolic parameters.

Results KS patients had a higher waist circumference and Body Mass Index in comparison to controls. Further on, decreased insulin sensitivity, higher levels of triglycerides and lipoprotein type a as well as lower concentrations of HDL-cholesterol were found in patients. Levels of high-resolution C-reactive protein were elevated in Klinefelter patients. Consequently, the prevalence of the Metabolic Syndrome according the harmonized criteria was markedly higher in Klinefelter men than in controls (52/130 vs 5/50). Corroboratingly, carotid artery intima-media thickness was increased and flow mediated dilatation of the brachial artery was decreased in patients vs controls. These differences were statistically significant. Metabolic disadvantages of patients were further enhanced by low testosterone concentrations and already present in the sub-cohort younger than 40 years.

Conclusion The EXAKT study revealed an unfavorable pattern of cardiovascular risk factors in KS in comparison to healthy male controls. This picture is already present in younger patients and enforced by testosterone deficiency.

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P102

Muenster-EXAKT: X-Chromosome Inactivation in 2 Klinefelter-twin Pairs

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Introduction Klinefelter's syndrome (KS) with an incidence of 1 in 500 males is one of the most frequent numeric chromosome aberrations, leading to infertility in 97% and clinically relevant metabolic patterns related to a pro-inflammatory status, resulting in a high prevalence of insulin resistance and cardiovascular impairment. Testosterone deficiency (primary hypogonadism) is a common feature in KS. Identical twins with KS have first been described in 1958. The twinning rate among KS patients was found to be between 5.4 and 8.8 %. The aim of this study was to elucidate whether epigenetic patterns are similar between twins or contribute to phenotypical variations.

Materials & Methods 2 twin pairs with karyotype 47,XXY and 48,XXXY, respectively were examined clinically by ultrasound of thyroid and testes, measuring bone density and skeleton age, and analysing semen and blood samples includinghormone parameters. The methylation profile of the genes XIST, FMR1 and KDM6A was evaluated by pyrosequencing and compared to results in 50 healthy male and 50 female controls.

Results Both twin pairs had sparse head and body hair growth, testicular volume was 1 ml each. The 48,XXY twins were mentally retarded. Besides different physical impairments and depression, testosterone deficiency was found in both twin pairs. After Testosterone therapy both twin pairs were mentally more ballanced and showed an increase in body hair growth and muscle mass. The 48,XXXY twins differed regarding phenotype and physical impairments and a differing methylation profile was found.

XIST methylation analysis in the 47,XXY twins revealed 68% methylation which equals the methylation profile of healthy women, but differed significantly from healthy men. For FMR1, a gene that is silenced due to the process of X-inactivation, 49 % methylation was found, which equals women-like methylation status. In the 48,XXXY twins XIST and FMR1 methylation differed from the 47,XXY twins and healthy female controls: XIST-methylation (50%) was lower in the 48,XXXY twins whereas the methylation of FMR1 (66,75 %) was higher compared to the 47,XXY twins and female controls. This finding is indicative to the presence of 2 inactivated X-chromosomes in the 48,XXXY twins. KDM6A, a gene escaping X-inactivation, showed a very low methylation status in all groups, indicating that this gene escapes X-inactivation in the 47,XXY and 48,XXXY twins.

Conclusion This is the first description of epigenetic patterns of the X-chromosome in two KS twin pairs with XXY or XXXY karyotype. X-inactivation did not differ among these karyotypes indicating that X-chromosome inactivation in these KS patients worked correctly. However, genes escaping X-inactivation could contribute to the observed phenotype.

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P103

The Muenster EXAKT Study: Microarray Gene Expression analysis in Blood Samples of Klinefelter Patients

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Introduction With an incidence of 0.2%, Klinefelter's syndrome (KS; karyotype 47,XXY) is the most common sex chromosomal disorder in men. Patients are mainly characterized by hypergonadotropic hypogonadism, and amongst other features, germ cell loss that results in infertility, is typical. However, KS patients exhibit a heterogeneous phenotype and many metabolic disturbances can be associated with this condition. The EXAKT study (Epigenetics, X-Chromosomal features and clinical Applications in Klinefelter's syndrome Trial) is a prospective project involving Klinefelter patients and their parents assessing a wide area of cardiovascular, inflammatory and metabolic factors as well as a broad range of genetic and epigenetic investigations. To elucidate a putative gene-dosage effect of the supernumerary X-chromosome as a cause for the Klinefelter phenotype, the global gene expression levels were analyzed in blood samples from KS patients and compared to healthy men.

Material & Methods RNA was isolated from blood samples of 35 KS patients and 15 healthy male controls. Microarray experiments were performed using the Affymetrix Gene 1.0 ST Chip. Data analysis was carried out using Partek Genomic Suite software and IPA (Ingenuity). Selected microarray results were validated by quantitative Real-Time PCR.

Results 36 genes were found to be differentially expressed between Klinefelter patients and healthy men. Among these genes, 9 are located on autosomes, whereas all other genes are located on the X and Y-chromosome, most of them in the pseudoautosomal region 1 (PAR1). The X-chromosomal genes that were upregulated in KS patients were predominantly genes, which escape the process of X-inactivation. Pathway analysis showed that most of the autosomal genes clustered in a network influenced by β-estradiol.

Conclusion The global gene expression analysis in blood samples of Klinefelter patients revealed that "escapee" genes are expressed higher in KS patients when compared to male controls. This indicates that these genes do escape X-inactivation as they do in healthy women, rendering them as candidate genes responsible for the altered phenotype observed in KS patients. The association of some differentially expressed autosomal genes with β-estradiol suggests a novel role of this hormone in the pathophysiology of Klinefelter Syndrome.

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Postersession 6: New Horizons in Andrology & Testicular Cancer

P104

Fibronectin on the Surface of Human Spermatozoa: A New Marker of Sperm Quality

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Introduction To identify a new marker on the surface of human spermatozoa used in sperm selection for assisted reproductive techniques (ART) and application of less invasive methods such asimmunomagnetic cell sorting method (MACS) for sperm selection. In this regard, presence of fibronectin (FN) on the surface of sperm was evaluated in correlation with sperm quality.

Methods Normozospermic semen was collected from 15 couples with female factor infertility. Normal sperm separated through density gradient centrifugation (DGC). The incubated sperm with rabbit anti FN antibody was loaded on MACS column. The three populations of sperm (crude, bounded and unbounded) were evaluated for fibronectin content, vitality, chromatin maturity (CMA3) and chromatin integrity (SCSA) by flow cytometry.

Results The MACS column increased FN-positive sperm significantly in bounded compared to unbound and crude fractions (77.34% ± 10.35% vs 2.36% ± 2.16% and 12.45% ± 10.38%, respectively; p = stained immature chromatin (25.4% ± 8.23% vs 41.13% ± 10.9%).

Conclusion This study shows that fibronectin on human sperm surface correlated with chromatin integrity and maturity; therefore, it can be considered as a positive marker of human sperm quality. Further comprehensive studies on FN in relation to sperm quality will reinforce its application in male infertility and ART in future.

P105

NAMPT is Expressed and Released by Human Spermatozoa Depending on Maturation Stage

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Introduction The underlying mechanisms of infertility in obese men are not completely understood. Nicotinamide phosphoribosyltransferase (NAMPT), a key enzyme of NAD^+ metabolism is implicated in the regulation of apoptosis and was found in spermatocytes and spermatides of chicken testis. Accordingly, we aimed to analyze the presence of NAMPT in human seminal plasma (SP) and in spermatozoa, the function of NAMPT in male reproduction and whether or not NAMPT levels depend on BMI.

Material & Methods Semen was analyzed according to the WHO guidelines 2010. In SP and serum of 96 healthy donors (age 35.1 ± 11.6 years, BMI 27.0 ± 5.55 kg/m^2). NAMPT concentrations were determined by ELISA. NAMPT activity was determined in SP by measuring the conversion of ^{14}C-nicotinamide to ^{14}C-nicotinamide mononucleotide (n = 4). Density gradient centrifugation was performed with human semen to obtain spermatozoa with different maturation stages. Subsequently, spermatozoa were incubated for 3 to 24 h to measure NAMPT in the respective supernatants (n = 10). NAMPT distribution in spermatozoa was analyzed by indirect immunofluorescence and western blot to detect NAMPT in lysates of mature and immature spermatozoa (n = 14).

Results NAMPT concentrations in SP were approximately 100-fold higher than in serum (194 ± 165 ng/ml vs 6.12 ± 14.7 ng/ml). However, NAMPT concentrations did not correlate with semen quality. No significant differences of NAMPT concentrations in SP between normal-weight and obese men were found. NAMPT was detected in supernatant of both mature and immature spermatozoa: After 12 h and 24 h NAMPT levels in supernatant of immature sperm were significantly higher than in the supernatant of mature spermatozoa (2.67 ± 3.65 ng/ml vs 0.29 ± 0.26 ng/ml and 3.31 ± 4.17 ng/ml vs 0.50 ± 0.42 ng/ml) whereas viability was significantly lower (68.2 ± 15.7 ng/ml vs 80.0 ± 11.2 ng/ml and 61.8 ± 17.6 vs 76.7 ± 10.7 ng/ml). In immature spermatozoa, NAMPT protein was localized in the tail and connecting piece. Immature spermatozoa had significantly more relative NAMPT protein amount than mature spermatozoa (1 ± 0 vs 7.07 ± 14.2 a. u.).

Conclusion The obtained results indicate NAMPT is released by spermatozoa which appeared to be independent of viability. Further experiments could clarify if NAMPT according to its regulatory effects on NAD^+ levels has a function in spermatozoa energy metabolism and therefore an impact on semen quality.

P106

Testicular Peritubular Cells Influence Spermatogonial Stem Cells via the Extracellular Matrix Proteoglycan Decorin and may Contribute to the Spermatogonial Stem Cell Niche in Man

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Question Spermatogenesis in man is fueled by lifelong proliferation and differentiation of spermatogonial stem cells (SSCs). These cells reside in a niche formed by Sertoli cells, on one side, and the basal lamina (BL), which separates them from peritubular cells, on the other side. Recent results, namely the production of GDNF by cultured human testicular peritubular cells (HTPCs), strongly suggested that these somatic cells contribute to the regulation of SSCs and may act in concert with Sertoli cells [Spinnler et al., Hum Rep 2010]. Testicular peritubular cells of the human testis are not well explored, yet the development of a culture model has allowed new insights. Thus they produce, for example, decorin (DCN). This proteoglycan has structural roles in the extracellular matrix and, importantly, can interact with growth factors (GFs) and furthermore can act as a ligand for GF receptors, including EGFR [Adam et al., Hum Rep 2011]. This aspect may be of importance for the regulation of SSC. In the present study we sought to explore whether DCN is indeed a major secretory product of HTPCs, examined its expression in the basal lamina of seminiferous tubules and tested whether it can affect a male germline model, the human seminoma cell line TCam2.

Methods Proteomic analysis (LC-MS/MS) was performed using conditioned culture media of HTPCs of 3 patients. A testicular biopsy was used for visualizing DCN at the EM level with cupromeronic blue (CMB). Expression of GF receptors by TCam2 were analyzed by PCR/sequencing and especially EGFR by Western Blot. The influence of DCN on viability (ATP-level), apoptosis (Caspase3/7-level) and proliferation (DAPI staining) was examined.

Results Proteomic results showed that DCN is among the most abundantly secreted factors of HTPCs (top 25). EM revealed that DCN is present in the BL of human seminiferous tubules. TCam2 cells expressed all receptors of the EGF family and other GF receptors that are known targets of DCN. DCN, while not altering ATP level, significantly reduced the activity of caspases 3/7 and increased the number of mitotic TCam-2 cells in a concentration and time dependent manner. We are currently studying whether this is due to a direct activation of EGFR by DCN or whether other GF receptors are involved.

Conclusion Our preliminary results show that the abundant peritubular cell-derived factor DCN promotes survival and proliferation of TCam2 cells and thus may contribute to the SSC niche in man.

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P107

Assessment of Sperm Concentration and Viability by Automated Image Cytometry

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Introduction An increasing number of couples have trouble conceiving a child. For a large proportion of these couples, poor semen quality is the major reason. Semen quality is typically evaluated based on several variables, where sperm concentration are one of the most predictive measurement of fertility chances. Sperm concentration measurements are, according to WHO's guidelines, performed by manual counting and are hence labor intensive.

Methods Samples from men from infertile couples attending the andrology outpatient clinic at Department of Growth and Reproduction, Rigshospitalet, Copenhagen and young volunteers from the general Danish population were included in the study. Sperm concentration and vitality/viability was determined manually according to WHO's 2010 guidelines and by the automated image cytometers NC-3000 and SP-100 from ChemoMetec A/S.

Results Initially, we investigated the impact of several factors (pipetting, mixing, round cell content, sperm concentration), which influence the read-out as well as inter-operator and -cytometer variation on the two different image cytometers. Secondly, we measured a large range of semen samples both by manual WHO assessment and by image cytometry and showed a tight correlation along the identity line; manual assessment = automated assessment. Finally, we showed by repeated measurements that image cytometry produces more robust and accurate measurements than manual counting of human sperm concentration. In addition, assessment of viability on a large range of semen samples showed that the viability determined by image cytometers correlated with traditional vitality assessment by eosin-nigrosin staining.

Conclusion We conclude that image cytometry provides an appealing substitute of

manual counting by providing accurate, robust and easy measurement of human sperm concentration and viability.

P108

Seminal, Ultrasound and Psychobiological Correlates of Metabolic Syndrome in Male Subjects of Infertile Couples

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Introduction Metabolic syndrome (MetS) is a diagnostic category increasing the risk of cardiovascular and metabolic diseases, erectile dysfunction and male hypogonadism. However, so far, no study is available concerning its impact on male infertility. We evaluated possible associations between MetS, semen parameters, scrotal ultrasound, and psychobiological and sexual characteristics in a cohort of men seeking medical care for couple infertility.

Methods Out of 367 consecutive subjects, we selected a subset of 351 men without genetic abnormalities (karyotype abnormalities, Y microdeletions or uni- or bi-lateral absence of vas deferens). MetS was defined according to AHA/NHLBI classification. Erectile and ejaculatory functions were assessed using the International Index of Erectile Function-15 erectile function domain (IIEF-15-EFD) and the Premature Ejaculation Diagnostic Tool (PEDT), respectively. Psychological symptoms were investigated using the Middlesex Hospital Questionnaire (MHQ).

Results Out of 351 patients, 27 (7.7%) fulfilled MetS criteria. In an age-adjusted model, MetS was associated with a stepwise decline in testosterone (T) and SHBG (adj. r = –0.212, p < 0.0001 and adj. r = –0.136, p = 0.021, respectively), without a concomitant rise in gonadotropins. All main sperm parameters (concentration, total count, progressive motility and normal morphology) were negatively associated with increasing number of MetS components, even after adjusting for age. However, when testosterone was introduced as a further covariate in the model, only sperm morphology was still associated with MetS (adj r = –0.199; p = 0.001). In the same logistic model, among scrotal ultrasound parameters, only testis inhomogeneity, but not testis volume, was positively related to MetS components (HR = 2.924 [1.205–7.100]; p = 0.018). The risk of erectile dysfunction (IIEF-EFD score < 26) increased as a function of number of MetS factors, being the association confirmed after adjusting for age and T (HR = 2.864 [1.119–7.326]; p < 0.0001). No association between PEDT score and MetS was observed. Finally, after adjusting for age and T, only severe depressive symptomathology (fourth quartile of MHQ-D) was associated with MetS (HR = 2.703 [1.037–7.050]; p = 0.042).

Conclusions In a cohort of subjects seeking medical care for couple infertility, MetS isassociated with hypogonadotropic hypogonadism, characterized by poorer sperm morphology, testis inhomogeneity, low T, erectile dysfunction and depressive symptoms. Recognizing MetS could help the patients not only to improve fertility but also sexual and overall health.

P109

Seminal Apoptotic M540 Bodies Originate from the Testis: A Study in a Cohort of Infertile Subjects

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Introduction We have recently reported the presence in semen of round anucleate elements named "M540 bodies", resembling apoptotic bodies. The aim of this study is to investigate, in a cohort of infertile subjects, associations between their detection, other parameters of semen quality, and sonographic alterations of the male genital tract.

Methods A consecutive series of 130 males with couple infertility were evaluated, during the same day session, for clinical, scrotal and transrectal colour-Doppler-ultrasound (CDU) characteristics, hormonal and semen parameters, including interleukin 8 (sIL-8) and M540 body levels.

Results The average percentage value of M540 bodies was 24.6 ± 18.3. M450 body levels were negatively correlated with sperm number/ejaculate, progressive motility, normal morphology and sIL-8 levels, even after adjusting for possible confounders (age, waist, calculated free testosterone and smoking habit) (adj.r = -0.455, p < 0.0001; adj.r = –0.464, p < 0.0001; adj.r = –0.430, p < 0.001; adj.r = –0.236, p < 0.05, respectively). In a subset of patients with a history of cryptorchidism (n = 8), M540 bodies were higher than in non cryptorchid men (40.5 ± 14.8% vs 23.6 ± 18.2%; p < 0.02). A negative correlation was found between M540 and ultrasound testis volume, even after adjustment for confounders (adj.r = –0.241, p < 0.05), whereas a positive association was found with testis inhomogeneity (HR = 1.06 [1.02–1.09]; p = 0.002) and hypoechogenicity (HR = 1.05 [1.01–1.08]; p < 0.02) along with FSH levels (adj. r = 0.309; p < 0.01). No relationships were found with any CDU characteristic of the prostate, seminal vesicles, epididymis and vas deferens. In a multivariate model, testis inhomogeneity and history of cryptorchidism were independently associated with M540 body levels (adj.r = 0.355, p < 0.01 and adj.r = 0.223, p < 0.05, respectively). Receiver operating characteristic (ROC) analysis demonstrated that at the threshold of 27%, M540 bodies discriminate subjects with testis inhomogeneity with a sensitivity of 72% and specificity of 73%.

Conclusions These results strongly suggest that semen M450 bodies originate from the testis, as a result of deranged apoptosis due to an impairment of spermatogenesis. Overall, M540 bodies may be considered a semen marker of testis apoptosis.

P110

Sperm Oxidative DNA Damage Increases with Age even in Highly Selected Nellore Bulls

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Introduction Sperm quality decreases with age, and may be related to an unbalance between a higher production of reactive oxygen species and a decline in antioxidant production [Pasqualotto and Pasqualotto, 2011]. Studies indicate that ageing of the male mammals reproductive system may be characterized by higher rates of sperm DNA damage. This damages are well documented in humans [Hammiche et al. 2011; Pasqualotto and Pasqualotto, 2011] and rats [Zubkova and Robaire, 2006]. Nevertheless, the impact on highly selected animals, like bulls in an Artificial Insemination Centre is unknown. The aim of this study was to evaluate the existence of a positive correlation between age, sperm quality and DNA damage in highly selected Nellore cryopreserved semen samples.

Material & Methods 3 ejaculates from 31 (total 93 samples) highly selected and routinely collected Nellore bulls (3.5–14.3 years) from an Artificial Insemination Centre were evaluated for: computer-assisted sperm analysis (CASA), simultaneous evaluation of plasmatic membrane and acrosome integrity, mitochondrial potential and DNA oxidation damages by detection of 8-OHdG production (OxyDNA Kit, Biotrin, Ireland, GB). The correlation coefficient was calculated for the selected parameters.

Results The semen traits showed a reduced quality in ageing animals. Parameters considered desirable, such as total motility (r = –0.45), progressive motility (r = –0.37), high mitochondrial membrane potential (r = –0.35) and simultaneous membrane and acrosome integrity (r = –0.36) were negatively correlated with age (p < 0.0001). DNA damage caused by guanine oxidation measured through 8-OHdG production, were positively related to bull age (r = 0.63; p < 0.0001) and can be observed on **Figure 20**.

Conclusion The results indicate that an oxidative unbalance caused by ageing may affect even highly selected animals, causing a decrease in semen quality and DNA integ-

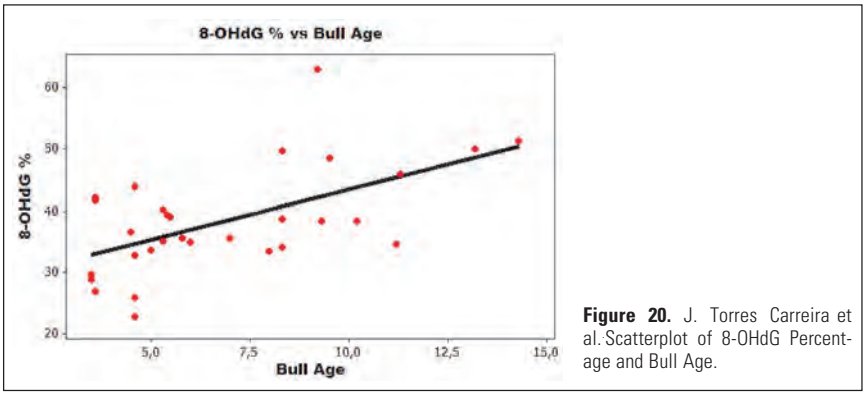

Figure 20. J. Torres Carreira et al. Scatterplot of 8-OHdG Percentage and Bull Age.

rity. Further studies are necessary to evaluate the impact of these damages on fertility.

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P111

Feasibility of the Testosterone-inducible CMG2 Protein as Biomarker for Prostate Carcinoma

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Introduction CMG2 (capillary morphogenesis gene 2) was identified as a gene, which is upregulated during vascularisation. High affinity of CMG2 protein to laminin and collagen type IV may influence development of basement membrane and morphogenesis of endothelial cells. The CMG2 protein occurs in 4 isoforms, 3 are membrane-bound and 1 is soluble and can be detected in serum. CMG2 was also identified as testosterone-inducible in the prostate carcinoma cell line HPr-1AR. Survival analyses of CMG2 mRNA expression revealed an association between a low CMG2 mRNA-level in the tumor and a decreased survival time of soft tissue sarcoma patients. The aim of this study was to measure CMG2 protein level in serum of prostate carcinoma patients (n = 260), other carcinoma patients (renal clear cell carcinoma and urothel carcinoma; n = 20) and control healthy men (n = 138), and to explore the feasibility as potential biomarker for prostate carcinoma as well as its diagnostic relevance.

Material & Methods The study was approved by the ethics committee of the university and study participants provided informed consent before entering the study. The level of soluble CMG2 protein in serum was measured by immunodetection with specific antibodies (ELISA, antibodies-online, Aachen, Germany). CMG2 protein expression was statistically correlated with clinical parameters. Correlation analysis was performed applying bivariate correlation according to Spearman-Rho, Chi-square-tests and Receiver Operating Characteristics. $P < 0.05$ was considered statistical significant.

Results The bivariate correlation according to Spearman-Rho showed a linkage between CMG2 protein expression in serum and general appearance of a tumor independent of prostate carcinoma ($p = 0.008$; $n = 418$). CMG2 was also correlated with metastasis of the prostate carcinoma ($p = 0.039$; $n = 418$). The Chi-Square-test confirmed these results, still the general occurrence of a tumor correlates with higher CMG2 serum protein level ($p = 0.015$). The Receiver Operating Characteristic (ROC) indicated the diagnostic relevance of CMG2 serum protein level as marker protein not specific for prostate carcinoma but for the general diagnosis of a tumor (AUC = 0.57; 95%-CI: 0.52–0.63).

Conclusion In this study we could not confirm CMG2 protein as serum biomarker for the specific diagnosis of prostate carcinoma. However, high CMG2 protein level correlate with the general occurrence of a malignant carcinoma and metastasis of prostate carcinoma.

P112

Molecular Influence of Androgens on Adipogenic Differentiation of human SGBS Preadipocytes

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Introduction Evidence-based studies demonstrate a clinically significant decrease in serum testosterone (T) with advancing age in males. This decrease is associated with a significant increase of adipose tissue mass and decreased insulin sensitivity. To understand the mechanism of action of testosterone on adipose tissue the effects of T on differentiation of progenitor cells to adipocytes are of special relevance. Several studies demonstrated the suppressive effect of the islet-specific miR-375 on adipogenic differentiation of mouse preadipocytes and its inhibiting action on the insulin release of human pancreatic beta cells and murine adiponectin receptor 2 (ADIPOR2). The aim of our study was to determine the effect of androgens on miR-375 and ADIPOR2 regulation in human preadipocytes.

Methods Human SGBS preadipocytes were cultured and adipogenic differentiation was induced for up to 14 d +/– T in a dose dependent manner. Dual-Glo® Luciferase Assay was performed to identify ADIPOR2 as a predicted target of the miR-375. mRNA expression of ADIPOR2 and miR-375 was measured by qRT-PCR. Western Blot analysis was performed for detecting protein expression of ADIPOR2.

Results We could demonstrate that ADIPOR2 is a direct target of miR-375. Cells were transfected with wild type (wt) and mutated (mt) 3´-UTR of ADIPOR2, and co-transfected with miR-375 mimics (25 and 50 nm). 25 nm of miR-375-mimic affected a decrease of about 40 % in relation to ADIPOR2 (wt). In contrast, transfection with miR-375 in relation to ADIPOR2 (mt) did not effect a change of the expression. Furthermore, qRT-PCR analyses revealed that T (100 nm) inhibits miR-375 expression (7-fold) in contrast to differentiated adipocytes without testosterone treatment, and increases ADIPOR2 expression (22-fold) in contrast to untreated adipocytes. Western Blot analyses exhibited similar effects on ADIPOR2 expression

Conclusion We conclude that T administration leads to decreased miR-375 expression and an increased ADIPOR2 expression in human preadipocytes and thereby inhibits adipogenic differentiation. These results may contribute to a better understanding of the mechanism of increase in visceral fat mass and the associated insulin resistance caused by testosterone deficiency.

P113

Androgen Receptor Expression in Stromal and Epithelial Prostate Cancer Tissue Specimen

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Introduction Prostate cancer (PCa) is one of the leading causes of tumor death in Western countries. Modifications in expression and functional alterations that involve the androgen receptor (AR) have been implicated in the progression of PCa and in the development of androgen independence; however, the role of AR in these processes is still debated, as contrasting results have been reported in several studies evaluating the relation between AR expression and disease pro-

Table 11. K. Ausmees et al. Age, Testicular size, BMI, Basic semen and Hormonal parameters in middle aged Males (healthy men vs male partners of infertile couples.)

	Healthy men (n = 61)	Male partners of infertile couples (n = 164)				
		Total (n = 164)		In couples with primary infertility (n = 95)	In couples with secondary infertility (n = 69)	
Characteristics	**Median (IQR)**	**Median (IQR)**	**p-value[1]**	**Median (IQR)**	**Median (IQR)**	**p-value[2]**
Age (years)	53.0 (49.0–56.3)	50.0 (49.0–54.4)	0.137	50.0 (49.0–53.0)	50.0 (49.0–53.0)	0.454
Testicular volume[3] (mL)	24.0 (22.0–25.0)	22.0 (18.0–25.0)	0.021	22.0 (18.0–25.0)	22.0 (18.5–25.0)	0.634
BMI (kg/m^2)	26.8 (24.7–29.5)	27.1 (24.9–29.3)	0.734	27.4 (25.0–29.9)	26.0 (23.9–28.2)	0.063
Basic semen parameters						
Semen volume (mL)	2.8 (2.1–4.3)	3.2 (2.0–4.5)	0.768	3.0 (1.7–4.4)	3.4 (2.3–4.7)	0.396
Total sperm count (×10^6)	241.5 (150.7–452.3)	97.8 (27.8–223.7)	< 0.00	96.9 (30.0–248.4)	98.8 (31.3–165.5)	0.914
Sperm concentration (10^6/mL)	89.0 (47.8–179.3)	29.5 (10.0–72.5)	< 0.001	33.0 (9.5–85.3)	26.0 (10.0–62.0)	0.550
Sperm A+B motility (%)	30.0 (18.8–32.3)	28.5 (16.0–42.0)	0.838	30.0 (17.3–42.8)	28.0 (13.8–41.3)	0.479
Normal sperm (%)	8.0 (2.8–9.3)	4.0 (1.0–7.5)	0.040	4.5 (1.0–10.0)	4.0 (1.0–6.0)	0.214
WBC in semen	0.0 (0.0–0.4)	0.1 (0.0–0.2)	0.768	0.1 (0.0–0.3)	0.1 (0.0–0.2)	0:621
Abstinence time (days)	5.0 (3.0–5.3)	4.0 (3.0–5.0)	0.207	4.0 (3.0–5.0)	4.0 (3.0–5.0)	0.197
Basic hormonal parametes						
Testosterone (nmol/L)	16.4 (12.7–20.6)	15.1 (11.2–19.8)	0.207	16.2 (11.0–25.5)	14.3 (11.5–18.9)	0.430
Estradiol (pmol/L)	145.0 (113.8–180.5)	94.3 (73.4–134.5)	< 0.001	101.0 (73.4–138.8)	90.5 (73.4–131.0)	0.848
FSH (IU/L)	5.1 (3.4–7.3)	5.3 (3.6–8.4)	0.150	5.3 (3.8–8.3)	5.2 (3.5–8.8)	0.693
LH (IU/L)	2.6 (1.9–3.7)	3.1 (2.2–4.6)	0.998	3.0 (2.2–4.6)	3.2 (2.1–4.6)	0.667
FSH/LH (IU/L)	1.8 (1.3–2.6)	1.9 (1.4–2.69)	0.386	1.9 (1.4–2.5)	2.0 (1.3–2.7)	0.952

IQR: interquartile range (25th–75th percentile); WBC: white blood cells; FSH: follicle stimulating hormone; LH: luteinizing hormone
[1] statistical difference between healthy and male partners of infertile couples (Mann-Whitney test); [2] statistical difference between male subjects in couples with primary and secondary infertility (Mann-Whitney test); [3] right+left testis/2

gression [Tamburrino et al, 2012]. Such evaluations are performed in PC specimens where, however, tumor tissue may be mixed to stromal and normal. There is now evidence in the literature that AR role in PCa may vary depending on its location. Indeed, studies performed in animal models [Niu et al, 2008] pointed out the different role of epithelial (protective toward a malignant phenotype) vs stromal (leading to tumor aggressiveness) AR in PCa. The present study was undertaken to evaluate AR expression in stromal and epithelial compartments of PCa specimens following careful microdissection.

Material & Methods Stromal and epithelial AR, EGFR, PSA and PTEN mRNA expression was analyzed in frozen in paraffin-embedded PCa specimens after laser microdissection of the stromal and epithelial compartments, according to a previously published protocol [Pinzani et al, 2008]. Microdissection has been performed collecting epithelial areas with different Gleason scores from the same specimens and the relative surrounding stromal tissue. As reference genes we used both RPL13a (coding for a ribosomal protein) and GAPDH. As positive and negative controls for AR we used mRNA extracted from LNCaP cells and HeLa cells, respectively.

Results So far we have analyzed 130 microdissected samples from 20 patients and further analyses are in progress. Preliminary results indicate that AR expression is correlated to that of EGFR in epithelial but not in stromal compartment. No correlation is found between AR and PSA in both compartments, although a trend to positive correlation is present in epithelial compartment for low levels of AR. PTEN expression tends to decrease and to become undetectable in high-grade tumors. AR expression appears to be lower in microdissected carcinoma areas with higher Gleason scores. In few patients with locally invasive tumors AR expression is higher in stromal respect to epithelial compartment.

Conclusions Evaluation of AR expression in microdissected PCa specimens may reveal new insights on the role of the steroid receptor in PCa progression.

P114

Reproductive Function in Middle-aged Males: Healthy Men versus Male Partners of Infertile Couples

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Introduction The issue of semen quality and fertility of middle-aged males receives increasing attention due to trends toward prolonged life expectancy and higher paternal ages in developed countries. Prior papers discuss about the lack of discriminating reproductive function between healthy aging males and those with chronic illnesses or risks of infertility. There are no previous reports in literature comparing possible differences of reproductive function in the abovementioned groups of males. Therefore, the aim of this study was to compare the reproductive parameters, including semen quality and hormonal status, of healthy men > 45 years of age with male subjects of the same age of infertile couples (with no known female risk factors).

Methods A total of 164 men of infertile couples with a preceding period of infertility of at least 12 months and 61 men attending a prostate health screening and considering themselves healthy. The semen samples, assessed according to WHO guidelines, were obtained by masturbation and ejaculated into a clean collection tube after 2–8 days of ejaculatory abstinence. Blood samples were collected for hormonal markers. Physical examination included assessment of body mass index (BMI) and genital pathologies, i.e. penis, testicles, groin and prostate.

Results According to WHO reference ranges at the time of investigation (WHO 1999), the subset of men with three normal variables (volume, sperm density and total sperm output) in semen analysis was significantly lower among men in infertile couples compared to healthy subjects (45.7% vs 72.0%, p = 0.001).

There were significant positive correlations between testicular volume and semen quality while negative correlations were observed between gonadotropin levels and sperm parameters in both groups. We did not find a significant influence of male age on semen quality and testis volume in the investigated groups. At the same time, the subject's age was in positive correlation with serum FSH in men of infertile couples (r = 0.156, p = 0.04) and with E_2 levels in healthy men (r = 0.348,

Table 12. K. Ausmees et al. Correlations of Age, Testicular volume, Hormonal parameters, BMI and Sperm Quality.

	Male partners of infertile couples (n = 164)				
Characteristics	**SEVOL**	**TSO**	**CONC**	**MOTIL**	**MORPHOL**
Age (years)	–0.020	–0.126	–0.135	–0.131	–0.080
Testicular volume (right + left/2, mL)	0.087	0.564[1]	0.517[2]	0.214[3]	0.210[4]
Testosterone (nmol/L)	–0.117	0.085	0.105	0.037	0.006
Estradiol (pmol/L)	–0.111	–0.008	0.006	–0.066	0.023
FSH (IU/L)	–0.071	–0.429[5]	–0.403[6]	–0.181[7]	–0.243[8]
LH (IU/L)	–0.075	–0.280[9]	–0.253[10]	–0.263[11]	–0.142
FSH/LH (IU/L)	–0.010	–0.339[12]	–0.340[13]	–0.031	–0.262[14]
BMI (kg/m²)	0.008	–0.094	–0.830	–0.070	–0.076
	Healthy Men (n= 61)				
Characteristics	**SEVOL**	**TSO**	**CONC**	**MOTIL**	**MORPHOL**
Age (years)	–0.029	–0.111	0.026	–0.217	–0.114
Testicular volume (right + left/2, mL)	0.103	0.412[15]	0.325[16]	–0.096	0.194
Testosterone (nmol/L)	–0.023	–0.24	–0.210	–0.245	–0.289[17]
Estradiol (pmol/L)	–0.079	–0.056	0.023	–0.189	–0.113
FSH (IU/L)	0.009	–0.239	–0.204	–0.175	–0.304[18]
LH (IU/L)	0.156	–0.347[19]	–0.414[20]	–0.079	–0.246
FSH/LH (IU/L)	–0.128	0.008	0.123	–0.051	–0.053
BMI (kg/m²)	–0.018	0.064	0.021	0.255	0.142

SEVOL: semen volume; TSO: total sperm output; CONC: sperm concentration; MOTIL: sperm motility; MORPHOL: sperm morphology; FSH: follicle stimulating hormone
[1, 2, 5–7, 10–14] $p < 0.001$; [3, 4, 19] $p < 0.01$; [8, 17, 18] $p = 0.02$; [9, 20] $p = 0.001$; [15, 16] $p = 0.01$

$p = 0.01$). As concerns hormonal parameters in male subjects of infertile couples, LH showed a positive correlation with testosterone ($r = 0.244$, $p = 2$ [$r = –0.192$, $p = 0.01$]) levels in blood serum while the latter was also in negative correlation with FSH levels in healthy subjects ($r = –0.366$, $p = 0.001$).

Conclusions Our study revealed that sperm quality and associated reproductive parameters are related not only to general male aging as described previously. There are some obvious differences in clinical parameters between men of infertile couples and healthy middle-aged males – differences in testicular volume, sperm quality and hormonal levels in blood serum. Further and more detailed investigations on a wider scale are required to determine the actual age-related risk factors for reproductive function, and whether those factors could be related to lifestyle, environmental or physiological conditions **(Tab. 11, 12)**.

P115

Impact of Childhood Maltreatment on Psychological State and Sexual Performance of Men Facing Timed Intercourse (TI)

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Background Child maltreatment, or abuse, is the physical, sexual, emotional mistreatment, or neglect of children. In the UK, there are approximately 30,000 children on Child Protection Registers at any one time [Government Statistical Service, 2003]. The experience of abuse in childhood was reliably associated with an increased risk of disease in adulthood including neurological, musculoskeletal, cardiovascular, and respiratory conditions [Arias, 2004]. There is now sound evidence that brain/neurobiological changes accompany the psychological manifestations following abuse and neglect [Glaser, 2000]. Boys are more likely to be victims of severe physical abuse, psychological abuse, and neglect, whereas girls are more likely to be victims of sexual abuse. During the fertile window of a woman's menstrual cycle, the impact of impending timed intercourse (TI) on male partners has only been recently investigated. We aimed to evaluate the effect of CM on men facing TI, compulsory sexual intercourse in nature, a stressful event.

Methods This prospective study consisting of 439 men was conducted during a 3-year period between July 1, 2008 and June 30, 2011. Various characteristics were evaluated, including the experience of childhood maltreatment (CM), newly acquired erectile dysfunction (ED), extramarital sex (EMS), daily intake of soft drinks (SDs), the Beck Anxiety Inventory (BAI), the Buss Perry Aggression Questionnaire (BPAQ), the levels of hormones, such as follicle-stimulating hormone (FSH), luteinizing hormone (LH), testosterone (T), prolactin (PRL) and estradiol (E2).

Results A total of 39 men (8.88%) experienced CM. Among them with CM, 29 men (74.4%) experienced ED with their spouses while 24 (61.5%) consumed soft drinks on a daily basis. Those who experienced CM tend to complain more ED, experience EMS and consume SDs on a daily basis. Among the hormones investigated, the levels of E2 was significantly lower in men with CM ($p = 0.0019$). Those with a history of CM also reported statistically higher level of BAI and subscales of BPAQ- anxiety, anger, hostility and violence. TI significantly raised subscales of BPAQ in men with CM.

Conclusions TI imposes a great deal of stress with more severe degree on men with a past experience of CM that apparently affects the basal level of BAI before and after TI, hence, hinders the men to achieve the required erection to perform TI. Physicians and clinicians should acknowledge the potential harmful effects of CM on men facing TI, a stressful condition.

P116

Metachronous Bilateral Testis Tumors with Different Histology – A Case Report

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Introduction Germ Cell Testis Tumors (GCTT) affect mainly young adults. The incidence increased 50% in recent years in developed countries, particularly the seminoma variant. They are solid tumors with very good prognosis, even in advanced disease and are the paradigm of the need of multimodal therapy. The known risk factors are cryptorchidism, trauma, testicular atrophy, prior testicular tumor, and infertility.

Case Report This is a patient who underwent right radical orchiectomy with placement of testicular prosthesis three months before, for Non Seminoma Germ Cell Testis Tumor (NSGCT), and proposed for surveillance, during which, he initiates a slight increase of B-HCG and develops hard painful lump. Physical examination and ultrasonography diagnosis was compatible with a tumor of the contralateral testicle. Regarding the size and location of the lesion he was first proposed for excisional biopsy and biopsies of the tumor bed. Histology revealed a Seminoma GCT with Tin on the tumor bed. Because he had normal Testosterone production and paternity was not an option anymore he was proposed for adjuvant radiotherapy.

Discussion The incidence of bilateral testicular tumors, synchronous and or metachronous is 2–3%. At the time of orchidectomy, the incidence of Cis in contralateral testis is 5% however it remains low the prevalence of contra-lateral tumors (1%), and therefore the screening of the contralateral testis is not instituted, although it is recommended self-assessment by physical examination regularly.

The incidence is also correlated with age at onset (early age) in seminoma and the type of histology. Treatment decisions in contralateral tumor should take into account the stage and treatment carried out for the first tumor, current stage of disease and hormonal status of the patient and his will of fatherhood.

Conclusion This work aims to highlight the need for a regular follow-up, rigorous and prolonged, and the complications inherent to this fact, making use of self-examination as an important part of it, and made aware that

the greatest risk factor for a contralateral tumor is a prior TCGT. It also calls into question the best choice of treatment for that specific patient.

P117

Pituitary Transforming Gene 1 (PTTG1) Expression in Seminoma

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PTTG1 is a securing and plays a critical role in mitosis by inibition of sister chromatid separation. PTTG1 is overexpressed in a variety of tumors. Moreover PTTG1, as a transcription factor, can directly and indirectly induce expression of genes involved in tumorigenesis and cancer development. PTTG1 also induced VEGF and MMP2, involved in tumor development and cancer metastasis.

In order to clarify the role of PTTG1 in testis tumor, the expression of PTTG1 was evaluated by immunohistochemistry on formalin-fixed and paraffin-embedded specimen testicular tissues from 53 male patients underwent to therapeutic orchidectomy.

PTTG1 staining was located in the nuclear and cytoplasm of neoplastic cells. Within the tumor we identify a peripheral zone as edge of neoplasia and neoplastic tissue up to 1 mm towards interior of the tumor and central zone as neoplastic areas further than 1 mm from the border of the tumor. In the peripheral area, PTTG1 immunoreactivity was detected mostly localized in the nucleus, while in the central area PTTG1 staining was evident more intensely in cytoplasm of positive elements.

PTTG1 expression was significantly lower in the central when compared with the peripheral area, with a greater number of positive cells in the borders of the tumor and Δ periphery/centre significantly correlate with the size of the tumor. In seminoma with tumor diameter > 25 mm PTTG1 expression was significantly higher in the peripheral area; instead in seminoma with tumor diameter ≤ 25 mm, different distribution of PTTG1 positive cells from peripheral to central area has not been found. When compared the percentage of PTTG1 positive elements in the same area between different subgroups, in the central area tumors with greater size showed less percentage of PTTG1 positive elements than the smaller one. In the peripheral area of both subgroups PTTG1 positive elements were higher in the subgroup B, but the difference was not significantly.

PTTG1 positive staining was also reported in the peritumoral region and properly in the areas of tumor infiltration and in the interstitial intertubular spaces. In these areas the PTTG1 positive cells were about 40% of neoplastic cells was observed. PTTG1 nuclear staining pattern was prevalent.

For the first time we described neoplastic PTTG-1 cells in seminoma. Our data support the idea that PTTG1 expression in the front of the tumor infiltration and in the intertubular areas might be involved in the invasion of surrounding tissue. When the tumor is very small, at the beginning of the carcinogenic process, all PTTG-1 positive cells are present in the centre of the lesion and, increasing the tumor, they might move to the periphery to facilitate neoplastic infiltration.

P118

Early Detection of Carcinoma In Situ of the Testis with a New Non-Invasive Method

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Introduction Testicular germ cell tumors (TGCT), i. e. seminomas and non-seminomas, are the most common malignancy in Caucasian men aged 15–45 years. The common precursor of all TGCT is Carcinoma in situ (CIS) of the testis. CIS arises during development of the fetal testis and, under the influence of testosterone, progresses after puberty into cancer.

The majority of TGCT patients require, in addition to surgery, also irradiation and/or chemotherapy for cure. In spite of an overall high cure rate (> 95%), exposure to radiotherapy or chemotherapy in these young men have significant long term effects, including cardiovascular disease, metabolic syndrome, infertility and even second malignancies. Treatment of CIS consists of a low dose irradiation with a cure rate of 100%.

Hence, early identification CIS is currently based on an open surgical biopsy as gold standard. Non-invasive methods for CIS detection are reported, like scrotal ultrasound and immunohistochemical detection of OCT3/4 (POU5F1) on semen. The aim of this study is development of an informative non-invasive detection method for CIS.

Material & Methods A pilot study composed of three groups was performed, including 2 patient groups (I and II) and one control group (III). Patients of group I included men with TGCT risk factors (infertility, testis atrofy, cryptorchism or previous unilateral TGCT). Patients of group II included men with the risk factors and presence of testicular microlithiasis (TM) on ultrasound. The control group (III) consisted of normospermic men from couples with reduced fertility without TGCT risk factors.

Between June 2009 and June 2012, in total 82 men (28 in I, 24 in II and 30 in III) were prospectively enrolled. Analysis was performed on scrotal ultrasound, semen diagnostic (OCT3/4) and additional clinical data.

Results The reproductive hormones luteinizing hormone (LH), follicle stimulating hormone (FSH) and inhibin were significantly different between the 3 groups ($p < 0.001$, $p = 0.001$ and $p < 0.001$ respectively). Inhomogeneous testicular parenchyma (group II vs I and III: $p = 0.002$) is, beside TM, a significantly ultrasound characteristic between the 3 groups. Semen analysis according to WHO classification (2010) is not significantly different between patient group I and II ($p = 0.113$). So far, the intermediate analysis of 25 out of 82 semen OCT3/4 stainings has been done, revealing four positive cases (1/8 group II and 3/10 group III).

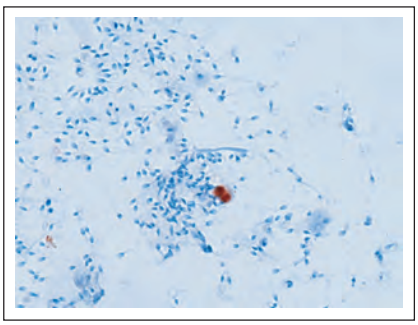

Figure 21. J.E. Elzinga-Tinke et al.

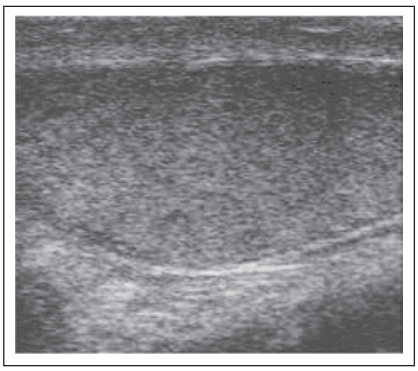

Figure 22. J.E. Elzinga-Tinke et al.

Conclusion An inhomogeneous testicular parenchyma, besides TM, is more common in men with multiple risk factors for TGCT. The semen diagnostic (OCT3/4) should be further analyzed and developed, but is promising as non-invasive detection method for CIS **(Fig. 21, 22)**.

P119

Testicular Cancer: Awareness and Testicular Self Examination in a Portuguese University Setting

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Introduction Testicular cancer (TC) is one of the most common malignancy in young population. The incidence of this malignancy appears to be increasing worldwide. The cure rate for TC now approaches 100%, but is negatively influenced by the delay in seeking medical attention. The lower public awareness to this type of cancer and the lack of testicular self-examination (TSE) are pre-

sumed reasons for the late presentation to medical care.

Our objective was to analyze and evaluate the awareness of TC and TSE in Portuguese men in an university setting.

Methods We performed a survey of 13 questions concerning the awareness of TC and the practice and perceived importance of TSE in 496 men from the university. The questionnaire was sent to the institutional email of students, professors and other workers and the data were collected and then analyzed.

Results Our population had a mean age of 30.7 ± 10.7 years. 49 (9.9%) participants were medical students. 389 (78.4%) of the participants reported that they had knowledge about TC, but only 49 (9.9%) answered all the questions correctly. 186 (37.5%) reported they performed self-examination, but only 39 (7.9%) did it every month. When asked about the importance of performing TSE in a Lickert-tipe scale (from 1–10), 464 (93.5%) of the population answered 5 or more, with 218 (44%) participants considering it extremely important (a value of 10 in the scale). 17 (3.4%) participants were both well informed about TC and had been performing TSE once a month as suggested. The medical students demonstrated higher level of knowledge when compared to the others participants ($p < 0.05$).

Conclusion The present study represents a first approach to characterize the awareness to testicular cancer in a Portuguese population. Despite a relatively high number of participants answered that they had knowledge about TC, only a relatively small number answered correctly to all the questions. A great number of participants considered that TSE was important, but less than 8% performed it.

This population has some awareness of testicular cancer, but still lack correct information about this disease and about testicular self-examination.

P120

Delayed Orchidectomy after Primary Chemotherapy for Metastatic Testicular Cancer

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Question To examine the outcome of delayed orchidectomy following primary chemotherapy in patients with metastatic testicular cancer.

Methods A retrospective analysis of patients who underwent primary chemotherapy without initial orchidectomy for testicular cancer between 1982 and 2006.

Patients were identified from the regional oncology cancer database in our tertiary referral hospital.

Case notes were reviewed regarding initial presentation, chemotherapy, clinical progress and pathological outcomes following surgery.

Table 13. R. Donat et al. Post Chemotherapy Orchidectomy Outcome and Survival Data

Group	n	Testis pathology	RPLND	Alive	Follow-up (yrs, mean*)	RIP
Early	13	Scar/necrosis	6	12	4.4	1
Early	3	Tumor	3	0	4.6	3**
Delayed	2	Scar/necrosis	0	2	8.4	1***
Delayed	3	Tumor	1	2	7.5	0

* Mean of available follow-up, patients usually discharged to general practice after 5 recurrence free years.
** Includes one death from secondary rhabdomyosarcoma (74 mo) and one death from Teratoma differentiated (82 mo).
*** Non-cancer death from bowel adhesions after radiotherapy + chemotherapy (75 mo)

Results 21 patients were identified with the majority diagnosed with non-seminomatous germ cell tumors and Marsden Stage III and IV disease.

16 patients underwent standard orchidectomy within 12 months of commencing chemotherapy, with five patients undergoing significantly delayed orchidectomy for a variety of reasons.

Orchidectomy in the standard group showed tumor necrosis or a scar in the majority of patients (n = 13) (81%), with differentiated or mature teratoma found in three patients associated with bulky poorly responsive retroperitoneal disease.

In the delayed orchidectomy group three patients had viable seminoma, of which two were associated with carcinoma in situ (CIS).

Conclusion Our study raises concerns as regards a potentially high risk of late tumor development in testis which are preserved despite apparent tumor resolution after chemotherapy.

The data support the standard recommendations for orchidectomy following chemotherapy and suggest the concept of a testis preservation policy in selected patients is not without risk **(Tab. 13, 14)**.

P121

Biological Hypogonadism and Abnormal Sperm in Males affected by Testicular Cancer

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Introduction Hypogonadism in males is a complex clinical syndrome defined by low level of testosterone and diminished sperm production. There is a high incidence of hypogonadism after cancer treatment in the childhood. In testicular cancer, post-treatment hypogonadism has been suggested to be more frequent if present already before cancer treatment. At our institution a longitudinal prospective study on fertility after testicular cancer is underway with the aims to improve the understanding of sexual behaviour and fertility in men who had survived testicular cancer. The preliminary results presented here concern the hormonal status of the patients and sperm quality.

Material & Methods Among 194 men who were referred for sperm cryopreservation before orchidectomy between January 2007 and December 2011, 100 men aged between 19 and 42 years accepted to participate in the study. Hormone assessment consisted of FSH, free and total testosterone (TT), SHBG and free testosterone index (ratio TT/SHBG). Additionally classical sperm analysis and sperm DNA fragmentation (TUNEL method) were determined immediately before or after orchidectomy (T0, n = 87), at one (T1, n = 60) and 2 years (T2, n = 67). All tumors were germ cell. Increased FSH (> 11 IU/L) indicated primary (1.) hypogonadism, and low FSH (< 0.7 IU/L) secondary (2.) hypogonadism.

Results At T 0, 1. and 2. hypogonadisms were frequent and equally represented, respectively 19.5 and 18.4 %. Patients with 2. hypogonadism were affected by more aggressive non-seminomatous tumors. At T1, all patients with 2. hypogonadism but one developed 1. hypogonadism. At T1 and T2, 1. hypogonadism was present in respectively 58% and 55% patients. At T0, T1 and T2, percentages of men with low testosterone (TT < 8 nmol/L) were respectively 17%, 16% and 6%. Sperm concentration < 15.10^6/mL was respectively 30%, 47% and 24%. Abnormal sperm DNA fragmentation (> 20%) was unchanged and high along the study at a 50% rate.

Conclusions These preliminary results confirm that hypogonadism is frequently observed in patients affected by testicular cancer even before any treatment toxic for germ cells. Secondary hypogonadism has no equivocal etiology whereas 1. hypogonadism may be explained by pre-existent gonadal dysfunction. Sperm quality is affected and remains poor with a 50% sperm DNA fragmentation at T2. Testosteron production is affected in about 6 to 15% patients. Beside recommended hormonal and sperm evaluation at semen cryopreservation, it might be reasonable to test testicular cancer survivors also at distance. Further studies will indicate us how long and with which regularity.

Table. 14. R. Donat et al. Patients Undergoing Delayed Orchidectomy > 12 Months after Commencing Chemotherapy (delayed group).

Presentation	Initial pathology	IGCCC	Marsden	Metastatic response	Testicular investigations	Reason for orchidectomy	Time (months)	Pathology (orchidectomy)
Back pain	Anaplastic seminoma	Good	3	Fibrosis only at RPLND for enlarging mass	Normal USS at presentation Exploration and frozen section normal 12 months post chemo	Right testicular pain, incidental finding of lesion on left on USS	44	Seminoma + CIS
Right loin pain, loin mass	Seminoma	Good	1	Resolution of mass on CT	Intra-abdominal testis likely primary site	Initially deemed too risky; reviewed later and excision recommended	68	Atrophic testis
Back pain, left retroperitoneal mass	Germ cell tumor likely seminoma	Inter	4	Partial response to second line chemo	Clinically normal left testis No documentation of USS	New left testis mass clinically	52	Seminoma + CIS
Abdo pain, weight loss	Teratoma (MTU)	Good	3	Partial response No RPLND – deemed irresectable	No mass clinically USS – 12 mm focus likely granuloma Patient declined surgery initially	Lesion increasing in size on USS	19	Seminoma
Loin pain, weight loss	Teratoma	Inter	4	Partial response	Left tumor on USS Exploration 24/12 – scar only	Increase in testis size clinically Hypoechoic area on USS Concerns despite negative exploration	68	Fibrous scar

P122

Prevalence of Intratubular Germ Cell Neoplasia in Testicular Biopsies of Infertile Iranian Men

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Objective Infertility is one of the components of testicular dysgenesis syndrome which defined as intratubular germ cell neoplasia (IGCN), cryptorchidism, hypospadias and low sperm counts. It is proved that these features could occur together. Previous studies in different parts of the world show various prevalence of CIS in infertile men (0–3.7%). There is no basic study about prevalence of CIS in Iranian infertile men, thus the aim of this study was to achieve prevalence in Iran.

Material & Methods We evaluate testicular biopsies of 1153 infertile men referring to pathology lab, from which 150 patients were suspicious for CIS. Then we applied immunohistochemistry for placental alkaline phosphatase (PLAP) marker for diagnosis of CIS.

Results Positive results were detected in 7 (0.6%) out of all 1153 patients of which 6 (1.1%) were positive out of 633 patients < 35 years.

Conclusion This study was the first one inIranrevealing prevalence in the range of other countries.

P123

Variations in Testosterone Pathway Genes and Susceptibility to Testicular Cancer in Norwegian Men

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Introduction Imbalance between the estrogen and androgen levelsin uterois hypothesized to influence testicular cancer (TC) risk. Thus, variation in genes involved in the action of sex hormones may affect an individual's susceptibility to TC. Mutations in testosterone pathway genes may alter the level of testosterone both in utero and later in life and may hypothetically alter the risk of developing TC. Luteinizing hormone receptor (LHR), 5α-reductase II (SRD5A2), and androgen receptor (AR) are key elements in androgen action. A case control study was conducted for investigation of polymorphisms in the LHR, SRD5A2 and AR genes and their possible association with TC.

Material & Methods 651 TC cases and 313 controls from the Norwegian population were included in the study. The LHR Asn291Ser and Ser312Asn as well as the SRD5A2 Ala49Thr and Val89Leu polymorphisms were analyzed by allele specific PCR and gel electrophoresis, the LHR InsLQ polymorphism was genotyped by sequencing and the number of AR CAG and GGN repeats was determined by capillary electrophoresis. The differences in genotype distribution between TC cases and controls were analyzed by binary logistic regression. P-values adjusted for multiple testing were calculated using the Bonferroni method, by multiplying the crude p-value by the number of markers analyzed (n = 7).

Results The controls were statistically significantly more often heterozygous for the LHR Ser312Asn polymorphism than the TC cases (OR = 0.66, 95%-CI: 0.48–0.89, P_{crude} = 0.007, P_{adj} = 0.049). None of the other investigated polymorphisms were associated with risk of TC. Furthermore, we found no statistically significant differences in genotype frequencies between the histological subtypes seminoma and nonseminoma after adjusting for multiple testing.

Conclusion Our results may suggest a possible association between genetic variation in the LHR gene and the risk of developing TC. The association should be verified in larger studies.

P124

Genetic Variations in Signal Transduction Pathways and Risk of Testicular Germ Cell Tumor in a Large Swedish-Norwegian Case-Parent, Case-Control Study

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Introduction Testicular germ cell tumor (TGCT) is the most common malignancy in young men. The aetiology is still unclear, but an imbalance between the sex hormone levels in utero is hypothesized to influence TGCT risk. Genome-wide association studies have identified single nucleotide polymorphisms (SNPs) in genes encoding proteins involved in several signalling pathways associated with TGCT risk. This study aimed to verify previously reported associations in a Swedish-Norwegian population, and investigate whether associations differed by parental sex and histologic subtype (seminoma/nonseminoma).

Material & Methods DNA from Swedish and Norwegian TGCT cases and their parents was genotyped using Sequenom MassARRAY iPLEX Gold. After sample and genotyping quality control; 98 SNPs tagging common variation in 831 case-parent triads, 474 dyads and 712 singletons were included. 3922 unrelated male controls were included from a population-based study of Swedish twins (TWINGENE). DNA was genotyped on an Illumina OmniExpress bead chip. Additional SNPs were imputed in both samples using BEAGLE and reference haplotypes from the 1000 genomes project. After imputation a total of 852 SNPs were analyzed. SNP-TGCT association in the combined case-parent, case-control study was tested using UNPHASED. Forward stepwise regression within each gene was applied to find independent association signals. Heterogeneity was tested for the markers in each gene most strongly associated with TGCT.

Results All genes were significantly associated with TGCT. The lowest unadjusted P-values (per gene) were; ATF7IP: 6.2e-06; BAK1: 2.1e-10; DMRT1: 6.7e-25; KITLG: 2.1e-48; SPRY4: 1.4e-29; TERT: 1.8e-18. Most genes had more than one independent association, and one marker in TERT (rs4975612) has to our knowledge not been reported previously. A significant difference by parental sex was observed for rs10463352 in SPRY4 ($p = 0.0008$, $OR_{mat} = 1.56$, $OR_{pat} = 1.03$). No evidence of heterogeneity between seminoma and nonseminoma was found.

Conclusion We validated previously reported associations in a large case-parent, case-control study. We also found a new, independent association in TERT. The genes within these loci are all biologically plausible candidates for TGCT predisposition and correspond to three distinct pathways; KITLG, SPRY4 and BAK1 are all involved in primordial germ cell development through the KIT/KITLG signaling pathway, TERT and ATF7IP are involved in telomerase regulation and DMRT1 is important for sex-determination. The case-parent design indicated one marker in SPRY4 interestingly associated with TGCT only when inherited maternally. Expression- and functional analyses will be required to identify the true causative variants and mechanisms of TGCT predisposition.

Postersession 7: Andrology along the Lifeline & Metabolic Syndrome and Reproductive Function & Hypogonadism

P125

Evaluation of Oxidative Stress in Diabetic Patients with Erectile Dysfunction

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Introduction Diabetes produces a number of physiological changes that affect the penile erectile ability, the synthesis of androgens, autonomic neuropathy, peripheral sensory and motor neuropathy, an increase in smooth muscle contractility, injury to the endothelium, in addition to psychological components (depression). Each of these components alone or together may be a cause of erectile dysfunction and/or perturbation of sexuality in diabetic patients. Prolonged hyperglycemia leads to decreased production of NO levels and increased formation of oxygen free radicals. Hyperglycaemia originates penile hemodynamic changes and decreases endothelium-dependent vasorelaxation via the NO/cGMP.

Objective Evaluation of oxidative stress on erectile dysfunction (ED) in human serum samples.

Methods We selected 60 patients (34 type 2 diabetics and 26 non-diabetics) with ED, in our institution. We performed a clinical evaluation and measurement of serum HbA_{1c}, lipid profile and total testosterone levels. Changes in libido, recent surgery, and active infections were exclusion criteria. Serum levels of GSH/GSSG were determined by high performance liquid chromatography.

Results Patients average age was 59 years. The mean duration of diabetes was 10 years. The mean duration of ED was 4 (diabetics) and 2 years (non-diabetics). The mean waist circumference was 99 in diabetic patients and 84 cm in non-diabetic. The mean International Index of Erectile Function Questionnaire score was 8 (diabetics) and 10 points (non-diabetics). 67% of diabetic and 38% of non-diabetic patients were treated with PDE5 inhibitor (sildenafil citrate). The success rate was 50% for diabetic patients and 54% for non-diabetics. The average GSH/GSSG ratio was 3 for non-diabetic patients and 1.5 for diabetics. In the subgroup of diabetic patients treated with PDE5 inhibitor without success the ratio GSH/GSSG ratio was 1.1.

Conclusions Diabetic patients have a generalized decrease in GSH/GSSG ratio. We found that for higher levels of prolonged hyperglycaemia there was a considerable decrease in GSH/GSSG ratio, more noticeable in the sub-group of diabetic patients with poor response to treatment with PDE5 inhibitor. The measurement of GSH/GSSG ratio may be an indicator of treatment efficacy with PDE5 inhibitor. The method used for determination of serum GSH/GSSG levels is relatively inexpensive with easy reproducibility. Prospective studies with larger samples are needed to corroborate these results.

P126

Effect of the Metabolic Syndrome on Male Hormonal Status and Sperm Function: A Case-Controlled Pilot Study

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Components of the metabolic syndrome (MetS) in males include central adiposity, hypertension, dyslipidaemia, glucose intolerance and hypogonadism. As a relationship between MetS and male subfertility has not yet been directly investigated, this case controlled pilot study aimed to assess the effect of MetS on testosterone, progesterone and semen parameters. Recruited males aged 24–67 years old (n = 54) had body mass index (BMI), waist-hip ratio (WHR), diastolic blood pressure (dBP) and systolic blood pressure (sBP) recorded. Fasting blood samples were analyzed for HDL cholesterol, triglycerides (TG) and glucose (Glu). Saliva samples were assayed for testosterone (T) and progesterone (P) concentrations, and semen samples were analyzed for sperm concentration, sperm motility, vitality, mitochondrial membrane potential (MMP), DNA fragmentation (DF) and leukocyte concentration. Participants on any hormonal therapy, with known reproductive system disorders or leukocytospermia ($> 10^6$) where excluded

from analysis. Based on the criteria for diagnosis of MetS, the participants were divided into a control group (n = 28) or the MetS group (n = 26). Predictable differences were found between the groups for BMI, WHR, dBP, sBP, HDL, TG and Glu. The MetS group showed significant reductions in sperm concentration (p = 0.003), total motility (p = 0.029), sperm vitality (p = 0.002), MMP (p = 0.004), DF (p = 0.029), T (p = 0.009) and P (p = 0.013). Overall, the clinical, biochemical and semen parameters correlated as expected. Age correlated positively with DF (r^2 = 0.35) and negatively with progressive (r^2 = –0.44) and total (r^2 = –0.35) motility, vitality (r^2 = –0.35) and FT (r^2 = –0.31). BMI correlated positively with MMP (r^2 = 0.41) and negatively with sperm concentration (r^2 = –0.38), total motility (r^2 = –0.3), vitality (r^2 = –0.39). WHR correlated positively with DF (r^2 = 0.43) and negatively with total motility (r^2 = –0.3) and vitality (r^2 = –0.36). dBP correlated negatively with sperm concentration (r^2 = –0.38), total motility (r^2 = –0.33), vitality (r^2 = –0.36) and P (r^2 = –0.34). TG correlated negatively with T (r^2 = –0.38). Glu correlated positively with DF (r^2 = 0.32) and negatively with total motility (r^2 = –0.3) and vitality (r^2 = –0.35). T correlated positively with P (r^2 = 0.58). Although limited by sample size, the results indicate that MetS males without leukocytospermia have compromised sperm parameters that may negatively affect fertility. A reduction in P suggests that steroidogenesis cascades may be compromised, resulting in hypogonadism. Although it can be hypothesized that chronic inflammation and oxidative stress associated with MetS may provide a novel explanation for these results, the mechanisms for this relationship also require investigation.

P127

Restoring Testosterone to Normal Levels in Elderly Men is Efficacious in Weight Reduction: A Follow-up-Study over 5 Years

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Introduction & Objectives Obesity is associated with reduced testosterone, and low testosterone induces weight gain. This study analyzed the effects of normalization of serum testosterone on anthropometric parameters in mainly elderly, hypogonadal men.

Methods Open-label, single-centre, cumulative registry study of 255 men (mean age 60.6 ± 8.0 years), with testosterone levels ≤ 3.5 ng/mL. 215 men were studied for at least 2 years, 182 for 3 years, 148 for 4 and 116 for at least 5 years. They received parenteral testosterone undecanoate 1000 mg/12 weeks after an initial interval of 6 weeks.

Results The following changes were observed: weight (kg) decreased from 106.22 ± 16.93 (minimum: 70, maximum: 139) to 90.07 ± 9.51 (min 74, max 115). The statistical significance was p < 0.0001 vs baseline and vs the previous year over 5 years indicating a continuous weight loss over the full observation period. Waist circumference (cm) declined from 107.24 ± 9.14 (min 86, max 129) to 98.46 ± 7.39 (min 84, max 117) (p < 0.0001 vs baseline and vs the previous year over 5 years). Body mass index (BMI, m/kg^2) declined from 33.93 ± 5.54 (min 21.91, max 46.51) to 29.17 ± 3.09 (min 22.7; max 36.71) (p < 0.0001 vs baseline and vs the previous year over 5 years). The mean per cent weight loss after 1 year was 4.12 ± 3.48%, after 2 years 7.47 ± 5.01%, after 3 years 9.01 ± 6.5%, after 4 years 11.26 ± 6.76% and after 5 years 13.21 ± 7.24%. At baseline, only 5% of men fell into the normal weight category (BMI ≤ 24.9). 24% were overweight (BMI 25–29.9), 57% obese (BMI 30–40) and 14% morbidly obese (BMI ≥ 40). At the end of the observation period, 95% of men had lost any weight, 90% had lost ≥ 5 kg, 76% ≥ 10 kg, 53% ≥ 15 kg, and 31% lost ≥ 20 kg. At baseline, only 4% of men had a normal waist circumference (< 94 cm), 27% had an increased waist circumference (94–101.9 cm), and 68% a substantially increased waist circumference (≥ 102 cm). 97% experienced any reduction in waist circumference, 86% lost ≥ 5 cm, 46% ≥ 10 cm and 7% ≥ 15 cm.

Conclusions Almost all hypogonadal men are overweight and the majority are obese. Normalising serum testosterone produced loss of weight, waist circumference and BMI. These improvements were progressive over the full 5 years of the study.

P128

Side Effect Profile of Long-term Treatment of Elderly Hypogonadal Men with Testosterone Undecanoate

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Introduction Testosterone therapy for hypogonadal men has been used for decades. However, there are still concerns regarding the safety of this treatment, particularly in elderly men.

Methods Prospective registry study of 255 men (mean age 60.6 ± 8.0 years), with testosterone levels between ≤ 3.5 ng/ml. They received parenteral testosterone undecanoate 1000 mg at day 1, week 6 and every 12 weeks thereafter for up to 66 months.

Results After 60 months the following changes were observed:

Erythropoiesis: haemoglobin increased from 14.44 ± 0.72 to 14.99 ± 0.45 g/dl (p < 0.0001 vs baseline). Haematocrit increased from 43.22 ± 2.84 to 48.78 ± 1.7% (p < 0.0001 vs baseline). Four patients had haematocrit levels > 52% which resolved without intervention.

Prostate: PSA increased from 1.77 ± 0.96 to 1.82 ± 0.96 ng/ml (p < 0.0001 vs baseline) with a plateau after 24 months. Prostate volume increased from 28.51 ± 11.2 to 30.23 ± 12.4 ml (p < 0.0001 vs baseline). 3/255 patients were diagnosed with prostate cancer following elevated PSA (< 4 ng/mL) at 18 weeks of treatment. Tumor grade was T2 in all three and Gleason score 3+3 in 2 and 3+2 in 1 patient, resp. They all underwent radical prostatectomy. The proportion was 1.18% with an incidence of 30.334 per 10.000 patient years. For comparison: in the PLCO trial with a 7-year follow-up, the proportion of prostate cancer was 7.35% with an incidence of 116 per 10.000 patient years [1], in the ERSPC trial with a follow-up of 11 years, 96.6 [2]. – The International Prostate Symptom score (IPSS) improved from 6.73 ± 4.21 to 2.83 ± 1.25 (p < 0.0001).

Liver enzymes: aspartate transaminase (AST) decreased from 43.05 ± 17.29 to 20.16 ± 3.21 U/L (p < 0.0001 vs baseline) reaching a plateau after 24 months, alanine transaminase (ALT) from 43.89 ± 18.11 to 20.54 ± 3.92 U/L (p < 0.0001 vs baseline) with a plateau after 36 months.

Conclusions The incidence of 3/255 patients with prostate cancer does not suggest an increased risk of prostate cancer in elderly men on long-term testosterone treatment. Long-term treatment with testosterone undecanoate with monitoring according to the guidelines is acceptably safe.

References:

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P129

"Late onset hypogonadism" is not an Isolated Condition – Comorbidities in Elderly Hypogonadal Men Presenting or Referred to Urological Institutions in Germany

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Introduction Serum testosterone declines with aging, not primarily determined by calendar age per se but rather by factors impairing the health of aging men, such as obesity, metabolic syndrome, diabetes mellitus and other diseases. We determined concurrent diseases in two cohorts of mainly elderly men with so-called late onset hypogonadism ("LOH").

Methods In 2 separate cumulative registry studies following identical protocols, 2 cohorts of 516 mainly elderly men were analyzed for concurrent diseases. Cohort A (Dr. Haider, Bremerhaven, Germany) consisted

of 255 men, cohort B (Dr. Yassin, Norderstedt, Germany) of 261 men. These men had either sought urological consultation or had been referred by other disciplines because of suspected hypogonadism. All men received treatment with injections of long-acting testosterone undecanoate.

Results The following comorbidities were encountered:
- Cardiology: hypertension: A: 40%, B: 45% ; coronary artery disease: A: 16%, B: 13%; condition post myocardial infarction: A 15%
- Internal Medicine: Diabetes mellitus: A: 31%, B: 26%; dyslipidemia: A: 18%, B: 33%.
- Gastroenterology: inflammatory bowel disease: A: 16%
- Urology: chronic prostatitis: A: 38%, B: 11%
- Dermatology: psoriasis: A: 5%
- Endocrinology: Klinefelter's syndrome: A: 9%, B: 2%.

In addition, there were a total of 14 patients with a history of maldescensus testis and 19 patients with a history of unilateral or bilateral orchiectomy following testicular cancer.

Orthopedics: osteoporosis: A: 14%, B: 6%.

Conclusions

1. The majority of middle-aged to elderly patients with hypogonadism have one or more comorbidities. For adequate treatment, hypogonadal men should be examined for concurrent diseases. Testosterone administration may be a significant element in their treatment.

2. With progression of their age elderly men will suffer increasingly from ailments and hypogonadism may be an element, so far not often diagnosed. Testosterone treatment may contribute to a better quality treatment.

3. 60/516 men had conditions which cannot be categorized as "LOH". Klinefelter's syndrome may still have been undiagnosed, and a history of maldescensus testis may be unknown. The term "LOH" should be used with caution, and the general term "hypogonadism" may be preferable.

P130

The Pharmacodynamics of Enclomiphene citrate (Androxal®) in Men with Secondary Hypogonadism Indicates that it Acts as a Restorative Therapy Rather than as a Replacement Therapy

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Objective To determine the pharmacodynamics (PD) of serum total testosterone (TT) and luteinizing hormone (LH) after daily oral enclomiphene citrate (Androxal®) in comparison to topical testosterone in men with secondary hypogonadism.

Methods & Subjects A randomized, single blind, two-centre phase II study evaluating Androxal® and AndroGel over 24-hours. 60 men were screened, 48 were admitted and 44 completed both PD arms. All subjects had TT < 350 ng/dL and low-to-normal LH (< 12 IU/L). Serum samples were taken every hour for 24hrs after 6.25 mg, 12.5 mg and 25 mg Androxal® or AndroGel. TT and LH was determined in a naïve population following a single oral capsule of one of three Androxal® doses or AndroGel (PD part 1) and after 6 weeks of continuous daily oral or topical treatment (PD part 2). The pharmacokinetic profile of Androxal® was also determined. Hormonal profiles were also assessed one week after treatment discontinuation.

Results Both treatments were effective after a single dose with AndroGel generating slightly higher TT levels initially. After 6 weeks of continuous use, the mean concentration of TT at time 0, C_{0hr} was 604 + 160 ng/dL for men taking the 25 mg dose of Androxal® and 500 + 278 ng/dL for those men treated with AndroGel. These values were higher than baseline but not different from each other. All three doses of Androxal increased C_{0hr}, C_{avg}, $C_{max,}$ C_{min} and C_{range} for TT. The pattern of TT over the 24-hour-period following 6 weeks was best described as a non-linear function with a mid-day trough and rising night-time level. Androxal was associated with elevations of LH, FSH with no effects on most other hormones, apart from TT. AndroGel decreased LH, FSH and was more inter- and intra-subject variability in TT elevation.

Conclusions The effects of Androxal® on TT appeared to occur in parallel with increases in LH independent of the peak of drug in the serum. We interpret this as a normalization of the mechanism by which LH brings about production of TT rather than an acute effect of drug. Indeed, TT, LH, and FSH all reached and/or were maintained in the normal range. The overall endocrine profile indicates that Androxal acts through a tonic stimulus of LH; as a result there is a restoration of gonadotropin activity consistent with normalization of TT, LH and FSH. This normalization or restoration persists for at least one week after drug treatment stops. This process is different from testosterone replacement seen with existing products and represents a new way to relieve low TT in men with a sluggish but intact hypothalamus-pituitary-testicular axis.

P131

Testosterone and Cardiovascular Risk in Patients with Erectile Dysfunction

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Introduction The relationship between cardiovascular (CV) diseases (CVD) and testosterone (T) levels in men has not been completely clarified. The aim of the study is to evaluate the association between T levels and CV risk in subjects with erectile dysfunction (ED) and to verify whether their body mass index might (BMI) represents a possible confounder in testosterone-related CV stratification.

Methods A consecutive series of 2269 male patients attending the Outpatient Clinic for ED was studied. The assessment of CV risk was evaluated using the engine derived from the Progetto Cuore study.

Results After adjustment and for BMI and associated morbidities, sex hormone binding globulin bound (SHBG) and unbound T levels decreased as a function of CV risk assessed thorough Progetto Cuore risk engine. In addition, a higher prevalence of hypogonadism related symptoms and signs were associated with a higher CV risk. Among factors included in the Progetto Cuore risk engine age, total and HDL cholesterol and diabetes were all significantly associated with CV risk-dependent modification of total and calculated free-T levels. When the relationship between SHBG bound and unbound testosterone and CV risk was evaluated as a function of obesity (BMI > 30 kg/m^2), all the aforementioned associations were confirmed only in non-obese patients.

Conclusions Hypogonadism could be associated either with an increased or reduced CV risk, depending on the characteristics of subjects. Low T observed in obese patients might represent the result of higher CV risk rather than a direct pathogenetic mechanism.

P132

Investigation on Psychological Symptoms Improves ANDROTEST Accuracy in Predicting Hypogonadism in Subjects with Sexual Dysfunction

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Introduction The role of psychological symptoms in recognizing late-onset hypogonadism (LOH) is still controversial. The aim of the study is to evaluate the association between LOH and specific psychological symptoms and to verify whether investigating intra-psychic domain improves the accuracy of a validated case-history tool (ANDROTEST) in detecting LOH.

Methods A consecutive series of 1009 subjects (mean age 49.23 ± 13.34) consulting for sexual dysfunction was studied. Intrapsychic symptoms were investigated by Middlesex Hospital Questionnaire (MHQ), a self-reported questionnaire for screening of mental disorders.

Results A minimum set of 2 MHQ items was identified through iterative ROC curve analysis, with assessment of sensitivity and specificity for hypogonadism (calculated free testosterone < 0.225 nmol/L) in an exploratory sample of 462 patients. Sensitivity

and specificity were verified in a validation sample of 547 subjects, in which the final 2-item version showed an accuracy of 58.4 ± 3.2% in detecting hypogonadism. The combination of the 2-item score with ANDROTEST increased the accuracy in predicting hypogonadism (0.741 ± 0.029; $p < 0.0001$), when compared to ANDROTEST (0.696 ± 0.018; $p < 0.0001$) and the 2-item score ($p < 0.05$) alone.

Conclusions Combining these two psychological symptoms with a physical scoring system improves its ability in detecting hypogonadism. The combination of the scores should be tested in other studies.

P133

Testosterone Supplementation Improves Adipose Tissue Function in Animal Model of Metabolic Syndrome

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Introduction We recently demonstrated that testosterone (T) dosing was able to ameliorate the metabolic profile and to reduce visceral adipose tissue (VAT) accumulation in a high-fat diet (HFD)-induced rabbit model of metabolic syndrome (MetS). We observed negative relationship between T plasma levels and VAT weight. We studied the effects of HFD and in vivo T dosing on adipose tissue function, and on the adipogenic capacity of precursor cells (rabbit preadipocytes, rPAD) isolated from VAT of the following rabbit groups: regular diet (rPAD-RD), HFD (rPAD-HFD) and T-treated HFD (rPAD-T).

Methods VAT of the all rabbit groups was studied by histomorphometric, Western blot and RT-PCR analysis. Isolated rPAD were exposed to adipocyte differentiating mixture (DIM) and adipogenic potential was evaluated by: quantitative analysis of triglyceride content (adipored assay), expression of adipocyte-specific genes, insulin-stimulated glucose uptake and GLUT4 membrane translocation.

Results Adipocyte size was significantly increased in VAT of HFD-rabbits compared to RD-rabbits, indicating adipocyte dysfunction, which was normalized by T dosing. Accordingly, we observed that perilipin, an antilipolytic protein, was significantly increased in HFD-rabbits when compared to all other groups. In VAT, androgen receptor expression was positively associated with expression of genes related to insulin signalling: GLUT4 (insulin-regulated glucose transporter) and STAMP2 (androgen-dependent, as seen in androgen-regulated prostate cells, and required for normal insulin signaling). Interestingly, STAMP2 mRNA expression in VAT of T-treated HFD rabbits was significantly increased, when compared to all other groups. Moreover, GLUT4 membrane translocation was significantly reduced in VAT from HFD-rabbits, as compared to RD-rabbits, which was increased in T-treated HFD rabbits. In rPAD-HFD, the capacity to accumulate triglyceride, when exposed to DIM, was reduced (66% over untreated cells) in comparison with rPAD-RD (534%). Moreover, glucose uptake ability and GLUT4 membrane translocation was also reduced in rPAD-HFD. Interestingly, DIM-exposed rPAD-T showed a triglyceride accumulation capacity (262%), a glucose uptake ability and GLUT4 membrane translocation comparable to that of rPAD-RD. These findings were confirmed in terms of mRNA expression of adipocyte-specific genes, which were significantly induced by DIM in rPAD-RD and -T, but not in rPAD-HFD.

Conclusion Our results indicate that T supplementation in MetS animal model may positively affect adipose tissue functions. This could reflect the ability of T in counteracting metabolic alterations, most likely restoring insulin sensitivity in experimental MetS.

P134

Is Psychological Stress Associated with Reduced semen quality? – A Cross-Sectional Study among 949 Healthy Young Danish Men

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Introduction Chronic stress is a very common condition and approximately 10% of Danish men report high or very high levels of subjective stress. Furthermore, more than 40% of young Danish men have sperm counts at a level that may indicate a prolonged waiting time to pregnancy.

Evidence from animal studies indicates that psychological stress might impair testicular function. Additionally, some epidemiological studies have described a negative association between self-reported stress or "stressful life events" and impaired semen quality. However, most studies have been conducted in infertile men, which makes it difficult to draw any firm conclusion due to potential inverse causation.

From an ongoing study of young Danish men from the general population (i. e. not selected due fertility status) we have obtained information on self-reported stress from 949 consecutively investigated men besides information of semen quality and reproductive hormones. The preliminary results are reported here.

Material & Methods 949 men (age 18–20 years) were included in this study. They were consecutively investigated from April 2008 to December 2011. All men completed a questionnaire, and delivered a semen sample assessed for volume, sperm concentration, total sperm count, and percent morphologically normal spermatozoa.

The men responded to four standardized questions about perceived stress during the past 4 weeks: "*How often have you had problems relaxing, been irritable, tense or stressed?*" The answer categories were; all the time (scoring 100%), large part of the time (scoring 67%), rarely (scoring 33%) or never (scoring 0%). A summarized stress score was calculated as the mean score of the four questions. Based on this, an individual stress level was calculated as low, moderate or high.

Preliminary Results & Statistics In total 296 (31%) reported low stress, 389 (41%) moderate and 264 (28%) high stress. The mean number of morphologically normal spermatozoa were 8.0%, 7.5% and 7.1% in the 3 groups, respectively ($p = 0.02$, stress score entered as a continuous variable in regression model). A tendency towards lower total sperm count and sperm concentration was seen in the high stress group ($p = 0.08$ and 0.04, respectively) in comparison to the low and moderate stress groups.

Preliminary Conclusion This study suggests a negative association between self-reported psychological stress and semen quality. The findings were most pronounced for morphology and less for sperm concentration and total sperm counts when results were analyzed using the stress score as a continuous variable. When stratified according to stress categories, the association between stress and the semen variables were strongest for the high stressed group vs. the lower stressed. Further analyses of our data are needed before a final conclusion can be reached.

P135

Comparative Efficacy of the Anastrasol for the Treatment of Idiopathic Infertility in Men with Normal BMI and Obesity

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Background Aromatase is the enzyme that converts testosterone to estradiol in the tissues. Anastrasol is one of the drugs used for inhibition of this enzyme. The aim of our study was to evaluate effectiveness of anastrasol for the treatment of infertile men with obesity and normal BMI.

Methods Data from 60 men with idiopathic infertility at a mean age of 33.2 years were combined. In the group 1 were included 30 men with obesity, in the group 2–30 men with normal BMI. They were managed with Anastrasol 1 mg once a day during 3 months. Analysis of complaints, history of the disease, physical examination, sperm analysis and laboratory investigations were performed. After treatment the sperm analysis and hormones level were investigated.

Results

Group 1: Mean level of estradiol decreased from 108.4 ± 4.2 till 72.3 ± 3.3 pg/ml (–33.3%), mean level of testosterone increased from

12.8 ± 0.6 till 21.9 ± 0.6 nmol/l (+70.7%), mean level of LH increased from 3.1 ± 0.3 till 5.3 ± 0.2 mEd/ml (+71.8%), mean level of FSH increased from 4.6 ± 0.6 till 7.4 ± 0.4 mEd/ml (+60.9%); the concentration of spermatozoa increased from 16.7 ± 4.3 till 30.1 ± 5.2 mln/ml, sperm motility (category A) increased from 13.1 ± 0.7 till 23.0 ± 0.9%, sperm motility (category B) increased from 16.7 ± 1.2 till 28.4 ± 0.8%, mean count of the normal forms of spermatozoa increased from 15.7 ± 1.4 to 24.8 ± 1.5%. All the differences were statistically significant.

Group 2: Mean level of estradiol decreased from 89.0 ± 6.2 till 70.3 ± 3.7 pg/ml (–21.0%), mean level of testosterone increased from 17.9 ± 1.3 till 21.8 ± 0.8 nmol/l (+21.8%), mean level of LH increased from 4.7 ± 0.2 till 5.5 ± 0.3 mEd/ml (+17.0%), mean level of FSH increased from 5.4 ± 0.5 till 6.3 ± 0.4 mEd/ml (+16.7%); the concentration of spermatozoa increased on 16.5%, sperm motility (category A) increased on 14.0%, sperm motility (category B) increased on 15.9%, mean count of the normal forms of spermatozoa increased on 13.1%.

Conclusion The inhibitors of aromatase more effective in men with idiopathic infertility and obesity. Positive effect may be explained by their influence on hormonal profile, decreasing the aromatization of testosterone in the visceral obesity tissue. Because of a small number of the patients further investigations are needed.

P136

Anti-Inflammatory Effect of Androgen Receptor Activation in Human BPH Cells

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Progression of benign prostatic hyperplasia (BPH) involves chronic inflammation and immune dysregulation. A loss of self tolerance, due to expansion of Th17 cells and overexpression of IL-17, which stimulates prostatic stromal cells to produce the potent growth factors (IL-8 and IL-6), is crucial in BPH development, linking a self-perpetuating autoimmune response to hyperplastic growth.

Preclinical studies have demonstrated that prostate inflammation and tissue remodeling are exacerbated by hypogonadism and prevented by testosterone (T) supplementation.

We investigated whether, in humans, hypogonadism was associated with more severe BPH inflammation and thein vitroeffect of the selective androgen receptor (AR) agonist dihydrotestosterone (DHT) on cultures of stromal cells derived from BPH patients (hBPH). Histological analysis of inflammatory infiltrates in prostatectomy specimens from a cohort of BPH patients, and correlation with serum T level was performed.

Histopathological examination of BPH specimens demonstrated the presence of prostatic inflammation in all cases. The inflammatory score (IS) was higher in hypogonadal (T ≤ 8 nM) as compared to eugonadal (T > 8 nM) patients ($p < 0.01$). Accordingly, hypogonadism increased the risk of prostate inflammation by a factor of 5, even after adjusting for age and BMI (HR = 5.7 [1.1–29.4], $p < 0.05$). In an age- and BMI-adjusted model, among the different factors composing the IS, the inflammatory infiltrate grade showed a significant, negative association with testosterone levels (adj. $r = –0.35$, $p = 0.03$).

Triggering hBPH cells by inflammatory stimuli (TNFα, LPS or $CD4^+$ T cells) induced abundant secretion of several inflammatory and growth factors (IL-8, IL-6, bFGF). Co-colture of $CD4^+$ T cells with irradiated hBPH cells induced secretion of Th1-inducer (IL-12), Th1-recruiting chemokine (IP-10) and Th2- (IL-9) and Th17- (IL-17) specific cytokines. Pretreatment with DHT inhibited NF-κB activation and suppressed secretion of several inflammatory/growth factors-with the most pronounced effects on IL-8, IL-6 and bFGF. Reduced inflammatory cytokines production by T cells, an increase in IL-10 and a significant reduction of T cells proliferation suggested that DHT exerted a broad anti-inflammatory effect on T cells. In conclusion, DHT exerts an immune-regulatory role on human prostatic stromal cells, inhibiting their potential to actively induce and/or sustain autoimmune and inflammatory responses.

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Is Metabolic Syndrome a Useless Category in Subjects with High Cardiovascular Risk? – Results from a Cohort Study in Men with Erectile Dysfunction

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Question Although several studies have demonstrated that MetS is associated with a two-fold increase in the risk of cardiovascular (CV) diseases, this risk does not appear to be greater than the sum of risks associated with each of its individual components. To determine the association of men with ED and individual components of MetS and their subsequent relationship to cardiovascular (CV) risk, and, more specifically whether the sum of the MetS components is greater than the individual components in predicting CV risk.

Methods We longitudinally studied a consecutive series of 1687 (mean age 52.9 ± 12.8; range 17–88 years) patients attending our clinic for ED and evaluated different clinical and biochemical parameters. Information on MACE was obtained through the City of Florence Registry Office.

Results 139 MACE, 15 of which were fatal, occurred during a mean follow-up of 4.3 ± 2.6 years. Subjects with MetS at baseline showed a higher incidence of MACE (HR = 1.77), after adjusting for age, however, the association disappeared in an alternative Cox model, adjusting both for age and for individual MetS components (HR = 1.525 [0.564–4.123]; $p = 0.408$). The 2 most predictive MetS components of CV risk were low HDL cholesterol and high triglycerides. Exploring possible interactions between individual components of MetS and their effect on CV risk using two alternative approaches indicates that the effect of MetS components on CV risk is additive, but not synergistic. Among subjects with hypertension, after adjusting for age, elevated glycaemia and low HDL-cholesterol confer relevant additional risk, while in subjects with high triglycerides, hyperglycaemia increased the risk of incident MACE.

Conclusions With regards to CV risk, the MetS construct seems to add little or nothing to the careful assessment of its components. Thus, there is no reason to recommend the use of MetS as a diagnostic category in patients with ED.

P138

Fat Boosts, while Androgen Receptor Activation Counteracts, BPH-associated Prostate Inflammation

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Metabolic syndrome (MetS) and benign prostate hyperplasia (BPH) are often comorbid. Chronic inflammation has been proposed as a putative link between the 2 conditions, as it is a determinant factor for BPH development and progression. This study was aimed at evaluating whether MetS is associated with BPH-related inflammation and at investigating thein vitroeffect of oxidized low-density lipoprotein (oxLDL) – the most relevant autoantigen described in dyslipidaemia – on cultures of stromal cells, derived from BPH patients (hBPH).

In a multi-centre cohort of BPH patients (n = 244), inflammatory infiltrates in prostatectomy specimens showed a step-wise association with the number of MetS factors ($p = 0.001$). After adjusting for age, reduced HDL cholesterol and elevated triglycerides were the only factors significantly associated with inflammatory score (IS). In the subset of patients in whom testosterone (T) evaluation was available (n = 92), increased IS was also significantly associated with hypogo-

nadism. In an age- and T-adjusted model, dyslipidaemia was still associated with IS. In addition, prostatic volume and the anterior-posterior (AP) diameter were positively associated with the number of MetS components. At logistic regression analysis, among MetS determinants, only dyslipidaemia (increased serum triglycerides and reduced serum HDL levels) was significantly associated with an increased risk of having a prostatic volume > 60 cm^3 (HR = 3.268, CI: 1.810–5.901, p < 0.001).

Triggering hBPH cells by oxLDL induced a huge secretion of proinflammatory factors promoting BPH cell growth, such as IL-8, IL-6, bFGF, and a significant increase of IL-7, together with a decrease of the anti-inflammatory cytokines IL-10 and IL-1RA. DHT markedly suppresses the oxLDL-induced IL-8 secretion and the expression of oxLDL receptor (LOX-1). In conclusion, fats could have a detrimental effect on prostate health, boosting prostate inflammation, a key factor in the development and progression of BPH/LUTS. Conversely, beneficial effects of DHT in counteracting lipid-induced IL-8 secretion, make testosterone more a friend than a foe of the prostate.

P139

Waist Circumference and Metabolic syndrome in Relation to Semen Quality and Serum Reproductive Hormone Levels among Estonian Fertile Men

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Introduction Body mass index (BMI) as a surrogate measure of body fat is the standard system for classifying obesity at a population level. It has been suggested that BMI, especially above 30, is associated with subfertility in men. At the same time validity of BMI to distinguish variability in body composition and body fat distribution, have been questioned [1]. In our study we employed more accurate surrogate measure of adiposity such as waist circumference (WC) to evaluate whether overweight and obesity are related to changes in semen quality and serum sex hormone concentrations. Futhermore, we investigated whether metabolic syndrome (MS) is associated with male reproductive parameters.

Methods During 2010 and 2011 male partners of pregnant women were invited to participate in this study. A total of 281 men were divided into 3 groups according to their WC: < 94 cm, 94–102 cm, ≥ 102 cm. Semen was collected by masturbation and sperm parameters were analyzed according to WHO criteria. Patient height, weight, WC and blood pressure were recorded. Body composition was determined using TANITA Corporation (TBF-300MA). Blood samples were collected for sex hormones and biochemical markers. MS was defined using the joint statement from a number of professional organizations [2]. Statistical analysis was done using STADA statistical software. Statistical significance was defined as p < 0.05.

Table 15. K. Ehala-Aleksejev et al. Clinical Findings of the Fertile Estonian Men.

Parameters	Mean ± SD	Median
Age (years)	32 (6.7)	31
Height (cm)	181 (6.2)	181
Weight (kg)	83.5 (13.2)	82
BMI	25.6 (3.8)	25
Testicular volume (ml)[a]	23.52 (4.8)	23.3
Genital and chronic diseases		
– Varicocele, (n%)	71 (25)	
– Cryptorchidism, n (%)	2 (0.7)	
– Cryptorchidism operated, n (%)	3 (1)	
– Testicular cancer operated, n (%)	2 (0.7)	
– *Chlamydia trachomatis*, n (%)	4 (1.4)	
– Ureaplasma urealyticum, n (%)	11 (3.9)	
– HIV, n (%)[b]	1 (0.4)	
– Diabetes, n (%)	1 (0.4)	
– Hypertension, n (%)	16 (5.7)	
– Hypothyreosis, n (%)	1 (0.4)	

[a] Mean of left and right testis, measured by use of Prader's orchidometer
[b] Patient is on the antiviral treatment

Results The mean age of the 281 men was 32 years. Clinical findings are shown in **Table 15**. There was a strong inverse relation between WC and total testosterone levels. Men with WC ≥ 102 cm had lower total sperm count than did men with a WC < 94 cm. WC was not related to estradiol, FSH and LH levels nor to sperm volume, concentration, motility or morphology. Men with MS had lower total testosterone levels. MS was unrelated to sperm parameters.

Conclusions Our preliminary results suggest that visceral adiposity (as assessed by increased WC above 94 cm) alone and as a component of the metabolic syndrome, is specifically associated with lower testosterone levels. Men with WC ≥ 102 cm are also increased risk of lower total sperm count.

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P140

Mechanism of Action of Phosphodiesterase Type 5 Inhibition in Metabolic-syndrome-associated Prostate Alterations: An Experimental Study

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Background Phosphodiesterase type 5 (PDE5) inhibitors improve benign prostatic hyperplasia (BPH)-related lower urinary tract symptoms (LUTS), often associated with metabolic syndrome (MetS). This study investigated the effects of PDE5 inhibition in the prostate of a MetS rabbit model, obtained by high fat diet (HFD) for 12 weeks. HFD-rabbits recapitulate human MetS (glucose intolerance, dyslipidemia, increased abdominal adiposity and hypertension) and develop hypogonadism and LUT abnormalities (prostate and bladder inflammation/ tissue remodelling).

Methods Gene expression was analyzed by quantitative RT-PCR. Morphological changes, inflammation and oxygenation of rabbit prostate were evaluated by immunohistochemistry.

Results HFD prostates showed increased PDE5 expression, suggesting a peculiar sensitivity of prostate to the action of PDE5 inhibitors during MetS. Accordingly, prostate PDE5 mRNA was negatively associated to plasma testosterone/estradiol ratio, whose reduction characterizes MetS, and positively with the expression in prostate of several genes exploring BPH/LUTS pathogenetic processes, such as inflammation, leukocyte infiltration and fibrosis/myofibroblast activation. Most of these genes was up-regulated by HFD, and significantly reduced by PDE5 inhibition, through either chronic (12-weeks) or, at a lower extent, acute (1-week) tadalafil dosing. Tadalafil was also able to reduce blood pressure and visceral fat in HFD rabbits, without changing any other MetS parameter. Interestingly, 1-week tadalafil administration to HFD rabbits, significantly blunted prostate inflammation (increased CD45 immunopositivity), fibrosis (reduced muscle/fiber ratio) and hypo-oxygenation, thus suggesting a potential curative effect of PDE5 inhibition on MetS-related prostate alterations.

Conclusions Our data demonstrate that PDE5 inhibition in an animal model of HFD-induced MetS may act at multiple levels in counteracting prostate tissue derangements, thus adding new insights into the comprehension of their mechanism of action in alleviating LUTS and supporting the multiple potentiality of this class of drugs as a useful therapeutic tool in MetS patients.

P141

Sperm Characteristics in an Animal Model of Metabolic Syndrome

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Introduction Metabolic syndrome (MetS) represents an important epidemiologic entity that potentially affects many aspects of human physiology. Recently an association between MetS and hypogonadotropic hypogonadism (hypo-hypo) possibly leading to male infertility was observed. To study the relationship between MetS and male infertility we used an animal model of male rabbits, fed with high fat diet (HFD) [Filippi et al., 2009], which showed the main characteristics of MetS and hypo-hypo. We evaluated several sperm parameters after sacrifice of these animals.

Material & Methods The groups of animals analyzed were: (1) control (fed by a regular diet); (2) HFD; (3) HFD+Testosterone (T); (4) HFD+Tamoxifen (Tam). As control, a group of animals was treated with GnRH analog, to simulate hypo-hypo induced by castration. Spermatozoa were extracted from epidydimis to evaluate number, morphology (by Diff-Quick staining), motility (by computer system analyzer [CASA] and microscopy), DNA fragmentation (SDF) (by TUNEL assay [Muratori et al., 2008]) and acrosome reaction (AR) induced by progesterone (P) (by lectin staining).

Results Sperm number and motility were decreased in HFD rabbits (number: mean ± sd = 38.3 ± 29.9; motility: mean ± sd = 36 ± 16.9) respect to control (number: mean ± sd = 71.3 ± 65.9; motility: mean ± sd = 76 ± 9.8; p = 0.01). CASA motility parameters were modified towards an early hyperactivated motility state, showing decrease of VAP, VSL, STR, LIN and increase of VCL and ALH. Treatment with T restored motility to control levels. The supplementation of Tam further reduced the sperm number (mean ± sd = 18.3 ± 6.1), increased the percentage of non-progressive motility (mean ± sd = 64 ± 2.8; p = 0.02) and augmented the shift to hyperactivation. Moreover, in HFD + Tam group, a significant increase of coiled tail sperm, consistent with exposure to a hyposmotic epididymal fluid, was observed. Rabbits treated with GnRH analog showed no alterations in sperm number or motility. Preliminary results demonstrated an increase of spontaneous AR and lack of response to P in HFD + Tam and GnRH-treated groups. No differences were observed in percentage of SDF among the various groups.

Conclusion Our data indicate the occurrence of slight alterations of seminal parameters in our model of MetS, which were restored by treatment with T. Of interest, our data suggest that treatments with Tam and GnRH shift epididymal sperm towards a early hyperactivated and capacitated phenotype possibly altering the timing of the 2 processes.

P142

Antioxidant Treatment with Edaravone or Taurine Ameliorates Diabetes-induced Testicular Dysfunction in the Rat

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Objectives Diabetes mellitus with the subsequent generation of reactive oxygen species represent a major risk factor for testicular dysfunction (TD). We tried to investigate whether administration of the antioxidants edaravone and taurine, could prevent type 1 diabetes-induced TD in the rat.

Materials & Methods 6-week-old male Wistar rats were divided into four groups. Group A was treated with citrate-phosphate buffer plus normal saline, whereas in the other three groups diabetes was induced by streptozotocin (50mg/kg intraperitonealy). Subsequently, the diabetic rats were treated for 4 weeks either with normal saline (Group B), edaravone (10 mg/kg/day, intraperitonealy; Group C) or taurine (500 mg/kg/day, intraperitonealy; Group D).

Body, testicular, and epididymal weight, serum glucose, malondialdehyde levels, testicular catalase activity and serum testosterone levels were determined. Histological examination and the Johnsen score were used to observe and evaluate respectively the morphological changes in the testes. TUNEL assay was used to examine DNA fragmentation. Mating studies were performed in order to evaluate the fertility potential of the male rats in each group.

Results Edaravone or taurine treatment prevented significantly the decreased body, testicular and epididymal weight induced by diabetes. Moreover, edaravone or taurine significantly decreased the diabetes-induced malondialdehyde levels, the morphological damage, and the number of apoptotic cells. Taurine but not edaravone increased significantly the testicular catalase activity. The antioxidant treatment had no effect on the fertility potential of the diabetic rats.

Conclusions The morphological damage, increased lipid peroxidation, and apoptosis in testicular tissue can be significantly relieved by edaravone or taurine treatment through suppressing the increased oxidative stress in the rat testis.

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Has Metabolic Syndrome any Effect on Reproductive Characteristics in Overweight Males?

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Introduction Metabolic syndrome (MeS) is a heterogeneous constellation of abnormalities including overweight, dyslipidaemia, hypertension, and hyperglycaemia. It is well known that in combination, these disturbances increase the risk of developing cardiovascular disease and diabetes. The influence of MetS on fertility and especially on male fertility, is less well explored. The aim of this study was to compare reproductive characteristics in overweight and obese men with MetS versus subjects without MetS.

Material & Methods 101 men (22–61 years) with BMI ≥ 25 kg/m^2 were investigated. Semen analysis was performed according to WHO recommendation. Levels of serum lipids (total cholesterol, LDL-cholesterol, HDL-cholesterol and triglycerides), micro-CRP, blood glucose and glycated hemoglobin, testosterone, SHBG and estradiol were measured in fasting blood samples. Blood pressure, waist circumference, percent body fat, general health status, medication, smoking, and physical activity were registered. MetS was defined using the National Cholesterol Education Program Adult Treatment Panel III criteria (ATP III). Free androgen index (FAI) was calculated from total testosterone and SHBG. Linear regression analysis was performed to evaluate the differences between men with and without MetS.

Results MetS was present in 63 men (62%). The group with MetS had significant higher BMI (median 34.1, range 27.5–62.6) than the group without MetS (median 29.2, range 25.0–54.2). Men with MetS had significantly lower testosterone (p = 0.012) as well as SHBG (p = 0.004) levels, when adjusted for age. We did not observe any significant difference in estradiol concentration or FAI between the two groups. There were no significant differences between the sperm characteristics investigated (sperm concentration, total sperm count, vitality, morphology) when adjusted for age. However, there was a tendency towards lower total sperm count in men with MetS. There was also a tendency towards more abnormalities in spermatozoa from men with MetS.

Conclusion Our study shows lower levels of testosterone and SHBG in men with MeS, and suggests that MetS may be associated with reduced semen quality. Further studies are necessary to clarify the effect of metabolic abnormalities on male reproductive function.

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Association between UGT2B17 Genotype and Testosterone Substitution Dosages?

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Introduction The excretion of testosterone glucuronide is partly dependent on the UGT2B17 genotype and recent studies have shown that deletion polymorphisms are strongly associated with significant lower levels of excreted urinary glucunidated testosterone in men [1–3]. The objective of this study was to investigate the association of the UGT2B17 gene polymorphism and the dosages of testosterone substitution in men with androgen deficiency. Our hypothesis was that the men having the UGT2B17 deletion would need lower dosages.

Material & Methods 229 men treated with Testosterone undecanoate (TU) (Nebido®) were retrospectively included. All men had been given 1000 mg TU per injection, with same intervals. At endocrine follow-ups blood samples were drawn 2 weeks, 6 weeks (2nd injection), 18 weeks (3rd injection), 30 weeks (4th injection) and 42 weeks (5th injection) after the initial injection.

Results The men were stratified according to their carrier status of the UGT2B17 gene. Thirty-one (13.5 %) had a homozygote deletions (group 1), 103 (45.0 %) were heterozygote (group 2) and 95 (41.5 %) were homozygotes for the wildtype (group 3). At the 3rd injection (18 weeks), testosterone levels did not differ between the 3 groups in total ($p = 0.065$): median 13.2 nmol/l, 12.7 nmol/l and 14.0 nmol/l in group 1, 2 and 3, respectively. Estradiol levels tended to be higher in group 1 (66,5 pmol/l) then in the 2 other groups (ins/del: 54 pmol/l and ins/ins: 52 pmol/l), though not statistically significant. LH, SHBG, the free androgen index (FAI), total cholesterol or haemoglobin did not differ between groups.

At follow-ups 2–3 years after initiation of treatment all patients had individual treatment regimes, but no association to genotype was detected.

Conclusion Serum testosterone levels did not depend on UGT2B17 genotype in hypogonadal men given standard treatment with TU. Thus, there is no need to consider this genotype as a marker of dosage or interval when initiating testosterone treatment.

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Postersession 8: Sexual Medicine & Non-obstructive Azoospermia

P144

Use of Ethnicity-specific Sequence Tag Site Markers for Y-chromosome Microdeletion Studies in Non-Obstructive Azoopsermic Males

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Introduction Microdeletions in the azoospermia factor region on the long arm of Y chromosome are associated with spermatogenic failure. There are many markers for the diagnosis of Y chromosome microdeletion analysis, but in routine practice only a limited set of markers can be tested.

Objective The objectives of this study were to determine the frequency of Y chromosome microdeletion in idiopathic cases of male infertility in India, to attempt genotype-phenotype correlation, and to evaluate whether markers to be tested for diagnosis of Y chromosom microdeletion should be ethnicity specific.

Methods Microdeletions in the Y chromosome were analyzed in 200 infertilemales. The six sequence tag site (STS) markers prescribed by the European Academy of Andrology (EAA) were used initially. Patients in whom no deletions were detected by use of these markers were tested by markers selected from other studies from India.

Results The STS markers prescribed by EAA detected deletions in only 6 (3%) of 200 infertile males. However, markers selected from previous Indian studies showed deletions in an additional 15 (7.5%) of infertile males. Overall, Y chromosome microdeletions were observed in 21 (10.5%) of 200 patients. Of these, 13 were cases of azoospermia and 8 were cases of severe oligospermia.

Conclusion The markers prescribed by EAA alone are not suitable for the diagnosis of Y chromosome microdeletions in infertile males. The protocol for identification of Y chromosome microdeletions in cases of non-obstructive azoospermia/severe oligospermia would have to include a different set of STS markers.

P145

Expression of Testosterone in Leydig Cells of Infertile Patients with Non-Obstructive Azoospermia (NOA)

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One of the most severe forms of male infertility is non-obstructive azoospermia (NOA). NOA is frequently characterized by a heavy damage of seminiferous tubules. However, there is a lack of data on changes of Leydig cells and the expression of testosterone in situ. Therefore, the aim of the current survey was to investigate Leydig cells in testicular biopsies (n = 120) of 2 groups of patients, i.e. controls and infertile men with NOA. Methods used were qualitative and quantitative histological analysis and immunohistochemistry. In addition, blood levels of gonadotrophins and testosterone were determined. Results of qualitative histological analysis demonstrated a kind of "mosaic" picture of regular and irregular Leydig cells in the NOA group. In average, there were 35% irregular Leydig cells and their number correlated with low testis volume and damaged spermatogenesis. Histological analysis indicated a significantly lower number of testosterone-producing cells ($p < 0.001$) **(Fig. 23, 24)**. The results of the study pointed out that the patients with NOA could suffer from a deficit of androgens in situ as well as a premature andropause.

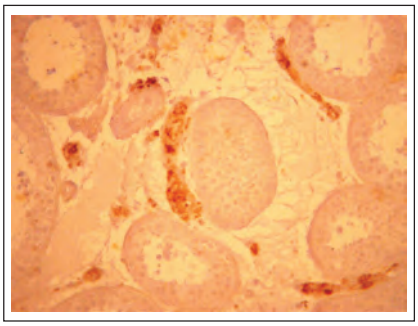

Figure 23. D. Jezek et al.

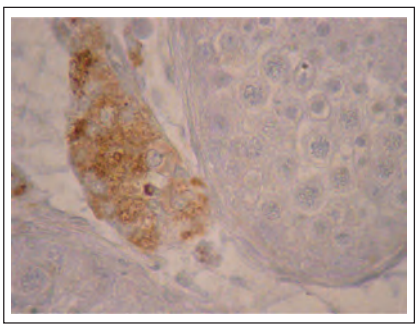

Figure 24. D. Jezek et al.

P146

Circumcision and Male Sexual health: A Predictive Analysis

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Introduction Circumcision is a widespread practice nowadays, although little is known about its impact on male sexual life.

Question To characterize the sexual life of the enrolled population and to evaluate possible associations of circumcision with several measures of sexual health.

Methods We conducted telephone surveys about sexual habits to patients that underwent circumcision (n = 62) and other urological surgical procedures (n = 68) in Centro Hospitalar V.N. Gaia/Espinho during 2011 (population characterization in **Table 16**). Odds ratios (ORs), adjusted in a multivariate analysis (to age, marital status, diabetes mellitus and chronic medication) explored associations between circumcision and present sexual dysfunctions.

Results The median age of first sexual intercourse was 17 years old (interquartile range [IQR] 16–18), the median number of sexual partners was 4 (IQR 2–8) and the median number of sexual intercourse per month was 8 (IQR 4.25–12). The presence of a significant organic dysfunction was more common in those circumcised vs uncircumcised (71% vs 45.5%, adjusted OR [OR_{adjust}] of 2.56, 95%-CI: 1.09–5.99; p = 0.030), particularly delayed orgasm (48.4% vs 12.5%, OR_{adjust} 4.40, 95%-CI: 1.72–11.24; p = 0.002). In circumcised patients, the complete satisfaction of sexual needs tends to be lower (40.3 vs 22.7%, with age-adjusted OR [OR_{age}] of 2.60, 95%-CI: 1.18–5.75; p = 0.018; OR_{adjust} 2.16, 95%-CI: 0.88–5.32; p = 0.097) and erectile dysfunction tends to be more frequent (25.8% vs 13.6%, OR_{age} of 2.74, 95%-CI: 1.05–7.14; p = 0.040; OR_{adjust} 1.47, 95%-CI: 0.46–4.67; p = 0.516). No significant differences were found regarding sexual desire, premature ejaculation or dyspareunia.

Conclusion Circumcision was associated with significant sexual dysfunctions, prompting a careful selection of patients in whom this procedure should be performed.

Table 16. J. Dias et al. Population Characterization

	Total Population (n = 128)	Circumcised (n = 62)	Non-Circumcised (n = 66)	
Age (yrs)	43.62 (± 14.10)	41.79 (± 15.4)	45.34 (± 12.62)	p = 0.158*
School attendance (yrs)				p = 0.144**
0–4	22.7%	19.4%	25.8%	
5–9	27.3%	24.2%	30.3%	
10–12	25.8%	27.4%	24.2%	
College	24.2%	29.0%	19.7%	
Martial status				p < 0.001**
Single	21.1%	32.3%	10.6%	
Married	70.3%	54.8%	84.8%	
Divorced	7.08%	11.3%	4.5%	
Widowed	0.8%	1.6%	0%	
Diabetes mellitus	14.1%	24.2%	4.5%	p = 0.002#
Duration (yrs)	6.5% (± 6.95)	6.87% (± 7.55)	4.67 (± 2.31)	
Smoking				p = 0.340**
Non Smoker	47.7%	46.8%	48.5%	
Former Smoker	23.4%	17.7%	28.8%	
Smoker	28.9%	35.5%	22.7%	
Pack-Year	13 [6–25]	30 [20–35]	19 [10–28]	p = 0.660##
Use of chronical medication				p = 0.083#
Yes	40.6%	48.4%	33.3%	
No	59.4%	51.6%	66.7%	
Follow-up (months)	11.08 (± 3.74)	11.49 (± 3.81)	10.70 (± 3.65)	p = 0.237*

(): standard deviation; []: interquartile range; * t-student test; # Chi square test; ## Mann-Whitey test

P147

Perceived Reduced Sleep-Related Erections in Subjects with Erectile Dysfunction: Psychobiological Correlates

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Introduction Perceived reduced sleep-related erections (PR-SREs), along with erectile dysfunction (ED) and hypoactive sexual desire, have been recently recognized as the most important symptoms characterizing late-onset hypogonadism in community-dwelling European men. However, the clinical correlates of PR-SREs have not been thoroughly investigated. The aim of the study is to evaluate the psychobiological correlates of PR-SREs in a large series of subjects consulting for ED.

Methods A consecutive series of 3,888 (mean age 51.6 ± 13.0 years) ED patients attending an outpatient ED clinic was retrospectively analyzed. PR-SREs were investigated using validated question #13 of structured interview on ED, which showed an accuracy of approximately 70% in predicting Rigiscan™ (Dacomed Corp., Minneapolis, MN, USA) parameters in a consecutive subset of 199 subjects. Clinical, biochemical, hormonal, instrumental (penile color Doppler ultrasound; PCDU), and intrapsychic (Middlesex Health Questionnaire) correlates were also evaluated.

Results PR-SREs were reported by 63.6% of patients. After adjustment for age, total, analog free, calculated free and calculated bioavailable testosterone (T) were significantly lower in subjects reporting more severe PR-SREs. After adjusting for T levels and other confounders, PR-SREs were still associated with higher body mass index, glucose, and triglyceride levels, as well as with an increased 10-year cardiovascular risk score. Accordingly, PR-SREs were more prevalent in subjects showing a reduced dynamic peak systolic velocity at PCDU or reporting severe ED. Among intrapsychic parameters, depressive and histrionic traits were significantly higher and lower, respectively, in subjects with any degree of PR-SREs.

Conclusions Our study indicates that investigating PR-SREs represents an important step during the andrological consultation. In fact,reduced SREs might indicate an endocrine, organic, and/or psychiatric ED background that might help in directing further investigation.

P148

Our Experience of MicroTESE in Men with Non-obstructive Azoospermia

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Introduction There are several different surgical procedures for spermatozoa retrieval when the patient has non-obstructive azoospermia. The aim of our study was to evaluate the effectiveness of TESE (testicular sperm extraction) and microTESE in men with non-obstructive azoospermia.

Materials & Methods Analysis of complaints, history of the disease, physical examination, sperm analysis and laboratory investigations were performed. All patients undergo genetic (AZF-factor, CFTR, cariotype) tests and hormone profile (FSH, LH, testosterone, estradiol, prolactine, tyroxine). In the period from march 2011 to april 2012 for 148 patients with non-obstructive azoospermia the different methods of surgical treatment were perfomed (92 TESE and 56 microTESE). In TESE group the multiple biopsies were performed.

Results There were no differences of FSH-level, Inhibin B-level, volume of the testis in

the groups. In TESE group spermatozoa were detected unilaterally in 34.8% (32 patients) and bilaterally in 39.1% (36 patients), in microTESE group – 46.3% unilaterally (26 patients) and 55.4% bilaterally (31 patients).

Conclusion MicroTESE is the most effective procedure for spermatozoa retrieval in men with non-obstructive azoospermia. This procedure could minimize the damage of testicular tissue and prevent vascular injury. Bilateral exploration of the testes is more beneficial.

P149

SIEDY Scale 3, a New Instrument to Detect Psychological Component in Subjects with Erectile Dysfunction

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Question We previously developed and validated a structured interview (SIEDY) dealing with the organic (Scale 1), relational (Scale 2) and psychological (Scale 3) components of erectile dysfunction (ED). The aim of this study is to identify a pathological threshold for SIEDY Scale 3 and to analyze Scale 3 score with biological and psychological correlates in subjects with sexual dysfunction.

Method A pathological threshold of SIEDY Scale 3 score in predicting subjects with a medical history of psychopathology and using psychiatric drugs was identified through receiver operating characteristic (ROC) curve analysis, in a sample of 484 patients (Sample A). Sensitivity and specificity, along with possible interactions with biological and psychological (Middlesex Hospital Questionnaire, MHQ-score) correlates were verified in a further sample of 1275 patients (Sample B).

Results In Sample A, 39 (8%) and 60 (12.4%) subjects reported a positive medical history for psychiatric disturbances or for the use of psychotropic medication, respectively. The association with both conditions was present in 28 (5.8%) subjects. ROC curve showed that SIEDY Scale 3 score predicts psychopathology with an accuracy of 69.5 ± 5.9% ($p < 0.002$), when a threshold of 3 was chosen. When the same threshold was applied in Sample B, it identified a higher ranking in MHQ-A (free-floating anxiety), MHQ-S (somatized anxiety) and MHQ-D (depressive symptoms) subscales, even after adjustment for age and Σ-MHQ (a broader index of general psychopathology). In the same sample, we also confirmed that pathological Scale 3 score was related to a higher risk of psychopathology at medical history or to the use of psychotropic drugs as well as with risky lifestyle behaviors, including smoking and alcohol abuse, and elevated BMI.

Conclusions SIEDY represents an easy tool for the identification of patients with a relevant intra-psychic component who should be considered for psychological/psychiatric treatment.

P150

Proteomic Analysis of Seminal Plasma from Normozospermic and None-obstructive Azoospermic (NOA) Men to Discover New Markers

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Introduction Seminal plasma is a complex mixture of secretions with a large collection of proteins arising from the testis, epididymis, prostate, and seminal vesicles. Azoospermia is the causes of 10–15% of male infertility that present as obstructive and none-obstructive azoospermia. Proteomics is able to determine the changes in proteins pattern following impairment of spermatogenesis that lead to abnormal sperm criteria or absence or limited focal spermatogenesis in testis. It can introduce new biomarkers for prognosis of presence of sperm in testis of NOA men.

Material & Methods Seminal plasma samples were collected from two groups of normozoospermic as control and none-obstructive azoospermic men contain three individuals in each group. Protein profiles of these samples were acquired using two dimensional gel electrophoresis technique and colloidal coomassie blue staining. Seminal plasma patterns of 2 groups (normal and azoospermia) were compared by using ImageMaster software.

Results 1179 proteins were detected on the average gel that significant expressional differences were observed in 7 proteins, 3 proteins had over expression in azoospermia while one protein was underexpressed and 1 protein was totally absent in seminal plasma of azoospermia and 2 newly expressed were determined.

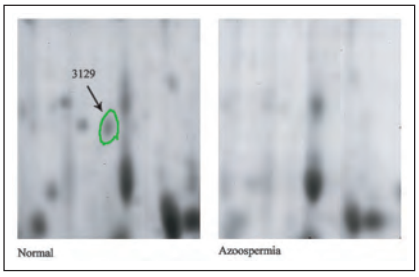

Figure 25. A. Meyfour et al. Representative images of colloidal coomassie blue stained 2-D gels from normal and azoospermia to highlight the spot demonstrating significant expressional difference between normal and azoospermia seminal plasma samples.

Conclusion Mass spectrometry identification of seminal plasma protein alterations by 2-D gel electrophoresis can lead us to better understanding of molecular mechanisms involved in male infertility and finding biomarkers for azoospermia to utilize them in clinical diagnosis **(Fig. 25)**.

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P151

Thyroid Hormones and Male Sexual Function

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Question The role of thyroid hormones in the control of erectile functioning has been only superficially investigated. The aim of the present study is to investigate the association between thyroid and erectile function in two different cohorts of subjects.

Methods The cohort derives from the European Male Aging Study (EMAS study), a multicentre survey performed on a sample of 3,369 community dwelling men aged 40–79 years (mean 60 ± 11 years). The second cohort is a consecutive series of 3203 heterosexual male patients (mean age 51.8 ± 13.0 years) attending our Andrology and Sexual Medicine Outpatient Clinic for sexual dysfunction at the University of Florence (UNIFI study). In the EMAS study all subjects were tested for thyroid-stimulating hormone (TSH) and free thyroxine (FT4). Similarly, TSH levels were checked in all patients in the UNIFI study, while FT4 only when TSH resulted outside the reference range.

Results Overt primary hyperthyroidism (reduced TSH and elevated FT4, according to the reference range) was found in 0.3 and 0.2% of EMAS and UNIFI study, respectively. In both study cohorts, suppressed TSH levels was associated with erectile dysfunction (ED). Overt hyperthyroidism was associated with an increased risk of severe erectile dysfunction (ED, hazard ratio = 14 and 16 in the EMAS and UNIFI study, respectively; both $p < 0.05$), after adjusting for confounding factors. These associations were confirmed in nested case-control analyses, comparing subjects with overt hyperthyroidism to age, BMI, smoking status and testosterone-matched controls. Conversely, no association between primary hypothyroidism and ED was observed.

Conclusions Erectile function should be evaluated in all individuals with hyperthyroidism. Conversely, assessment of thyroid function cannot be recommended as routine practice in all ED patients.

P152

Gorlin-Goltz Syndrome – A Rare Cause of Male Infertility

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Introduction The Gorlin-Goltz syndrome is a rare autosomal dominant hereditary disease defined by major and numerous minor criterias. The most important features are multiple basal cell carcinomas, odontogenic keratocysts, palmar or plantar hyperkeratosis, calcifications of the falx cerebris and skeletal abnormities. However, an association of Gorlin-Goltz syndrome and disorders of the genitourinary tract are rarely known.

Methods We report on a 34 year old male with a diagnosted Gorlin-Goltz syndrome with anvoluntary childlessness, who was admitted to our department for further diagnostics. We present data of a systematic literature analysis on “Gorlin Goltz”, “cryptorchidism” and “fertility”.

Results The urological anamnesis revealed a bilateral cryptorchidism with a right sided orchiectomy during puberty.

The clinical examination showed a hypotrophy of the left testis including a varicocele testis 1°. The spermiogram showed an azoospermia and a hypospermia. The elevated serum FSH (26.2 IU/l) and LH (13.3 IU/l) indicated a hypergonadotrophic hypogonadism. The sperm retrieval was performed as a trifocal-TESE (testicular sperm extraction) in general anesthesia.

The histopathological examination showed a germ cell aplasia in 50% of the tubuli and an early maturation arrest (Johnson-score 4). Thus assisted reproductive techniques were unfortunately not successful.

The association of Gorlin-Goltz syndrome and infertility has not been discussed in literature until now.

Conclusions The Gorlin-Goltz syndrome can lead to male infertility due to cryptorchidism and hypogonadism. Although the genitourinary disorders normally affect the patients only by minor symptoms the issues can restrict male reproductive capacity tremendously.

P153

Ultrastructure of Cultured Spermatogenic cells in Testicular Materials of Non-obstructive Azoospermic Patients

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Introduction Spermatogenic arrest is the pause of spermatogenesis in some seminiferous tubules during spermatocyte or spermatid phase. In vitro spermiogenesis is an application to maintain the post-meiotic differentiation in spermatogenic arrest patients. Recently, it became an interest of many researchers because it can be used as a pretreatment in assisted reproductive techniques, however, remained to be unknown.

We searched in vitro spermiogenesis of testis materials dissected from spermatogenic arrest patients by culturing their biopsy materials and investigated their ultrastructure under transmission electron microscopy (TEM).

Materials & Methods Testicular biopsies of non-obstructive azospermic patients (n = 18) diagnosed as maturation arrest were dissected mechanically AND discontinuous density gradient of 40% and 80% was applied for isolation. Isolated cells were counted and statistically analyzed using Dunn’s Test.

0.5 ml pre-cultured cell suspensions from both gradients were washed and prepared for transmission electron microscopy investigation. Other 0.5 ml cell suspension from 40% gradient was washed and cultured with G-IVF medium supplemented with FSH and testosterone for six days. Half of the cultured cells were centrifuged, smeared and stained with May-Grünwald Giemsa. The other half were also prepared for TEM and examined under Jeol JEM 1011 TEM.

Results Cell counts isolated from 40% gradient contained significantly higher numbers of round cells than 80% gradient). When isolated cells before the culture were examined under TEM, round spermatids (Sa) with condensing nuclei were observed, as parallel to light microscopic results. However, some spermatids have had damaged morphology and were apoptotic. Nuclei had apoptotic bodies and cytoplasms were scattered. Connective tissue cells and collagen bundles and elastic fibers were shown in some samples **(Fig. 26)**.

TEM results showed the elongated spermatids (Sd) among the cultured cells, as seen under the light microscope. However, ultrastructure of some spermatids were damaged, had apoptotic bodies, degenerated membrane and acrosomes **(Fig. 27)**.

Conclusions It is a controversial issue that mechanical dissection of seminiferous tubules of azospermic patients and cell isolation procedures for in-vitro maturation studies can be hazardous for cell homeostasis. In this study, we showed that apoptosis is induced in both pre- and post-cultured cells due to mechanical forces arised from centrifugation. In spite of application of the ideal culture conditions with FSH and testosterone conditioned medium (corresponding to the literature), since spermatogenic cells are delicate and un-protected, isolation procedures may affect them negatively. Low culture success may be due to the damaged ultrastructure of germ cells. Thus, we conclude that alternations in the physiological conditions of cells may result in damaged morphology.

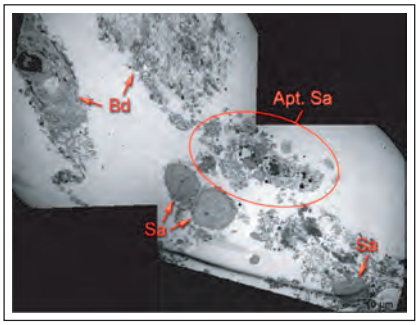

Figure 26. E. Yaprak et al.

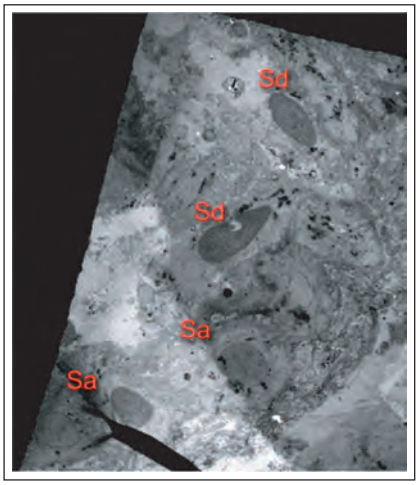

Figure 27. E. Yaprak et al.

P155

Multifactorial Etiology of Erectile Dysfunction in Patients with Prostate Carcinoma Treated by Radiotherapy

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Erectile dysfunction (ED) is a frequent problem in patients treated for prostate carcinoma, profoundly affecting quality of life. It has been reported that neurovascular mechanisms can be affected by radiotherapy; however, ED can recognize a more complex multifactorial etiology, including ageing per se, patient comorbidity, metabolic and endocrine factors. Hypogonadism can also represent a biochemical and clinical entity; but hormones, other than testosterone (T) can influence sexual function; we previously demonstrated an increased estradiol concentration in patients affected by venous leakage ED.

In order to evaluate the endocrine component, we have evaluated a group of 24 patients, aged 51–76 ys., treated by radiotherapy and antiandrogen pharmacological therapy (bicalutamide), with ED, studying: metabolic parameters (glycemia, total HDL LDL cholesterol, triglycerides, uric acid, albumin), hormones (T, LH, FSH, estradiol, dihydrotestoterone, SHBG, IGF-1, PRL, FT3, FT4, TSH, insulin), evaluation of international index of erectile failure (IIEF); main vascular and neurological factors were excluded on the basis of basal Doppler evaluation and vibration threshold.

7 patients, previously treated with LHRH analogues, were still hypotestosteronemic (mean ± SD 0.93 ± 0.59 ng/ml), while in the other patients testosterone exhibited normal values (6.74 ± 2.62 ng/ml). Testosterone/estradiol molar ratio markedly different in the two groups (0.59 ± 0.23 in hypo-T patients and 1.56 ± 0.26 in normo-T patients); IIEF were significantly lower in hypo-T than in normo-T patients (4.2 ± 7.4 vs 10.4 ± 10.8). Metabolic parameters were markedy worse in hypo-T vs normo-T (HOMA index 5.42 ± 6.6 vs 2.41 ± 1.4).

These preliminary data suggest that, despite similar radiotherapic schedule treatment, sexual function is strictly related to hormonal milieu and metabolic status. A systematic approach to evaluate ED is mandatory in such patients, since improving some components ED can be positively influenced by personalized therapy.

P156

Inhibition of Circulating Angiogenic Cells from Healthy Men is associated with a TNF-α-dependent Activation of Caspase Cascade and to Mitochondrial Depolarization

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Introduction Soluble factors in the serum of men with erectile dysfunction (ED) and vascular risk factors (VRFs) inhibited cultured mononuclear circulating cells (MNCs) of healthy men to differentiate to circulating angiogenic cells (CACs), putatively involved in endothelial damage repair [Pelliccione et al. Int J Androl, 2012]. Here we explored molecular mechanisms potentially involved in a reduced differentiation of CACs from MNCs of healthy men, focusing on tumor necrosis factor-α (TNF-α).

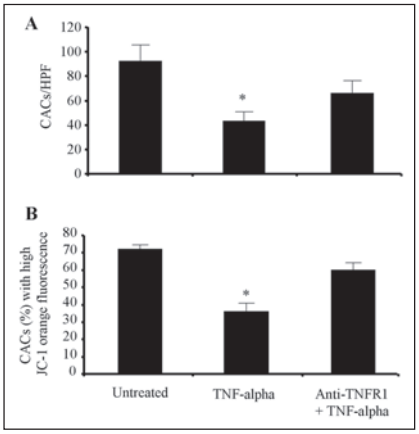

Figure 28. S. Francavilla et al. Effect of TNF-α (10 ng/ml) with or without 100 mg/ml competitive anti-type 1 TNF-α receptor (anti-TNFR1) monoclonal antibody on differentiation **(a)** and mitochondrial membrane potential **(b)** of circulating angiogenic cells (CACs).
(a): *p = 0.01 vs all the others (Tukey HSD test). HPF = high power field. (b): *p = 0.001 vs untreated and p = 0.008 vs Anti-TNFR1+TNF-α (Tukey HSD test).

Material & Methods After 4 days of culture of MNCs from healthy men, adherent cells were maintained for further 3 days with or without hrTNF-α (10 ng/ml), with or without a 15 min pre-exposure to a competitive anti-type 1 TNF-α receptor (TNFR1) mAb (100 mg/ml). After culture, CACs were identified by uptake of acetylated low-density lipoprotein and binding of *Ulex europaeus* agglutinin I. Mitochondrial membrane potential (ΔΨm), was assessed by flow cytometry with JC-1, which emits orange or green fluorescence in the presence of high or low ΔΨm, respectively. Caspase activation was evaluated by flow cytometry using permeable FITC-conjugated peptides (IETD-FMK, LEHD-FMK and DEVD-FMK), which irreversibly bind to the activated caspase-8, -9 and -3, respectively, in apoptotic cells.

Results The mean number of CACs from healthy men was significantly reduced after culturing MNCs with hrTNF-α compared to standard medium. TNF-α treatment also significantly decreased cellular ΔΨm compared to control medium (36.0 ± 7.7% vs 71.8 ± 3.8%, $p < 0.05$) **(Fig. 28)**. TNF-α treatment was associated to caspase-8 (27.3 ± 18.4%), caspase-9 (33.2 ± 25.4%) and caspase-3 (31.0 ± 23.7%) activation (controls: 7.9 ± 3.6%, 6.0 ± 1.3%, 5.4 ± 4.0%, respectively, $p < 0.05$) **(Fig. 29)**. The effects of TNF-α on CACs differentiation, ΔΨm and caspase activation were prevented by the anti-hTNFR1 mAb.

Conclusion Increased circulating levels of TNF-α, reported in men with ED and VRFs, could adversely impact CACs differentiation from MNCs by triggering both death-receptor pathway and mitochondrial pathway of apoptosis. Current study are analysing the contribution of TNF-α in the inhibition of CACs from healthy men by serum of men with ED.

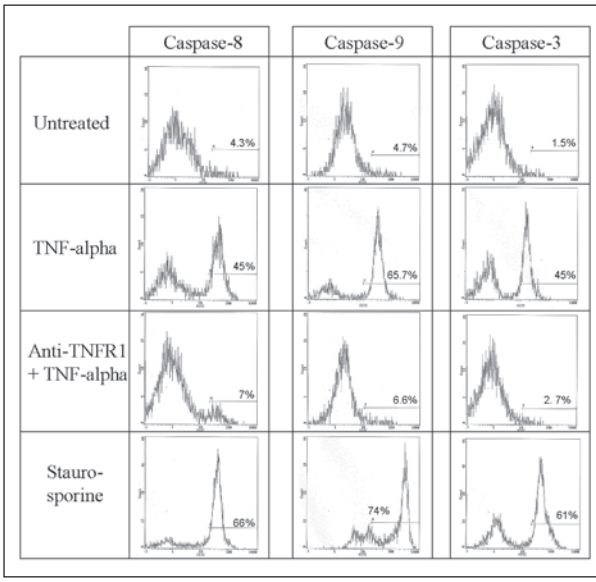

Figure 29. S. Francavilla et al. Histograms of fluorescence for activated caspases at flowcytometry. Staurosporine (1 mM) was used as positive control for caspase activation.

P157

Ejaculatory Disorders as a Result of Antituberculous Therapy

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Introduction Majority of patients with pulmonary TB (PTB) are young men for whom sexual function is very important. The aim was to estimate the frequency of ejaculatory disorders in men suffering from tuberculosis and to determine the effect of TB treatment on the ejaculation.

Material & Methods 98 PTB patients were enrolled in study. The intravaginal latency time before onset of TB was estimated retrospectively and in 3 months of anti-TB therapy.

Results Before anti-TB therapy 14.3% of PTB patients had ejaculatory disorders: 10.2% had premature ejaculation, and 4.1% delayed ejaculation. The rest 85.7% had normal ejaculation. After three months of the therapy with 4 anti-TB drugs (isonazid, rifampicin, pyrazinamid and streptomycin) the share of patients with normal ejaculation decreased to 61.2%. Frequency of premature ejaculation increased twice (20.4%), and delayed ejaculation – in 4.5 times (18.4%).

Conclusion Proportion of ejaculatory disorders in patients with pulmonary TB before a start of anti-TB therapy was the same as in population as whole. So, tuberculosis as a disease does not damage an ejaculatory function. Three months of standard anti-TB therapy with four drugs significantly worsened the ejaculatory function of patients. The high growth of delayed ejaculation may be explained by neurotoxicity of anti-TB drugs. So, tuberculosis as a disease does not damage an ejaculatory function, but the treatment of tuberculosis does it. There is necessary a special pathogenetic therapy to prevent this complication.

P158

Sexual Health of Siberians

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Objectives To obtain a greater understanding of sexual status, behavior and habits among men in Siberia to offer special approach of treatment for sexual dysfunction.

Design and Methods A population-based study was conducted among men in Siberia. 1280 men filled in special detailed questionnaire on sexual health.

Results 23% were healthy; others had chronic diseases. 84% are rare consumer of the alcohol. 29% were non-smokers, others smoked – from "sometimes" (29%) up to "more than one pack per day" (16%). 42% used to exercises, 4% did it rarely, and 54% of the men were not engaged in sports at all.

In 3% the first coitus was in 14; the latest debut of sexual life was in 24. 81% had only one constant sexual partner. The self-estimation of the erection has appeared is low. Only 33% estimated their erection as "excellent", 27% as "good", 20% "middle", 13% "poor" and 7% "very poor". Exactly the same proportion was revealed in the self-estimation of life success, career etc. 33% men counted their life success as "excellent", 27% thought it was "good", 20% "middle", 13% "poor" and 7% "very poor".

Conclusion A status of sexual health is satisfactory. We have revealed high direct correlation between level of the erection and life success. It is the additional evidence that erectile dysfunction is both medical and social problem.

P159

Women as a Cause of Male Sexual Dysfunction

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Background Sexual function is an action of a couple, but each participant demonstrates it in different style.

Material & Methods Population study on 1280 Russian men (mean age 36.0 years) and 768 women (mean age 35.8 years), who filled in special questionnaires. Mostly they were from Siberia – coldest region of Russia.

Results Life span in Russia is 58.6 years for men and 72.4 years for women. In our study 76% men and 84% women consumed an alcohol rarely, and 19% and 9% accordingly – did not drink it at all. 71% women and only 30% men were non-smokers. Both sexes had one constant partner in 81%. 57% women and 43% men used condoms for contraception, but only 29% women and 17% men did it in sex with unknown partner, in casual sexual affairs. In 5-score scale sexual function was "great" in 34% women and 33% men, poor – in 7% and 20% accordingly. We have found direct correlation between male sexual function and their successfulness in whole, but there was no this phenomenon in women. Only 22% woman under 40 considered the size of the penis is important, 28% believed it is an average important and a half of them unimportant. None did think the size has great importance. After 40 the proportion has changed. 9% believed the size is very important, 18% important, 44% mild important and 29% unimportant. Meanwhile a size of the penis was very important for 60% young men and twice less (31%) for men older 40.

Conclusion There are severe gender differences in life style, sexual function, contraception etc, and these differences should be taken into account not only sexual medicine specialist, but any doctors and social workers.

P160

Ejaculatory Disorders in Southeners and Siberians

E. Kulchavenya, E. Brizhatyuk
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Background Ejaculatory disorders are one of the most frequent sexual dysfunction. The aim was to evaluate that problem in different climatic regions.

Material & Methods 417 Russian men (149 from South region – "Southerners", and other 268 – "Siberians") were enrolled in study. Duration of IELT, level of testosterone, co-morbidity chronic prostatitis were estimated.

Results Young men were 34%, older 50 years 18.2%. 59.2% of young men had normal ejaculation, but only 20% in age after 50, when delayed ejaculation predominated. 43.6% of southerners and 35.8% of Siberians had premature ejaculation, and accordingly 6.1% and 14.6% delayed ejaculation. 26.7% southerners and 29.3% Siberians were hypogonadal. Among men with normal testosterone normal ejaculation was in 74.6%. Among hypogonadal patients 51% had premature ejaculation and 25.5% – delayed ejaculation. 69.9% of all men had chronic prostatitis. Only 46% of patients with chronic prostatitis had normal ejaculation, 43.3% had premature ejaculation and 10.7% had delayed ejaculation.

Conclusion In cold climate delayed ejaculation is more often sexual dysfunction, than in South, where premature ejaculation predominates. Low level of testosterone resulted in ejaculatory disorders, as well as chronic prostatitis. Thus, there are severe differences in ejaculation between young and old men, southerners and Siberians, eugonadal and hypogonadal. It is impossible to speak about "normal" in copulative act without taking into account age, co-morbidity, morality, region of living of the patient etc.

P161

Poor Response to Alprostadil ICI Test is Associated with Arteriogenic Erectile Dysfunction and Higher Risk of Major Adverse Cardiovascular Events

G. Rastrelli[1], G. Corona[1, 2], M. Monami[3], C. Melani[3], D. Balzi[3], A. Sforza[2], G. Forti[1], E. Mannucci[3], M. Maggi[1]
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Introduction Intracavernous alprostadil injection (ICI) test has been considered useless in assessing the vascular status of subjects with erectile dysfunction (ED). The aim of the study is to analyze the clinical correlates of ICI test in patients with ED and to verify the value of this test in predicting major adverse cardiovascular events (MACE).

Methods A consecutive series of 2,396 men (mean age 55.9 ± 11.9 years) attending our outpatient clinic for sexual dysfunction was

retrospectively studied. A subset of this sample (n = 1,687) was enrolled in a longitudinal study. Several clinical, biochemical, and instrumental (penile color Doppler ultrasound; PCDU) factors were evaluated. All patients underwent an ICI test, and responses were recorded on a 4-point scale ranging from 1 = no response to 4 = full erection.

Results Among the patients studied, 16.4%, 41.2%, 40.2% and 2.2% showed grade 4, 3, 2, and 1 ICI test response, respectively. After adjusting for confounders, subjects with grade 1 ICI test response showed reduced perceived sleep-related, masturbation-related, and sexual-related erections when compared with the rest of the sample. In addition, a worse response to ICI test was associated with a higher prevalence of hypogonadism-related symptoms and signs along with lower testosterone levels. The prevalence of both diabetes mellitus and metabolic syndrome was inversely related to ICI test response. Accordingly, dynamic and basal peak systolic velocity (PSV), as well as acceleration at PCDU, decreased as a function of ICI test response. In the longitudinal study, after adjusting for confounders, grade 1 response was independently associated with a higher incidence of MACE (hazard ratio = 2.745 [1.200–6.277]; $p < 0.05$). These data were confirmed even when only subjects with normal PSV (> 25 cm/s) were considered.

Conclusions Our results demonstrate that poor ICI test response is associated with several metabolic disturbances and higher incidence of MACE. We strongly recommend performing ICI test with alprostadil in all ED subjects.

P162

Inhibitors of 5α-reductase-related Side Effects in Patients Seeking Medical Care for Sexual Dysfunction

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Introduction Despite their efficacy in the treatment of benign prostatic hyperplasia (BPH) the popularity of inhibitors of 5α-reductase (5ARIs) is limited by their association with adverse sexual side effects. However, the real impact of 5ARIs on sex hormones and sexual function is controversial. The aim of the study is to investigate the role of 5ARIs therapy on hormonal parameters and sexual function in men already complaining of sexual problems.

Methods A consecutive series of 3837 men (mean age 63.5 ± 12.8 years) attending our outpatient clinic for sexual dysfunction was retrospectively studied. Several clinical, biochemical and instrumental (penile color doppler ultrasound; PCDU) factors were evaluated.

Results Among the patients studied, 78.7% reported erectile dysfunction, 51.1% hypoactive sexual desire (HSD), 86.7% perceived reduced sleep-related erections (PR-SREs) and 19.1% premature ejaculation. The use of 5ARIs was associated with an increased risk of HSD and PR-SRs whereas no relationship was found with erectile dysfunction and ejaculation disturbances. Subjects using 5ARIs also more frequently had gynaecomastia along with reduced SHBG and higher calculated free testosterone levels. All these associations were confirmed in a case-control study comparing 5ARIs users with age-body mass index-smoking status and total testosterone matched controls.

Conclusions Our data indicates that use of 5ARIs in men with sexual dysfunction does not significantly exacerbate pre-existing ejaculatory or erectile difficulties, but can further impair their sexual life by reducing sexual drive and spontaneous erection.

P163

Two Unconventional Risk Factors in Estimating Risk of Major Adverse Cardiovascular Events in Subjects with Erectile Dysfunction: Low Education and Reported Partner's Hypoactive Sexual Desire in Comparison with Conventional Risk Factors

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Introduction The classification of subjects as low or high cardiovascular (CV) risk is usually performed by risk engines, based upon multivariate prediction algorithms. However, their accuracy in predicting major adverse CV events (MACE) is lower in high-risk populations, since they take into account only conventional risk factors. The aim of the study is to evaluate the accuracy of Progetto Cuore risk engine in predicting MACE in subjects with erectile dysfunction (ED), and to test the role of unconventional CV risk factors, specifically identified for ED.

Methods A consecutive series of 1,233 men (mean age 53.33 ± 9.08 years) attending our outpatient clinic for sexual dysfunction was longitudinally studied for a mean period of 4.4±2.6 years. Several clinical, biochemical, and instrumental parameters were evaluated. Subjects were classified as high- or low-risk, according to previously reported ED-specific risk factors.

Results In the overall population, Progetto Cuore-predicted population survival was not significantly different from the observed one ($p = 0.545$). Accordingly, Receiver Operating Characteristic (ROC) analysis shows that Progetto Cuore has an accuracy of 0.697 ± 0.037.

Conclusion Overall, Progetto Cuore is a proper instrument for evaluating CV risk in ED subjects. However, in ED, other factors as low-education and partner's HSD concur to risk profile. At variance with low-education, Progetto Cuore is not accurate enough to predict MACE in subjects with partner's HSD, suggesting that the latter effect is not mediated by conventional risk factors included in the algorithm.

P164

Testosterone/Estradiol Ratio Regulates NO-induced Bladder Relaxation and Responsiveness to PDE5 Inhibitors

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Introduction Although originally developed and marketed for erectile dysfunction (ED), phosphodiesterase type 5 inhibitors (PDE5i) have also been investigated in a variety of other potential medical applications, including pulmonary hypertension, low urinary tract symptoms (LUTS) and the management of sexual dysfunctions in women. However, PDE5i failed to demonstrate any consistent effect in women with sexual arousal disorder. The biological underpinning of the gender-asymmetry in the efficacy of PDE5i has not been elucidated. The aim of the present study is to investigate and directly compare PDE5 expression and biological activity in female and male bladder, which is the less dimorphic urogenital structure.

Results The nitric oxide-donor, sodium nitroprussiate (SNP) is almost 3-log unit less potent in relaxing the male bladder than the female one. On the contrary, the PDE5 resistant cGMP analog SP-8-Br-PET-cGMPS induces a dose-dependent relaxation that is identical among genders. The effect of the selective PDE5i vardenafil in potentiating SNP-induced bladder relaxation is almost three-times more pronounced in male than in female rat bladder. Accordingly, the cGMP-hydrolyzing activity of PDE5 is two-fold higher in male vs. female homogenates. To further investigate the effect of changing sex steroid milieu on PDE5, we ovariectomize female rats and alternatively replace with estradiol, progesterone, testosterone or testosterone + letrozole, to completely abrogate testosterone-induced estrogen formation. Masculinization of female rats – by the simultaneous administration of testosterone and letrozole in ovariectomized rats – decreases by a factor of two responsiveness to SNP, that is even lower than in untreated males, while dramatically increases the activity of vardenafil in potentiating SNP-induced relaxation. Interestingly, vardenafil activity in potentiating SNP-induced relaxation in bladder is tightly associated with the increased testosterone/estradiol ratio.

Conclusion This study demonstrates that PDE5 biological and catalytic activity is more pronounced in male as compared to female bladder, and that testosterone/estradiol ratio positively regulates responsiveness to PDE5 inhibitors, thus suggesting that male bladder could be regarded as a more suitable target for PDE5i than the female counterpart.

P165

"It Takes 2 to Tango": The Relational Domain in a Cohort of Subjects with Erectile Dysfunction (ED)

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Introduction The relational domain of Erectile Dysfunction (ED) could be difficult to investigate in a clinical setting. We developed and validatedSIEDY, a 13-item structured interview, which assesses the organic, relational and intra-psychic domains of ED. SIEDY Scale scores 1 and 3 were previously validated to evaluate, respectively, organic and intra-psychic domain in subjects with ED.

Aim Aim of the present study is to identify a pathological threshold of SIEDY Scale 2 score to assess the relational impairment in patients with Erectile Dysfunction (ED).

Method A non-consecutive series of 2992 heterosexual males with ED was retrospectively studied with structured interview SIEDY.

Main Outcome Measure We assumed that conflict within the couple, and/or the presence of extramarital affairs, reflects the presence of an impaired relationship in a sample of 844 patients studied without systematically applying a psychometric questionnaire (A).

Results In sample A: 246 (29.2%) and 56 (6.6%) subjects reported conflicts within the couple or extramarital affairs, respectively. Scale 2 score predicts couple impairment with accuracy of 62.0 ± 2.2% ($p < 0.0001$), showing a sensitivity of 53% and specificity of 66%, when a threshold of ≥ 2 was chosen. In Sample B (2148 patients studied applying a psychometric questionnaire), when the same threshold was chosen, a pathological Scale 2 Score was associated with a higher risk of pathological MHQ-A, and MHQ-D score, with higher prevalence of psychopathology and higher Scale 3, even when adjusted for confounders. In the same sample, a Scale 2 score ≥ 2 was associated with reduced intimacy during sexual intercourse, as well as with worse sexual functioning.

Conclusions Until now, no instrument has been available to identify and quantify the marital domain of ED. The validation of a threshold of SIEDY Scale 2 score represents an easy tool for the identification of ED patients with a relevant marital impairment.

Late Abstract

Influence of Sexual Steroids on Short-Term and Long-Term Memory

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In recent years, sexual steroids have been arising as unignorable influence factors for a better understanding of human cognition. Among various cognitive processes the focus in this presentation will be on human memory, which has been gaining increasing significance in our aging society. Previous studies indicated that testosterone improves memory performance by modulating the hippocampus, a relevant structure for memory consolidation. Estrogen and progesterone play a crucial role in memory processes in women. The modulating effects of these steroid hormones can be explained by the high number of steroid hormone receptors in the hippocampus.

These findings are intriguing enough to explore sexual steroid-dependent functional architecture of memory. Up-to-date there is little known about neural activities interacting with various sexual steroids in men and women during memory processes.

In this ongoing study, we investigate the influence of sexual steroids on verbal and non-verbal short-term and long-term memory performance as well as their gender specific neural activities, using functional magnetic resonance imaging (fMRI). Male (n = 29, mean age 30.1 ± 4.4), female (n= 22 mean age: 26.6 ± 3.4) and hypogonadal subjects (n = 8 mean age: 33.6 ± 2.9) were included in the study so far. Hypogonadal men served as a model for low testosterone and impact on memory performance. Neural activities, reaction times and error rates were assessed. At this congress we present gender specific behavioral results in relation to (1) the stimulus materials (2) the encoding and retrieving processes and (3) hormonal levels. We found a tendency of a better performance in men compared to women and hypogonadal men in non-verbal tasks. In contrast, hypogonadal men and women performed better than men in verbal tasks.

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