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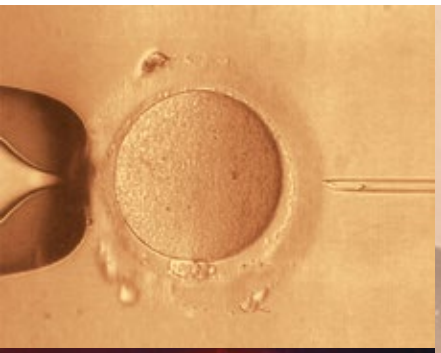

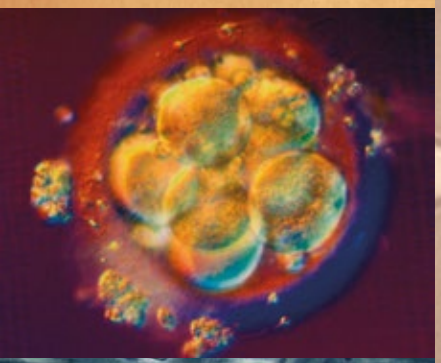

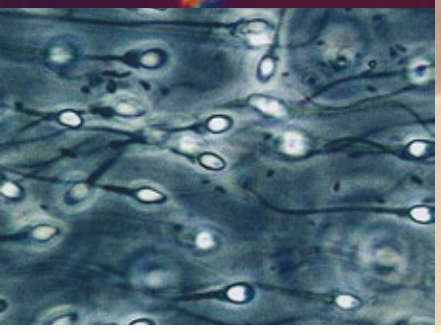

26. Jahrestagung der Deutschen Gesellschaft für Andrologie (DGA) e.V. - 18.-20. September 2014, Gießen (Abstracts)

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26. Jahrestagung der Deutschen Gesellschaft für Andrologie (DGA) e. V.

18.–20. September 2014, Gießen

Abstracts*

Freie Vorträge zu aktuellen Themen der Andrologie

FV1

Einzelzellmarkierung und -isolierung potenzieller humaner spermatogonialer Stammzellen

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Einleitung Humane spermatogoniale Stammzellen (hSSCs) könnten therapeutisch eingesetzt werden. Um unkalkulierbare Effekte bei einer medizinischen Anwendung, aber auch bei einer In-vitro-Vermehrung der Zellen einzugrenzen, ist die hochreine Isolierung der Zielzellpopulation ohne somatische Kontaminationen unumgänglich. Einen potenziellen Marker für hSSCs stellt der bei nicht-differenzierenden A-Spermatogonien (SPG) vorkommende Oberflächenrezeptor FGFR3 (Fibroblastenwachstumsfaktor-Rezeptor 3) dar [von Kopylow et al., 2010; 2012a; 2012b], mit dem SPG spezifisch isoliert werden könnten.

Methodik Wir haben eine Methode entwickelt, mit der die FGFR3-positiven SPG aus einer reiskorngroßen Hodenbiopsie unter Einsatz eines FACS-Antikörpers gegen FGFR3 (mouse monoclonal IgG #MAB766, R&D Systems) zuerst markiert und danach durch magnetische Zellisolierung mittels Dynabeads® (Invitrogen Dynal AS) angereichert werden. Die Bead-gebundenen Zellen werden dann aus der Positivfraktion einzeln mit einem Mikromanipulator gepickt. Die Analyse der isolierten Zellen erfolgte durch immunhistochemische Färbung (IHC) gegen den Puripotenzmarker UTF1 (Undifferentiated embryonic cell Transcription Factor 1) und Lebend-Tot-Färbung.

Ergebnisse Mit der beschriebenen Methode wird eine 100%ige Anreicherungsquote der FGFR3-positiven SPG ohne somatische oder jegliche andere zelluläre Kontamination erreicht. Auf diese Weise kann in der Zellkultur z. B. die ungewollte Differenzierung der potenziellen Stammzellen verhindert werden [Kanatsu-Shinohara & Shinohara, 2013]. Mit Hilfe der IHC-Färbung konnten wir nachweisen, dass die Zellen eine nukleäre Markierung mit UTF1 aufweisen. Dies demonstriert die Isolation richtigen Zellpopulation, da bekannt ist, dass FGFR3 und UTF1 in den gleichen humanen spermatogonialen Zellen exprimiert werden [von Kopylow et al., 2012b]. Die Ergebnisse der Lebend-Tot-Färbung zeigen, dass die Zellen vital sind.

Schlussfolgerung In der derzeitigen Praxis mangelt es an methodischen Ansätzen zur Isolierung reiner hSSC-Populationen ohne somatische Kontaminationen. Die von uns entwickelte Methode zur Isolierung von FGFR3-positiven SPG liefert eine Voraussetzung für den therapeutischen Einsatz von hSSCs, die beispielsweise prepubertären männlichen Patienten, die noch keine eigenen Spermien besitzen, nach erfolgreicher Krebstherapie retransplantiert werden könnten. Außerdem stellen die Zellen potenzielles Ausgangsmaterial für eine In-vitro-Spermatogenese oder die regenerative Medizin dar. Des Weiteren können sie zur Grundlagenforschung, z. B. zur Aufklärung des Mechanismus der humanen Spermatogoniogenese, verwendet werden.

Grants: Supported by DFG (FOR 1041)

FV2

miR-130/301 Cluster Regulates Adipogenesis-Associated Target Genes in a Testosterone-Dependent Manner

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Introduction Obesity is a growing public health challenge in industrialized nations. Besides supernutrition, one causal cofactor can be late-onset hypogonadism (LOH) in elderly men, which is characterized by a significant decrease in serum testosterone (T). LOH is frequently associated with an increase in visceral adipose tissue mass. Several studies demonstrated that specific miRs are important posttranscriptional gene regulators in the adipogenic differentiation. Androgens in turn may regulate these miRs, resulting in a decreased adipogenic differentiation of precursor cells. The aim of our study was to identify miR-130/301 cluster as a potential gene regulator in adipogenesis and to determine the effect of testosterone and dihydrotestosterone (DHT) on this miR and their specific target genes.

Methods Reporter Luciferase assays were performed in human osteosarcoma cells (Saos-2) to validate that AR, Leptin, ADIPOQ, PPAR-gamma, ARHGEF 12, RASD 1 and TNF-alpha are regulated by miR-130/301 cluster. The seed region of the miR binding site on the mRNA of these genes was mutated (mt) to reinforce potential results. Furthermore, after cultivation and induction of adipogenic differentiation in human SGBS preadipocytes for up to 14 d ± T (100 nM) or DHT (30 nM), mRNA-, miR- and protein expression was measured.

Results In Reporter Luciferase assays transfection with miR-130a-mimic (50 nM) displayed a decrease of luminescence at the wildtype (wt) 3`UTR of AR, ADIPOQ, PPAR-gamma and TNF-alpha, whereas the luciferase expression of mt constructs was not affected. Accordingly we demonstrated that AR, ADIPOQ, PPAR-gamma and TNF-alpha are direct targets of the miR-130/301 cluster. Moreover, T or DHT supplementation to differentiating preadipocytes inhibits the mRNA expression of ADIPOQ, PPAR-gamma and TNF-alpha and promotes the mRNA expression of AR in contrast to differentiating preadipocytes without androgene supplementation. miR-130/301 cluster showed a low expression during the adipogenic differentiation, but an increased expression with addition of T or DHT to differentiating adipocytes. T or DHT supplementation to differentiating preadipocytes promoted the protein expression of AR. The protein expression of ADIPOQ did not change with addition of T or DHT to differentiating preadipocytes.

Conclusion We conclude that a decreased serum testosterone level is associated with a decreased miR-130/301 cluster expression, leading to an elevation of the mRNA expression of its target genes in human preadipocytes. These genes promote adipogenic differentiation and thereby support the increase of visceral adipose tissue mass. Our results may contribute to a better understanding of the molecular mechanisms of hypogonadism-associated obesity.

FV3

Involvement of peroxisomes in steroid synthesis in Leydig cells and their role for male fertility

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Male infertility is a complex pathology, the genetic and molecular causes of which are

* Ein alphabetisches Verzeichnis der Erstautoren finden Sie auf Seite 210.

still elusive. A number of men are given this diagnosis, without an exact explanation of etiology and the majority of cases are idiopathic. Also male patients with peroxisomal diseases show a range of testicular pathologies leading to male infertility, the molecular pathogenesis of which is not yet understood. Peroxisomes are ubiquitous organelles, which are now known to play a crucial role in human physiology. Besides other metabolic pathways, they are involved in ROS metabolism as well as cholesterol synthesis and the side-chain modification of cholesterol. Steroid synthesis in Leydig cells is largely dependent on both cholesterol availability as well as ROS metabolism. In this study we therefore analyzed the effects of peroxisomes on steroidogenesis.

We characterized Leydig tumor cell line (MLTC-1) for its capability to function as model system for analysis of the peroxisomal compartment and steroid synthesis. We established a transient transfected *Pex13* shRNA knockdown model using microporation to study the consequences of peroxisomal deficiency in MLTC1 cells. Stimulation of steroidogenesis revealed a decrease of the intramitochondrial 30 kDA mature form of StAR protein in cells transfected with *Pex13* shRNA in comparison with cells transfected with control shRNA, suggesting that StAR protein expression is blocked in part at the level of mitochondrial import and processing. To elucidate the mechanism of inhibition of StAR in peroxisome deficient MLTC-1 cells, we analyzed the intracellular ROS levels by staining with dihydroethidium, as well as performing GSH-Glo™ Glutathione Assay. Both experiments revealed that ROS were increased in peroxisome deficient MLTC-1 cells. Additionally, Western blot analyses revealed an induction of antioxidant enzymes such as SOD2, SOD1, GSR and a reduction of the mitochondrial respiratory chain complex III protein in MLTC-1 cells with *Pex13* knockdown, indicative of mitochondrial dysfunction. We found also an up-regulation of aromatase and estrogen receptor alpha expression. Moreover, RNAi-mediated knockdown in primary Leydig cells has been done to analyze for eventual differences in the alteration of steroidogenesis due to peroxisomal dysfunction. We indeed could show, that the knock down of *Pex13* in MLTC-1 cells and in primary Leydig cells leads to the inhibition of progesterone, testosterone and estrone synthesis whereas, an induction of estradiol production occurs. To summarize, a knockdown of peroxisomes leads to oxidative stress, mitochondrial dysfunction and impaired steroid synthesis with androgen-estrogen imbalance.

FV4

Luminal Segmentation of the Epididymis

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The epididymal duct transports sperm over a distance of several meters within a few days, mediated by epididymal mechanisms. The luminal content flows through the duct and passes by distinct epithelial surfaces in each segment. Depending on surface interactions the spermatozoa are modified in a specific manner.

Segmentation of the epididymis has been described previously based on (i) distinct epithelial morphology, (ii) distinct gene expression pattern, and (iii) restriction of the interstitial space by connective tissue septa (CTS). The epididymal duct is a continuous tube with the luminal space considered to be without boundaries. However, our experiments with perfusion of the luminal space using tracer-suspensions revealed obstacles along the epididymal duct, suggesting a kind of valves along the duct correlating with the localization of segment boundaries.

Epididymal segments are able to resist high luminal pressure without influencing nearby segments when perfusing with a constant flow rate in an antero- or retrograde manner. Time lapse investigations of intraluminal tracer injection impressively showed a constant swelling of perfused segment without penetrating following segments. The valve eventually releases a surge of tracer-suspension only after a dramatic increase of pressure, resulting in filling up the entire adjacent segment within minutes.

The morphological and/or functional basis of these "valves" is unknown so far. We speculate, however, whether (i) the duct is narrowed by connective tissue at the CTS, (ii) the duct has a specific folding, (iii) valve function is due to contractions. Our data suggest new concepts of luminal segmentation along the epididymal duct which could be relevant for sperm maturation and ascending infections.

Grant: Land Hessen LOEWE MIBIE, A3

FV5

Verbesserung von anthropometrischen Parametern, erektiler Funktion und Lebensqualität unter Langzeitbehandlung mit Testosteron-Undecanoat-Injektionen (TU) bis zu 7 Jahren: Beobachtungsdaten einer Register-Studie

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Hintergrund und Fragestellung Gewichtsabnahme unter TU unter 5-jähriger Behandlung wurde bereits beschrieben [Saad et al., Obes 2013; 21: 1975–81; Haider et al., Obes Res Clin Pract 2014, in press]. Hier analysieren wir, ob die Effekte auch über 7 Jahre bestehen. Da Adipositas mit Beeinträchtigungen von Sexualität und Lebensqualität verbunden ist, untersuchen wir Effekte auf erektile Funktion und Lebensqualität.

Material und Methoden Prospektive Registerstudie. 340 Männer (Alter: 57,37 ± 7,03 Jahre) mit Testosteron ≤ 12,1 nmol/l erhielten TU 1000 mg alle 12 Wochen nach einem Anfangsintervall von 6 Wochen bis zu 7 Jahre. Gewicht und Bauchumfang, Internationaler Index für Erektile Funktion-EF-Domäne (IIEF-EF) und Aging Males' Symptoms-Fragebogen für Lebensqualität (AMS) wurden bei jedem Besuch erhoben.

Ergebnisse 7 % der Patienten waren normalgewichtig, 23 % übergewichtig und 70 % adipös. Das Gewicht fiel progressiv von 104,04 ± 16,39 auf 88,16 ± 8,7 kg. Der mittlere Gewichtsverlust betrug 18,37 ± 0,43 kg (16,67 ± 0,44 %). Der Bauchumfang fiel von 105,88 ± 8,61 auf 96,8 ± 7,01 cm, der Body-mass-Index (BMI) von 33,26 ± 5,35 auf 28,29 ± 2,79 kg/m^2. Der IIEF-EF verbesserte sich von 19,89 ± 4,83 auf 25,83 ± 3,09. Der AMS verbesserte sich von 51,67 ± 10,75 auf 17,09 ± 0,57.

Schlussfolgerungen Die Testosteron-Ersatz-Therapie bei hypogonadalen Männern führte zu nachhaltigen Verbesserungen von Gewicht, Bauchumfang, erektiler Funktion und Lebensqualität. Diese Effekte stehen in enger Beziehung zu einander und sollten gemeinsam untersucht werden.

FV6

The Muenster EXAKT study: Expression of Escapee Genes in the Testis of KS Patients

S. Werler[1], N. Terwort[1], F. Tüttelmann[2], M. Zitzmann[1], S. Kliesch[1], J. Wistuba[1], J. Gromoll[1]
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Introduction Klinefelter syndrome (KS; 47,XXY) is with an incidence of 0.2% the most common sex-chromosomal aberration in men. Patients are characterized by hypergonadotropic hypogonadism, small firm testis and infertility. Although known for decades, the underlying molecular mechanisms of the syndrome remain poorly understood. Within the EXAKT study, a study aimed at a phenotype/genotype comparison in KS patients, we investigated a possible gene-dosage effect of the supernumerary X-chromosome by analyzing the global gene expression in blood samples of KS patients and comparing them to 46, XY men. 36 genes were found to be differentially expressed, mainly X-chromosomal genes that are known to escape the process of X-inactivation in women. To further investigate a possible role of these genes in the manifestation of the testicular phenotype of KS patients we determined the expression of 25 of these genes in the testis by custom made PCR arrays.

Subjects and Methods Gene expression analysis was carried out in 16 testicular samples of KS patients. Azoospermic patients with Sertoli-cell-only (SCO) syndrome (n = 14, 46,XY) served as a control group. Histological evaluation of the testicular biopsies of the KS patients revealed absence of germ cells. According to the testicular morphology of the KS testis we divided the 16 testicular samples according to histological subtypes

into two groups: Group I: tubular ghosts 80–100%, SCO tubules 0–20% (n = 8); Group II: tubular ghosts 0–20%, SCO tubules 80–100% (n = 8). The SCO control group was grouped accordingly (group I: n = 8, group II: n = 6).

Results Among the 18 tested escapee genes, we could detect an increased expression in the testis of KS patients for 12 genes (fold-change 1.3–1.9) in group I. Biggest differences could be found for *KDM5C, ASMTL, ZBED1, GTPBP6, HDHD1A* and *PNPLA4*. In group II blood expression pattern of the escapee genes were paralleled in the testis for only 7 genes (fold change 1.2–3.9) with the most significant differences for the genes *ZFX* and *HDHD1A*. Among the 6 autosomal genes, found to be differentially expressed in blood of KS patients, 3 (*DOCK7, GSTM2, HERC2P2*; group I) and 5 (*DOCK7, GSTM2, HERC2P2, SLC22A5, ZFYVE9*; group II), respectively, showed a comparable expression pattern in the testis.

Conclusions Our analysis of testicular gene expression indicates that genes escaping X-inactivation and found to be overexpressed in blood samples of KS patients resemble a similar expression pattern in the testis as well. This observed expression pattern of escapee genes in the testis could potentially lead to a dosage related malfunctioning of the somatic and germ cell compartment of the testis and ultimately be involved in the germ cell loss of KS patients. Further analyses are required to characterize to which extent the observed mRNA expression is indicative for the cellular protein expression pattern of these genes, moreover to resolve the differences between group I and II. Nonetheless we suggest a major role of the escapee genes in the pathophysiology of KS patients.

Grants: This study was supported by DFG [WI 2723/4-1] and IZKF Münster [CRA 03/09]

FV7

Raman Mapping shows UVB Causes Sperm DNA Fragmentation but the Associated Mitochondrial Dysfunction does not

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Introduction The past two decades have seen a surge in interest surrounding nDNA fragmentation, a hitherto little investigated sperm abnormality which has been associated with poor embryo quality, decreased implantation rates and increased miscarriages. The current hypothesis is nDNA damage is caused by oxidative stress resulting from reactive oxygen species (ROS) of mitochondrial origin. Our aim was to investigate the extent and location of sperm nDNA fragmentation after differing UVb dosages which caused increasing levels of mitochondrial dysfunction and oxidative stress. In this way we sought to determine whether location of nDNA damage was consistent with mitochondrial ROS leakage and if seminal plasma acted as a protectant.

Materials and Methods Semen samples were collected from 30 patients attending the Andrology laboratory of CeRA. All samples underwent routine "swim up" and were placed in one of 6 groups: PBS, 20%, 40%, 60%, 100% seminal plasma. Aliquots of each group were then UVb irradiated for 15s, 30s, 45s, 60s, 120s, 240s. Sperm motility, viability were assessed (WHO criteria), nDNA status determined by acridine orange test, lipid peroxidation with BODIPY (latter two by flow cytometry). Samples were left for 15 mins, irradiated for an identical time and again assessed. DNA damage was localized using Raman microspectroscopic mapping of the sperm head and midpiece (one spectrum every 50nm).

Results Short UVb exposure was sufficient to significantly decrease sperm motility and viability, however nDNA integrity remained unaffected. DNA damage was seen at high dosages which were associated with sperm death. At levels where sperm were immotile but not dead (i. e. mitochondria had been damaged but the cell survived) no difference was seen in nDNA integrity. Increasing concentrations of seminal plasma were found to ameliorate the actions of UVb with as little as 40% capable of providing maximum protection. Raman spectral mapping showed fragmented nDNA only in the proximal region of the head, specifically under the acrosomal cap. The findings are consistent with UVb damaging the region with the least nDNA content. In contrast, the areas near and adjacent to the mitochondrial sheath showed no nDNA fragmentation.

Conclusion No evidence was found that damaged mitochondria and their proposed ROS leakage caused any nDNA damage (neither the quantity nor location). In light of our findings and the accumulating evidence from other studies, doubt exists if the current hypothesis truly reflects the origin of naturally occurring sperm nDNA fragmentation.

FV8

A prospective Evaluation of Intratesticular Perfusion by Colour-Coded Duplex Sonography (CCDS) in TESE-Patients with Azoospermia

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Introduction Scrotal ultrasonography as well as detecting the testicular perfusion by Colour-Coded Duplex Sonography (CCDS) has a key role for a non-invasive investigation of the testis. Our objective was to assess whether CCDS might predict the outcome of testicular sperm retrieval in patients with azoospermia. Furthermore, we evaluated potential sonographic alterations of the testicular structure before and after the standardized TESE (testicular sperm extraction) procedere (trifocal and M-TESE) performed as a combined testicular sperm retrieval technique.

Methods 61 patients (median age 33) were enrolled prospectively: 12 with obstructive azoospermia (OA) and 49 with non-obstructive azoospermia (NOA). 24 of 49 patients in the NOA group had a negative surgical sperm retrieval. Testicular volume, standard hormonal parameters, and sonographic findings were compared before and after TESE procedures. CCDS was performed at three different locations of the testis (upper, median, lower pole), the peak systolic value (PSV) was measured in all regions pre- and post-operatively. Testicular histology was investigated in all patients.

Results Testicular histology (spermatogenesis score count) was significantly correlated with the intratesticular PSV in the corresponding sonographic position ($p < 0.001$). The intratesticular PSV of the azoospermia patients with obstruction was significantly higher than in men with negative surgical sperm retrieval in the non-obstructive azoospermia group ($p < 0.05$). Pre-operative comparison of NOA vs. OA patients demonstrated increased FSH levels in NOA patients ($p < 0.05$), but testosterone levels indicated no statistical differences. Testicular volume and epididymal head cross diameter were significantly higher in OA patients ($p < 0.05$). Testicular volume decreased significantly in all patients postoperatively in the follow-up after 6 weeks ($p < 0.05$). Endocrinologically, FSH and LH increased in the follow-up, testosterone levels decreased. Overall, the PSV was significantly increased ($p < 0.05$) in all patients 24 h after surgery, with a normalization after 6 weeks in the follow-up.

Discussion CCDS reveals differences in patients with OA and NOA. It is also valuable to assess pathological changes in the follow-up after surgery. Since intratesticular PSV is correlated with testicular histology, the assessment of PSV might help to identify those patients with successful sperm retrieval.

FV9

The Human Sperm Glycocalix – Alterations caused by Acrosome Reaction

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Introduction Infectious epididymitis or epididymo-orchitis in men, a frequent entity in urological outpatient settings, is commonly caused by bacteria ascending the genitourinary tract. One of the most prevalent pathogens associated with epididymitis is *E. coli*. Eradication of pathogens occurs via antibiotic treatment, but in 50–60% of acute epididy-

mitis patients, fertility is permanently impaired. The sperm glycocalix is generated during spermatogenesis and substantially modified during epididymal maturation. Glycans are essential for fertility, e. g. as trigger for the sperm acrosome reaction as well as for sperm-zona pellucida binding and interaction. Moreover, alterations of the glycoprofile can predispose damaged spermatozoa for recognition and phagocytosis by immune cells. The sialic acid content of mammalian sperm correlates positively with protection from phagocytosis, but negatively with the capacity of sperm to bind to the zona pellucida of the ovum. Premature acrosome reaction caused by *E. coli* infection in an *in vivo* epididymitis mouse model prompted us to investigate the possible changes in sperm glycoprofile in mice and men.

Methods and Results In acrosomally reacted human sperm significant alterations in sialylation status pending on acrosome status were detected by HPLC as well as mass spectrometry based analytical applications both in monosaccharide moieties (including 50% reduction in sialic acid amount in following acrosome reaction) and a remarkable decrease in complex sialylated N-glycan structures. These data were confirmed by an enzymatic desialylation assay. Furthermore, the N-glycome of human sperm was comprehensively determined resulting in detection of a number of novel structures including bi- and tri- antennary sialylated N-glycans in sperm which were absent in acrosomally reacted sperm.

Conclusion These findings propose a massive redecoration in sperm glycocalyx induced by normal or premature microbial-stimulated acrosome reaction particularly in sialic acid content.

Poster

Männliche Infertilität – Grundlagenforschung

P1

Vergleich unterschiedlicher Färbungen zur Überprüfung der Membranintegrität von bovinen Spermien

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Fragestellung Ziel dieser Untersuchung war es, verschiedene Verfahren zur Untersuchung der Membranintegrität von bovinen Spermien zu vergleichen. Hierbei sollte auch die Variabilität von Ergebnissen verschiedener Untersucher sowie die Bedeutung von halbangefärbten Spermien berücksichtigt werden.

Methoden Es wurden jeweils 40 Ejakulate von 21 Bullen verwendet. Für jedes Ejakulat wurde der Anteil vorwärtsbeweglicher Spermien geschätzt, sowie die Spermienkonzentration und der Anteil toter Spermien mit dem NucleoCounter® (integriertes Fluoreszenzmikroskop, Fa. Chemometek, Dänemark) gemessen; bei zehn Ejakulaten wurde eine Doppelmessung durchgeführt. Von allen Ejakulaten wurden mit den Färbungen Eosin (E), Eosin-Nigrosin (EN) und Bromphenol-Nigrosin (BN) jeweils zwei Ausstriche angefertigt. Jeder der beiden Ausstriche wurde von drei verschiedenen Untersuchern zweimalig ausgewertet. Rot (E, EN) bzw. blau (BN) angefärbte Spermien wurden als „tot" bewertet. Halbgefärbte Spermien wurden gesondert gezählt.

Ergebnisse Die Doppelmessungen mit dem NucleoCounter® zeigten eine geringe Varianz (Variationskoeffizient 6,5 %). Bei der zweifachen Beurteilung desselben Ausstrichs durch die Untersucher lag der Variationskoeffizient bei 18,2 % (E), 17,5 % (EN) und 21,8 % (BN). Innerhalb aller Färbungen konnte eine Untersucherabhängigkeit nachgewiesen werden. Die Beurteilung der Proben war bei der Eosinfärbung aufgrund des schlechten Kontrastes am schwierigsten, während die BN-Färbung die beste Beurteilbarkeit aufwies. Diese Färbung wäre daher auch zur Beurteilung der Spermienmorphologie geeignet. Im Vergleich zum NucleoCounter® zeigte die Eosinfärbung bei Betrachtung der relativen mittleren Abweichung keinen signifikanten Unterschied (E 10,4 %, EN 34,1 %, BN 31,1 %, p = 0,58). Allerdings wies die Eosin-Färbung im Vergleich zur EN- und BN-Färbung die größte Streuung der Werte auf (E 21 %, EN 13,1 %, BN 14,8 %). Halbgefärbte Spermien konnten bei allen Färbungen beobachtet werden (Mittelwerte E 13,5 %, EN 14,2 %, BN 13,5 %). Bei der EN- und BN-Färbung führte die Addition des Anteils halbgefärbter Spermien zu den gefärbten Spermien zu einer geringeren Abweichung zum NucleoCounter®. Allerdings führte dies dann im Mittel zu einer Überschätzung des Anteils toter Spermien (EN –7,9 %, BN –15,4 %).

Schlussfolgerung Bei Verwendung von Lebend-Tot-Färbungen scheint der Anteil toter Spermien im Ejakulat im Vergleich zu fluoreszenzmikroskopischen Methoden eher unterschätzt zu werden. Die Untersuchungen lassen den Schluss zu, dass bei Verwendung der EN- und BN-Färbung halbgefärbte Spermien evtl. als „tot" beurteilt werden sollten.

P2

Peroxisomes are Essential for Regular Spermatogenesis

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Introduction Peroxisomes are cell organelles with important functions in the metabolism of lipids and reactive oxygen species. In germ cells, they have only recently been described by our groups. Their role for spermatogenesis has not been characterized in detail yet.

Methods and Results We have established a mouse model with a conditional knockout of *Pex13* in pre-meiotic germ cells to analyse the functions of peroxisomes for development and differentiation of male germ cells. The peroxisomal membrane protein *Pex13* is part of the translocation machinery required for import of peroxisomal matrix proteins into the organelle. The inactivation of *Pex13* leads to a biogenesis defect of peroxisomes with loss of all metabolic functions. Based on the *Cre*-lox technique, floxed *Pex13* mice were crossed with transgenic mice expressing cre recombinase under control of the *Stra8* promoter for inactivation of *Pex 13* in pre-meiotic germ cells.

Conclusions Histological analysis of knockout mice revealed a severe disturbance in germ cell differentiation with generation of multinucleated giant cells and post-meiotic arrest of spermatogenesis. Depending on their differentiation state, the multinucleated cells were TUNEL-positive. As a result of the peroxisomal dysfunction in germ cells we found a significant accumulation of lipid droplets within the germinal epithelium. On the ultrastructural level we could observe acrosome formation in multinucleated spermatids. However, several nuclei frequently shared one acrosome.

P3

Nachweis testikulärer und posttestikulärer Transkripte in humanen Spermatozoen – Potenzielle Modulatoren der männlichen Fertilität?

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Fragestellung Humane Spermatozoen enthalten eine komplexe Mischung unterschiedlicher RNA-Spezies, darunter auch mRNA, obwohl sie selbst transkriptionell inaktiv sind. Unter den mRNAs finden sich „Markertranskripte" testikulären und posttestikulären Ursprungs. Wir untersuchen, auf welchem Wege diese Transkripte in Spermatozoen gelangen.

Methoden Genexpressionsprofile humaner Ejakulate/Spermatozoen wurden aus der GEO-Datenbank (NCBI) heruntergeladen und in einer „Cross-Plattform-Cross-Labor"-Studie miteinander verglichen. Die Fraktionierung humaner Ejakulate in Seminalplasma (1), Zellpellets (2) bzw. reine Spermatozoen (3) erfolgte durch Zentrifugation bzw. Dichtegradientenzentrifugation. Genexpression wurde mit quantitativer PCR (qPCR) untersucht.

Ergebnisse In Ejakulaten und gereinigten Spermatozoen-Populationen wiesen wir mit Microarray-Analysen eine hohe Anzahl unterschiedlicher Transkripte nach. Bei einem Großteil handelte es sich um Keimzell-Transkripte aus dem Hoden, die als „Überbleibsel" der Spermatogenese anzusehen sind (z. B. PRM1, 2). Daneben fanden wir in geringerer Anzahl Transkripte, die innerhalb des männlichen Genitaltraktes als spezifisch für Epididymis, Seminalvesikel, Prostata oder Blutzellen gelten (z. B. DEFB129, SEMG1, MSMB, IL8). Dabei korrelierte die Expression der Transkripte, die auf jeweils ein Organ hinweisen, durch alle Proben. Ein Grund hierfür könnte sein, dass Transkripte aus den Rundzellen (z. B. abgeschilferten Epithelzellen) in Spermatozoen überführt werden und diese so die Rundzell-Zusammensetzung des Ejakulates widerspiegeln. Dafür spricht, dass wir aus dem Seminalplasma erhebliche Mengen an zellfreier RNA isolieren und mit qPCR spezifischeTranskripte aus dem Hoden und anderen Geweben (PRM2, SEMG1) nachweisen konnten.

Schlussfolgerungen Zellfreie RNA könnte eine Rolle beim mRNA-Transfer von den Epithelzellen des männlichen Genitaltraktes zu den reifen Spermatozoen spielen. Dabei wird die RNA entweder von Spermatozoen aufgenommen, oder sie ist auf der Spermatozoen-Oberfläche gebunden. Wir planen, die zelluläre Lokalisierung einiger Markertranskripte mit In-situ-Hybridisierung an Schnitten von Spermatozoen zu untersuchen. Erste Hinweise auf den mRNA-Transfer von den Rundzellen zu den Spermatozoen wollen wir durch Korrelation der Expression im Gesamtejakulat, reinen Spermatozoen sowie zellfreiem Seminalplasma erhärten. Ein Bezug der Seminalplasma-Transkripte zum Befruchtungspotenzial von Ejakulaten ist nicht unwahrscheinlich.

P4

Role of AKAP-PKA Complexes on Fertility in the Testis

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Introduction Temporal and spatial regulation of PKA activity is essential for vigorous sperm motility and for the resumption of meiosis in oocytes, which are required for successful fertilization. The phosphorylation of specific substrates by the C subunit of PKA is regulated in part by the subcellular localization of the PKA holoenzyme through the binding of its dimerized R subunits to the scaffolding A-kinase-anchoring proteins (AKAPs). AKAPs also interact with other signaling proteins, including other kinases, phosphodiesterases, and protein phosphatases, to form a signaling complex that imparts spatial localization and controls the timing and substrate specificity of protein phosphorylation.

ROS (Reactive oxygen species) generation is to damage organelles and initiate an intrinsic apoptotic cascade, as a consequence of which spermatozoa lose their motility, DNA integrity, and vitality. Peroxisomes are found to resist oxidative stress generated elsewhere in the cell. Thus the AKAPs which are localized in peroxisomes in testis are more valuable to study.

Methods and Results According to a scanning of all the AKAPs sequnces by computer programming and online-tool, Mm_AKAP1, Mm_AKAP4, Mm_AKAP10 and Rn_AKAP11 probably have PTS1 (Peroxisome targeting signal 1) terminus. After checking the expression distribution of these three AKAPs, all of them are very highly expressed in testis.

It is already known that AKAP1 enhances steroidogenesis by directing the synthesis and activation of STAR at the mitochondria in response to cAMP. Moreover, the PPARγ (Proliferator-Activated Receptor γ) agonist show increased AKAP1 mRNA levels. From a knockout lesson of AKAP4, which is a major component of the fibrous sheath in sperm, AKAP4 KO did result in infertility as well as structural changes in the flagellum. So the others might also have such significant role in fertility. But the information related to peroxisome is lacking, and AKAP11 which was shown in peroxisome also could be a question.

Conclusions To solve these problems, AKAPs cloning is going for subcellular localization confirmation and their collaborate partners will be checked by affinity purification. A Northern blotting is kept going for AKAP11 distribution and its alternative splicing forms conformation.

P5

Assembly line of Sperm Maturation – The Segmented Epididymis

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Background The epididymal duct is a continuous tube coiled into segments divided by connective tissue septa (CTS) that form segments in the epididymis. The blood epididymal barrier (BEB) is located in the epididymal epithelium and mediated by tight junctions. The BEB is essential to maintain a distinct milled in the luminal content. Never less, CTSs act as diffusion barriers in the interstitial space and are involved in creating segment specific interstitial milieu, representing a second type of barriers in the epididymis.

Methods We conducted functional studies of the dissected epididymis in mouse and rat using tracer application in organ culture and cell culture. Segmentation was studied by dissection and histological staining. Moreover, we characterized the epididymal epithelium using immunofluorescence and immunohistochemistry.

Results We show that the tightness of the BEB is regulated by interstitial factors. Furthermore, we demonstrate that CTS are diffusion barriers, not only in caput as described previously, but also in corpus and cauda. The CTS define zones of distinct morphology in the epithelium that are homogenous within each segment. We show that proteins of the epithelial surface can vary dramatically between adjacent segments, especially in the distal caput, which is of importance for aspects of sperm maturation. Specific interaction with the surface of the epithelium lining the epididymal duct is crucial for sperm maturation.

Conclusions We demonstrate that segmentation embodies both, the interstitum and the epididymal duct. Furthermore, we show that also human epididymis is segmented. Overall, our data suggest new concepts of compartmentation and barrier function in the epididymis with clinical relevancy for the invasion of epididymitis.

P6

Ouabain Interactions with the α4 Isoform of the Sodium Pump Stimulate Expression of the Tight Junction-Proteins Claudin-1 and Claudin-11 in Sertoli cells

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Interaction of the cardiotonic steroid (CTS) ouabain with the testes-specific α4 isoform of the sodium pump triggers activation of the c-Src/c-Raf/Erk1/2 signaling module and of the transcription factors CREB and ATF-1 in the Sertoli cell line 93RS2. Since CREB-inducible transcription in Sertoli cells is essential for the survival of spermatocytes and the production of mature spermatozoa, our further investigations focussed on the identification of ouabain- and CREB-stimulated expression of proteins that might be critical for male reproduction. The CRE promoter regulates the expression of claudin-1 and -11. Both proteins belong to the family of tight junction (TJ)-forming proteins that are critical for the formation of the blood-testis barrier (BTB), for the maturation of spermatocytes, and for male reproduction. Thus, the current investigation addressed the effects of ouabain on the expression of these claudins and on the formation of TJ.

RT-PCR experiments demonstrate that treatment of 93RS2 cells for 48 h with 10 nM ouabain leads to a clear increase in the expression of claudin-1- and claudin-11-specific mRNA/cDNA, while in untreated cells the

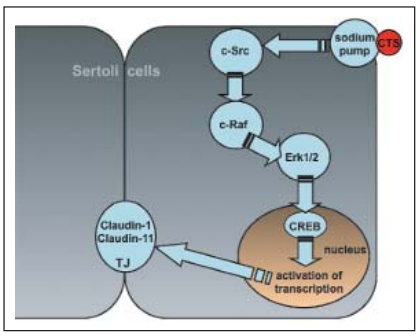

Figure 1. R. Dietze, et al (P6). Activation of claudin-1- and claudin-11-expression and TJ formation by ouabain.

detection of these mRNAs/cDNAs is negligible. The expression of claudin-1- and claudin-11-specific mRNAs/cDNAs correlates with the expression of the corresponding proteins, as demonstrated by immunofluorescence. Ouabain-induced stimulation of claudin-1 and claudin-11 expression leads to a significantly decreased diffusion of FITC-coupled Dextran MW4 through Sertoli cell monolayers grown in ThinCert inserts. In addition, transepithelial resistance (TER) increases significantly when cells are treated with the steroid under similar conditions. These experiments clearly show that ouabain-induced stimulation of claudin-1 and claudin-11 expression at the boundaries of adjacent Sertoli cells stimulates TJ formation between neighboring cells **(Fig. 1)**. Taking into consideration that ouabain and other CTS are thought to be produced endogenously, the demonstrated induction of claudin-1 and claudin-11 expression by ouabain and the influence of CTS on the formation of TJ might be of physiological significance for the formation of the BTB and male fertility.

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P7

Clusterin Inhibits Testicular Apoptosis and is Important for Meiosis

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Background Clusterin (ApoJ, SGP2, TRPM -2, CLI) is a ubiquitous glycoprotein participating in a plethora of important cellular functions, such as cell-cell interactions, lipid transportation, cell survival, DNA repair, and tumorigenesis. The main source of clusterin appears to be the Sertoli cells and its concentration is several folds higher in testis than in normal serum. However, besides clustering of Sertoli cells, clusterin function still remains largely unknown in testis. The present study investigates localization, secretion and the potential role of clusterin in testis.

Methods Protein synthesis and secretion by immortalized Sertoli cells and testicular tubules were measured by ELISA and Western blot analysis. Inhibitory/stimulatory effects of recombinant clusterin (rCLU) on apoptosis were determined by apoptosis detection assays. Paraffin sections of human testis with histologically normal spermatogenesis and impaired spermatogenesis were incubated with various antibodies against clusterin and its localization was quantified by immunohistochemistry.

Results Analysis of immortalized Sertoli cells and tubule culture supernatants confirmed that clusterin was efficiently secreted. Transforming growth factor-betas (TGF-βs) exerted a stimulatory effect on clusterin secretion in Sertoli cells, which was completely abrogated by blocking TGF-β/Smad signalling. Treatment of tubules with rCLU significantly reduced staurosporine and TNF-α-induced apoptosis. rCLU treatment also led to an increased expression of Stimulated by Retinoic Acid Gene 8 (Stra8) and v-myb myeloblastosis viral oncogene homolog-like 1 (MYBL1) proteins, which are essential for successful meiosis and normal spermatogenesis. In addition, rCLU also significantly enhanced secretion of bone morphogenetic protein 15 (BMP15), a marker of spermatocytes, whereas the amount of total BMP15 protein remained unchanged in the tubular cells. Furthermore, localization of clusterin in the human testis was found mainly in the cytoplasm of Sertoli cells and could not be detected in the nucleus. Interestingly, a basal-to-luminal gradient of clusterin localization was found in testicular biopsies from men with histologically normal spermatogenesis which is not detectable in biopsies with impaired spermatogenesis.

Conclusion Our data suggest clusterin secretion by Sertoli cells and testicular tubules, which seems to be regulated by TGF-βs. Apart from anti-apoptotic effects of clusterin, upregulation of meiotic proteins in tubules together with altered localization of clusterin in testicular biopsies from men with impaired spermatogenesis indicate its essential role in testis.

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P8

Differential Activation of Inflammatory Pathways in Testicular Macrophages Provides a Rationale for Their Subdued Inflammatory Capacity

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Spermatogenic cells express cell-specific molecules that have the potential to be seen as "foreign" by the immune system. In respect to the time difference between their appearance in puberty and the editing of the lymphocyte repertoire around birth, local adaptations of the immune system coined immune privilege are required to confer protection from auto-attack. Testicular macrophages (TM) play an important role in maintaining testicular immune privilege and display reduced pro-inflammatory capacity compared to other tissue-specific macrophages. However, the molecular mechanism underlying this macrophage phenotype remained elusive. We present evidence that TM have a lower constitutive expression of Toll like receptor pathway specific genes compared to peritoneal macrophages (PM). Moreover, in TM stimulated with lipopolysaccharide (LPS) the classical pro-inflammatory NF-κB signaling pathway is blocked due to lack of IκBα ubiquitination and, hence, degradation. Instead, challenge of TM with LPS or poly I : C induces MAP kinase, AP-1 and CREB signaling pathways which leads to production of pro-inflammatory cytokines such as TNF-α, though at much lower level than in PM. Pretreatment of TM with highly specific inhibitors for MAP kinases p38 and ERK1/2 suppress activation of AP-1 and CREB signaling pathways and attenuates LPS induced TNF-α secretion. High IL-10 levels produced by LPS stimulated TM indicate a regulatory macrophage phenotype. Our results suggest that TM maintain testicular immune privilege by inhibiting NF-κB signaling by impairing IκBα ubiquitination and a general reduction of TLR cascade gene expression. However, TM do maintain some capacity for innate immune responses through AP-1 and CREB signaling pathways.

P9

Inflammatory Prostatitis Syndrome in Fertile and Infertile Men – Role of Estrogen Receptors, Mast Cell Activity and Inflammation of Seminal Pathways

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Objective Inflammation of the prostate gland (prostatitis) is a common health problem affecting many young and middle-aged men, which can lead to prostate cancer. Recent clinical studies evidenced that prostatitis can also affect the male fertility parameters. However the underlying mechanisms are not well understood. This study aimed to analyze mast cells and the epigenetic status of estrogen receptors, and cytokines, which could modulate prostatic inflammation.

Materials and Methods Three prostate cancer cell lines *PC-3*, *DU145* and *LnCAP*, sperm cells and whole blood from prostatitis patients were subjected to CpG-promoter methylation analyses by COBRA (combined bisulfite restriction analysis) and gene expression analyses by qRT-PCR. Tissue samples from PCa (prostate carcinoma) and BPH (benign prostatic hyperplasia) and urine samples (first and second urine) were taken for immunohisto/cytochemical analyses. Additionally, we analyzed the mRNA expression of cytokines in urine samples of prostatitis patients.

Results We observed higher numbers of tryptase-activated mast cells in PCa compared to BPH stromal tissues. ER-beta expression was observed in the glandular prostate epithelium of BPH patients. Based on available literature, 44 inflammatory factors could be selected as involved in prostatitis. In-silico-analyses revealed that 9/44 have CpG-island promoters (CCL5, CXCL12, ER-alpha, IL4, IL12b, IL 13, CXCR4, HIF1-alpha, ER-beta) among which 5 (CCL5, IL13, CXCL12, ER-beta, CXCR4) exhibited in PCa cell lines hyper-methylated promoters and down regulated expression, respectively. Furthermore we observed a different methylation pattern of the same cytokines in blood and sperm cells within one patient. Studies on urine samples from prostatitis patients revealed that mRNA level of cytokines in first and second urine is similar. Interestingly, in some cases prostatic massage led to an intense increase of cytokine-mRNA levels in urine.

Conclusion Our study indicates that mast cells are activated in PCa and BPH patient samples and cytokines are affected in epigenetical manner in prostatitis patients.

P10

The piRNA Pathway in Mammalian Testis – Expression Patterns of HENMT1

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Purpose HENMT1 is an RNA methyltransferase involved in the stabilising 2'-O-methylation of the 3' end of small RNAs in plants, Drosophila and Zebra fish. This protects piRNAs in the testis from quick degradation, enabling them to contribute to translational and transposon control via the piRNA pathway. The relevance of HENMT1 in mammals is not yet fully understood but it is presumed to have a critical role in fertility as it does in other clades. Homozygous mutant male mice (Hen1P/P) show a severely impaired spermatogenesis, resulting in sterility.

Methods The phenotypic consequences of this mutation in mice were charecterized on ultrastructural level by electron microscopy. Furthermore, human HENMT1 expression was analysed on mRNA level by RT-PCR of testis homogenates and after laser microdissection of testis biopsy sections. Testicular HENMT1 and PIWIL1 expression on protein level was investigated by immune electron microscopy, immunohistochemistry and Western Blot.

Results Analysis of the mutant mice on ultrastructural level revealed that spermatogonia and spermatocytes do not show any alterations and spermatids develop normally until the elongating stage. Elongating and elongated spermatids revealed numerous phenotypic abnormalities in the HENMT1 mutant compared to wild type mice, such as changes in chromatoid body development, large cytoplasmic vesicles of unclear origin, a cytoplasmic accumulation of mitochondria including abnormal assembling around the axoneme, multinuclear spermatids and an abnormal development of nuclear manchette. In the human, HENMT1 protein was localized over mitochondria clusters in spermatogonia on ultrastructural level. This fits well with the immunohistochemistry staining showing protein expression mainly in spermatogonia. First RT-PCR results show that HENMT1 is expressed in normal spermatogenesis as well as in Sertoli cell only syndromes, indicating that expression is not limited to germ cells. However, the highest expression is found in tubules with normal spermatogenesis. RT-PCR after laser-assisted microdissection of the different cell populations within the tubule shows that HENMT1 mRNA expression is higher in spermatogonia than in spermatocytes and also present in Sertoli cells.

Conclusion Our data show that HENMT1 is expressed in the human testis. The mitochondrial location found in the immune EM matches the literature where piRNA pathway components are repeatedly reported in the vicinity of mitochondria and in chromatoid bodies which are interestingly both affected in the HENMT1 mutant mouse. The mouse data makes clear that normal HENMT1 function is essential for correct spermatogenesis and further work will show how human male infertility phenotypes are related to HENMT1 expression and function.

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Männliche Infertilität – Klinische Forschung

P11

Cytokine Networks in Human Testis – Differential Expression Patterns associated with Neoplasia and Disturbed Spermatogenesis

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Introduction Pre-invasive carcinoma in situ (CIS; syn.: testicular intraepithelial neoplasia) and seminomas almost invariably contain extensive inflammatory infiltrates mainly consisting of lymphocytes. However, the impact of tumor infiltrating lymphocytes (TILs) and accompanying other immune cells on testicular tumor immunosurveillance, progression and growth is still unknown. The present study tries to elucidate immunopathology and its key regulators in seminoma and CIS of the human testis in comparison to disturbed spermatogenesis with special emphasis on cytokine networks.

Material and Methods Cryopreserved human testicular tissue specimens were grouped as follows according to the analysis of corresponding HE-stained sections from routinely fixed material: 1.) hypospermatogenesis associated with inflammatory infiltrates (ly) (non-cancer samples, n = 12), 2.) CIS associated with ly and seminoma samples (cancer samples, n = 11). Measurements of transcripts encoding different cytokines were made using qualitative and quantitative RT-PCR after RNA extraction from cryopreserved material and cDNA synthesis. Cytokines analysed can be grouped due to their functional characteristics: 1.) pro-inflammatory cytokines (IL-1b, IL-6, IL-17a, TNF-α), 2.) anti-inflammatory cytokines (IL-10, TGF-β1), 3.) chemokines (CXCL-10, CXCL-13, CCL-5), 4.) cytokines important in T helper cell (Th) type 1 immune responses (IL-2, IFN-γ, IL-12a, IL-12b + IL-1b, IL-6, TNF-α, IL-10), 5.) cytokines important in Th2 immune responses (IL-4, IL-5, IL-13, IL-23a + IL-10) and 6) cytokines expressed by Th17 cells (IL-17a).

Results With regard to testicular mRNA expression, cytokines showing a significantly increased expression in cancer samples have strong pro-inflammatory characteristics and suggest the potential involvement of Th1 cells in testicular immune reactions directed towards neoplastic cells (IL-1b, IL-6, TNF-α, CXCL-10, CXCL-13, CCL-5, IL-2 and IFN-γ significantly increased in cancer-samples). In contrast, the cytokine expression pattern detectable within non-cancer samples seems to be highly influenced by Th2 and Th17 cells (IL-5, IL-13 and IL-23a significantly increased in non-cancer samples).

Conclusions Our data suggest that immune responses towards testicular neoplastic cells take place in a cytokine microenvironment that is highly influenced by pro-inflammatory factors and cytokines known to originate from Th1 cells. This finding leads to the hypothesis that testicular cancer, i.e. seminoma, is another type of malignancy in which an adaptive and specific immune response occurs. On the other hand, findings in non-cancer samples, i.e. hypospermatogenesis of unknown aetiology, reveal immunoregulatory and immunosuppressive factors that can be linked to the involvement of Th2 and Th17 cells, and thus, features of autoimmunity.

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P12

Antioxidative Supplementierung der Männer verbessert die Entwicklung der Blastozysten bei IVF/IMSI-Behandlung

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Fragestellung Die Zeugungsfähigkeit eines Mannes hängt eng mit der Qualität seiner Spermien zusammen. Begleitend zu einer Kinderwunschbehandlung wurde zur Verbesserung der Spermienqualität bei subfertilen Männern in den vergangenen Jahren immer wieder der Einsatz antioxidativer Supplemente diskutiert. Obwohl Verbesserungen der Spermienqualität in vielen Studien beobachtet wurden, so wurde doch immer wieder kritisch diskutiert, inwieweit und ob sich eine solche Therapie positiv auf den Erfolg einer Kinderwunschbehandlung (IVF/IMSI) auswirken kann.

Methoden In der vorliegenden Studie untersuchten wir den Einfluss einer begleitenden antioxidativen Behandlung auf den Therapieerfolg bei 92 Paaren, die in unserem Zentrum IVF/IMSI-Behandlungen hatten. Dabei wurde während eines ersten Behandlungszyklus eine Spermienprobe analysiert, die Daten zum Behandlungsergebnis wurden protokolliert. Die durchschnittlich ermittelten Werte der Samenproben lagen im Bereich einer Normozoospermie. Diese Daten dienten als Kontrolle und wurden mit den Ergebnissen einer zweiten Spermienprobe während einer zweiten Kinderwunschbehandlung verglichen. In diesem zweiten Behandlungszyklus erhielten die Männer für die Dauer von 3–6 Monaten eine antioxidative Supplementierung (Fertilovit® M). Die Auswertung der Spermienprobe erfolgte dabei sowohl nach den WHO-, als auch nach MSOME- (motile sperm organelle morphology examination-) Kriterien. Zur Beurteilung des Behandlungserfolges wurden die Daten zur Befruchtungsrate (FR), Blastozystenrate (BR), Top-Blastozystenrate (tBR), Schwangerschaftsrate (PR) und andauernde Schwangerschaftsrate (oPR) evaluiert und zwischen beiden Zyklen verglichen.

Ergebnisse Nach Supplementierung fanden wir eine leichte Verbesserungen der Spermienqualität nach WHO und eine signifikante Verbesserung der Spermienqualität nach MSOME (Anstieg des prozentualen Anteils von Spermien ohne Vakuolisierung (Klasse-I-Spermien, $p < 0{,}01$). Obwohl sich naturgemäß das durchschnittliche Alter der weiblichen Partner signifikant erhöhte (36,8 Jahre gegenüber 38,1 Jahre, $p < 0{,}03$), was erwartungsgemäß eher mit einem schlechteren Behandlungsergebnis einhergeht, beobachteten wir während des zweiten Behandlungszyklus nach antioxidativer Supplementation einen Anstieg der Blastozystenrate. Die Top-Blastozystenrate stieg sogar signifikant an ($p < 0{,}05$). Zudem beobachteten wir eine deutliche Verbesserung der Schwangerschaftsraten, sowohl im Hinblick auf eine biochemische Schwangerschaft als auch bei der später per embryonalem Herzschlag bestätigten Schwangerschaft.

Schlussfolgerungen Zusammenfassend zeigte sich, dass eine begleitende antioxidative Supplementation der Männer auch mit durchschnittlichen Spermiogramm-Werten im Rahmen einer Kinderwunschtherapie die Spermienparameter weiter verbessern und somit auch zu einer Steigerung des Behandlungserfolges beitragen kann (**Tab. 1**).

Tabelle 1: J. Wogatzky, et al. (P12).

Treatment without Supplementation		With Supplementation		
Oocytes (total)	1127	Oocytes (total)	1092	p = 0.7
Oocytes (mean)	12.4 ± 5.9	Oocytes (mean)	12.1 ± 5.7	
2 PN (total)	672	2PN	659	
2 PN (mean)	7.3 ± 3.9	2PN (mean)	7.3 ± 4.3	
Blastocysts (total)	267	Blastocysts (total)	288	
Blastocysts (mean)	2.9 ± 2.4	Blastocysts (mean)	3.1 ± 2.7	
Top-Blastocysts (total)	37	Top-Blastocysts (total)	56	
Top-Blastocysts (mean)	0.4 ± 1.1	Top-Blastocysts (mean)	0.6 ± 1.0	
Age female (mean)	36.8 ± 4.2	Age female (mean)	38.1 ± 3.9	p = 0.03
FR %	59.6	FR %	60.4	p = 0.73
Blasto Rate %	39.7	Blasto Rate %	43.7	p = 0.14
Blasto Rate Top %	5.5	Blasto Rate Top %	8.2	p = 0.03
PR %	34.8	PR %	44.5	n. s.
oPR %	32.6	oPR %	39.1	n. s.

P13

Der histologische Befund der Hodengewebs-Biopsie bei Männern mit Klinefelter-Syndrom ist kein absoluter Prädiktor für den Erfolg einer TESE/ICSI-Therapie

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Einleitung Das Klinefelter-Syndrom (47,XXY) ist die häufigste nachgewiesene chromosomale Ursache der männlichen Infertilität. Liegt bei betroffenen Patienten eine Azoospermie vor, ist die beidseitige Hodenbiopsie mit nachfolgender testikulärer Spermienextraktion (TESE) in Verbindung mit der intrazytoplasmatischen Spermieninjektion (ICSI) die Therapie der Wahl zur Erfüllung eines unerfüllten Kinderwunsches. Die Chancen einer erfolgreichen TESE liegen bei Patienten mit Klinefelter-Syndrom nach aktueller Evidenz bei 53 % [Dávila Garza et al., Curr Opin Obstet Gynecol 2013; 25: 229]. Die Histologie des Hodengewebes wird als der stärkste Prädiktor einer erfolgreichen Spermienextraktion angesehen [Bernie et al., Basic Clin Androl 2013; 23: 5].

Fallbericht Ein Patient, bei dem 1986 im Alter von 6 Jahren ein Klinefelter-Syndrom (47,XXY) diagnostiziert wurde und der seit 1994 ununterbrochen eine Testosteronsubstitutionstherapie erhielt, stellte sich im Alter von 31 Jahren in unserer Sprechstunde mit unerfülltem Kinderwunsch vor. Bei dem Patient wurde eine Azoospermie diagnostiziert. Die Testosteronsubstitutionstherapie wurde daraufhin nach entsprechender Aufklärung abgesetzt. Bei persistierender Azoospermie wurde 6 Monate später eine beidseitige Hodenbiopsie vorgenommen. Die histologische Untersuchung durch einen erfahrenen Pathologen zeigte keine Spermien oder Spermatiden im Hodengewebe mit einem maximalen Holstein-Score von 5. In der Probe-TESE im reproduktionsmedizinisch-andrologischen Labor wurden hingegen vereinzelte testikuläre Spermien gefunden. Im November 2011 wurde eine ebenfalls erfolgreiche TESE aus den kryokonservierten Hodengewebs-Proben des Patienten mit anschließender ICSI-Therapie durchgeführt. Beim ersten ICSI-Versuch trat eine klinische Schwangerschaft ein und letztendlich wurde im Juli 2012 ein gesundes Mädchen (46,XX) geboren. Bei noch nicht abgeschlossener Familienplanung sind in diesem Jahr eine erneute Hodenbiopsie und ggf. eine zweite TESE/ICSI-Therapie geplant.

Diskussion Der histologische Befund der Hodengewebs-Biopsie ist bei Männern mit Klinefelter-Syndrom kein absoluter Prädiktor für die Erfolgschancen einer TESE, weil durch die Histologie, im Gegenteil zu der TESE im reproduktionsmedizinisch-andrologischen Labor, nur ein relativ kleiner Teil der Gewebsproben untersucht wird. Eine negative Histologie schließt daher eine erfolgreiche TESE/ICSI-Therapie nicht aus und Patienten mit Klinefelter-Syndrom und unerfülltem Kinderwunsch sollten entsprechend beraten werden.

P14

Entwicklung eines multimodalen MRT-Protokolls zur funktionellen Charakterisierung des Hodens bei nicht-obstruktiver Azoospermie

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Zielsetzung 10 % aller infertilen Männer leiden an nicht-obstruktiver Azoospermie. Aktuelle Erfolgschancen von (M-)TESE lie-

gen bei lediglich 60 %. Die multimodale MRT soll der funktionellen Beurteilung und Lokalisation einer residuellen Spermatogenese dienen, um eine gezielte Hodenbiospie für die Spermiengewinnung zu ermöglichen.

Material und Methoden Gesunde und Patienten mit Azoospermie (Einteilung mittels Spermiogramm) wurden bei 3T mit einer 4-Kanal-Oberflächenspule untersucht. Das Studienprotokoll umfasst T2-gewichtete Sequenzen zur morphologischen Beurteilung, eine Diffusionssequenz zur Bestimmung der Zellularität, eine Perfusionsmessung nach intravenöser Kontrastmittelinjektion (T1-gewichtete Sequenzen) inklusive eines automatischen T1-Mappings, um Schrankenstörungen oder vaskuläre Pathologien darzustellen. Bisherige Studien [1, 2] ergaben, dass insbesondere die Metabolite Phosphocholin und Kreatin verlässliche Marker für die Spermatogenese darstellen. Somit gilt der MR-Spektroskopie ein besonderes Augenmerk.

Ergebnisse Das Kollektiv umfasst 21 Patienten mit NOA-Verdacht, ein Patienten mit Infarkt sowie 35 Gesunde. Das mittlere Alter der Patienten betrug 35, das der Probanden 28 Jahre. Die Bildqualität war stets gut. Befunde wie Atrophie, Leistenhoden, Inflammation und Varikozele wurden detektiert. Bezüglich der Volumetrie zeigt sich im MRT (Simpson-Methode) ein höheres Volumen als in der Sonographie (Ellipsoidformel) sowie ein deutlich geringeres Volumen bei den Patienten. In der MR-Spektroskopie ließen sich Cholin und Kreatin nachweisen. Die mittels ADC-Map ermittelten Diffusionswerte zeigten eine Diffusionsrestriktion bei den Patienten. Für eine ganzheitliche Analyse wurden urologische Daten wie Peakfluss der Arteria testicularis, Hormonstatus und Patientenhistologie hinzugezogen.

Diskussion Die ersten Ergebnisse zeigen, dass sich Hodenvolumen, -perfusion sowie -diffusion verlässlich mittels der MRT bestimmen lassen. Es finden sich bereits bei kleinem Kollektiv Unterschiede bei Volumen und Diffusion. Kleine Hodenvolumina erschweren die Durchführung der Spektroskopie. Es wurde ein Quotient Cholin/Kreatin gebildet. Hier dient eine Verringerung als Zeichen einer gestörten Spermatogenese. Insgesamt ist das multimodale MRT eine erfolgversprechende nicht-invasive Methode zur morphologischen und funktionellen Beurteilung des Hodens.

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P15

Zytokinprofile im Ejakulat – Funktionelle Analysen bei Inflammation und Störungen der akzessorischen Sekretion

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Fragestellung Zytokine sind zelluläre Botenstoffe, die bei einer Vielzahl von biologischen Prozessen eine essenzielle Rolle spielen. Die Bestimmung einzelner Zytokine im Seminalplasma ist vor allem Teil der Diagnostik entzündlicher Prozesse im Genitaltrakt. Umfassendere Zytokinprofile und ihr möglicher (immun-) pathologischer Stellenwert wurden bisher jedoch nicht systematisch untersucht.

Methoden Seminalplasma von 240 Männern (Infertilität 59; Epididymitis 56; HIV 22; Metabol. Syndrom 29; Vasektomie 23; Gesunde 52) wurde im Multiplexverfahren (Cytometric Bead Assay, BD Biosciences) auf 21 Zytokine untersucht (IL-1α, -1β, -2, -4, -6, -8, -9, -10, -12p70, -13; TNF-α, IFN-γ, VEGF, FGF, GCSF, GMCSF, MIP-1α, MIP-1β, RANTES, Eotaxin, IP-10). Die gleichzeitige Ejakulatanalyse nach WHO 2010 schloss Peroxidase+-Leukozyten (PL) und Granulozyten-Elastase ein.

Ergebnisse PL und Elastase zeigten signifikante positive Korrelationen untereinander sowie mit den Zytokinen IL-1α, IL-1β, IL-6, IL-8, IL-10, TNF-α, IFN-γ, GCSF, MIP-1α und MIP-1β und (stets $p < 0{,}001$). Außer GCSF waren erhöhte Level dieser Zytokine mit einem erhöhten pH-Wert und einem reduzierten Fruktose-Gehalt assoziiert, abgesehen von GCSF und IL-8 auch mit einer verminderten Zink-Konzentration ($p < 0{,}001$). Hingegen fand sich kein Effekt auf die α-Glukosidase. Eine negative Korrelation konnte zwischen IP-10, GMCSF sowie FGF und der Spermienkonzentration nachgewiesen werden ($p < 0{,}001$), nicht jedoch zwischen Zytokinen und Motilität oder Morphologie.

Schlussfolgerungen Die Multiplexmessung konnte spezifische Zytokine identifizieren, die mit etablierten Inflammationsmarkern und einer Störung der akzessorischen Sekretion assoziiert sind. Ein klinisch relevanter Einfluss auf die Spermienparameter Konzentration, Motilität und Morphologie ließ sich jedoch nicht feststellen.

P16

Inflammatory Prostatitis Syndrome in Fertile and Infertile Men

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Objectives Literature researches reveal a variety of studies that concentrate on a potential link between the chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS) and infertility of men [1]. To verify the assumed correlation, this study compares "fertility parameters" based on spermiograms in patients with CP/CPPS and infertile men.

Material and Methods The spermiogram according to the WHO [2] includes diverse parameters. This study is focused on the ejaculate volume (ml), sperm concentration (mio/ml), motility (PR/PR+NP, %) and pH values. Additional the evaluation of the NIH-CPSI [3] is considered. The cohort consists patients with CP/CPPS.

Results Spermiogram data were analyzed and compared according to the WHO [2]. The normal range of the progressive motility (PR, %) is > 32%. In contrast, the cohort of prostatitis patients (n = 47) showed that 25.5% are below the normal range. For the total motility score (PR+NP, %) with a reference of > 40%, 21.3% of the prostatitis patients are found under this rate. The optimal sperm concentration is 15–250 mio/ml [2]. The prostatitis patients (n = 43) showed that 20.9% were below this value. 83.7% of the investigated patients (n = 49) had an abnormal ejaculate volume compared to the WHO reference of 1.3–1.9 ml/ejaculation. 61.2% showed an increased volume over 1.9 ml. Within the analysis of the pH values we found that 14.3% of the prostatitis patients (n = 49) had a decreased pH compared to the norm of 7.2–8.0 [2]. The CPSI total score, which classifies the clinical symptoms of prostatitis patients, is on average 20 [4]. Compared to that, 56.6% of the analyzed patients (n = 69) had a higher score than 20.

Conclusions The results show that up to one quarter of the CP/CPPS patients have abnormal spermiogram parameters. In the future all the results are going to be correlated with the general prevalence and a healthy control group.

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P17

A Proteomic Approach to Identify Epididymis and Testis Specific Proteins in Seminal Plasma

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Introduction In patients presenting with non-obstructive azoospermia a definitive exclusion of an additional obstruction remains clinically challenging. Currently available parameters (e.g. α-glucosidase FSH, inhibin B and testicular size) have only a limited value as predictors for sperm retrieval in non-obstructive azoospermia. Thus, the aim of this study is the detection of epididymis and testis specific seminal plasma proteins for an improved preoperative assessment of patients with azoospermia using a proteomic approach.

Materials and Methods This study was approved by the institutional review board and written informed consent was obtained from all subjects. A comparative proteome analysis of seminal plasma was performed in 10 men requesting vasectomy. Sampling was done before vasectomy and six weeks after vasectomy. After protein extraction, 2D electrophoresis was performed separately with 100 µl seminal plasma for each sample. Gel images were acquired with a typhoon 9200 laser scanner and analysed with PdQuest software. Comparison between groups (before vs. after) revealed 84 significant different spots.

Results Protein identification revealed 27 different proteins. Of those, none was testis specific, while three epididymis specific candidates were identified: CRISP1 (Cystein-Rich Secretory Protein 1), FAM12B (Human Epididymis-specific protein 3-beta) and LTF (Lactotransferrin). ELISA validation detected only CRISP1in seminal plasma, but without any significant differences between both groups, while FAM12B and LTF were below detection limit in the corresponding ELISAs. Measurement of testis specific proteins revealed only LDHC in seminal plasma, while TEX101 was again below detection limit.

Discussion Contributions to the seminal plasma arise from different parts of the male genital tract. In our proteomic approach we could identify of current ducts possible candidate biomarkers for obstruction. There is an ongoing debate that a positive sperm retrieval in NOA patients is influenced significantly by a clinical non detected obstruction. However, more studies are needed to identify and establish specific proteins as clinical biomarkers to predict the spermatogenesis situation in patients with azoospermia before surgery.

Reproduktionsbiologie und -genetik

P18

Transfection of Rat Sertoli Cells with Human Androgen Receptor leads to Alterated Gene Expression without Androgen Incubation

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Introduction Androgens (i. e. testosterone [T] and dihydrotestosterone [DHT]) play an important role for the development of male fertility and also gained increasing interest as growth and survival factors for certain types of cancer. T/DHT act via the androgen receptor (AR), a ligand-activated transcriptional factor. The classical mechanism of AR action require (1.) binding of T/DHT, (2.) translocation into the nucleus, (3.) binding to certain androgen responsive elements and (4.) subsequent transcriptional activation or repression. As functional mechanisms of androgen action and AR reaction are in the focus of interest, cell culture systems were established, i. e. for human breast cancer, adrenocortical carcinoma and prostate carcinoma. Additionally, AR-deficient cell lines have been used and were – after stable transfection with AR – treated with T/DHT (for example [1]).

Results There are no studies whether gene expression analysis in transfected and stimulated cells may be distorted by the fact, that transfection with AR alone leads to an altered gene expression. Therefore, we introduced a full length human AR into the expression vector pcDNA 6.2 C-EmGFP and transfected AR-deficient rat Sertoli cells (93RS2) by electroporation. Transfection of animal cells with human transcripts have been shown to be functional [2]. Transfection success was assured by Western Blotting, immunofluorescence staining and RT-PCR. For microarray analysis, transfected and also non-transfected 93RS2 cells were used in biological and technical triplicates each. Microarray analysis revealed 676 significantly regulated genes with 202 genes showing up- and 474 genes showing down-regulation comparing transfected Sertoli cells with non-transfected control cells. Using DAVID gene database, regulated genes can be classified into four main groups, i. e. development, hormone response, immune response and metabolism. For 24 candidate genes, showing either up- or down-regulation comparing transfected and non-transfected cell lines, microarray results were confirmed by quantitative RT-PCR analysis.

Conclusions Our data indicate, that transfection of cell lines with AR itself has a measurable effect on gene expression, even without T/DHT stimulation. Alterations in AR-dependent gene expression in transfected cell lines, subsequently incubated with T/DHT, have to be thoroughly checked regarding effects of transfection itself. Such studies furthermore should include the original non-transfected cell line as negative control to avoid false-positive results (DFG KFO181, BE1016/7-1).

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P19

Induction of Piwi-like Gene Expression by 5'-azadeoxycytidine through DNA Methylation dependent Mechanisms

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Background Understanding the mechanisms regulating expression of genes during development and in germline stem cells will be critical for successful spermatogenesis. During differentiation, chromatin remodeling through histone deacetylation and DNA methylation are a potential mechanism for silencing genes normally maintaining the stem cell phenotype. The Piwi-gene family (P-element induced wimpy testis) is the germ-line expressed subclade of the Argonaute proteins. Piwi-genes are expressed in pre-pachytene and pachytene stages of spermatogenesis where they act in germ cell development and silencing of retrotransponsons to maintain genomic integrity. Their expression is typically repressed in late stages of spermatogenesis. We investigated DNA methylation as potential mechanism for regulating expression of human Piwi-like genes 1–4 in PCa cell lines as easy accessible cell system.

Methods Basal methylation status of Piwi-like 1–4 promoters in PCa cell lines Du145, PC3 and LNCaP was measured by bisulfite conversion of unmethylated cytosines. PCR primers for bisulfite sequencing were designed for bisulfite converted DNA using Methprimer (http://www.urogene.org/methprimer). PCR products with less than 95% C to T conversion of non-CpG cytosine residues were excluded from analysis based on incomplete bisulfite conversion. To investigate the expression of Piwi-like 1–4 mRNA, cells were treated with 1 µM of the demethylating agent 5'-azadeoxycytidine (5azadC) alone and in combination with 100nm of HDAC inhibitor Trichostatin A (TSA) for three days. RNA was isolated and mRNA expression of Piwi-like 1-4 was analyzed by quantitative RT-PCR. GAPDH was used as reference.

Results Bisulfite sequencing revealed a broad variation in methylation levels of Piwi-like genes 1–4 in PCa cell lines. LNCaP shows low levels of Piwi-like 1–3 promoter methylation. However, Piwi-like genes are

frequently methylated in Du145 and PC-3. Piwi-like 4 is hypermethylated in each cell line. Piwi-like 2 and 4 mRNA is basal expressed and levels rise up after incubation with 5azadC. mRNA of Piwi-like 1 and 3 is weakly detectable even after stimulation with demethylating agents. Treatment with TSA has no additional impact on mRNA expression of Piwi-like genes in PCa cell lines.

Conclusion We report that it is possible to reactivate Piwi-like transcription in PCa cell lines by treatment with 5azadC to different extents. Piwi-like 2 mRNA expression is strongly induced by demethylation. Piwi-like 1 and 3 expressions are slightly altered. Our results further indicate that Piwi-like 4 expression is weakly regulated by promoter methylation. Differential activation of Piwi-like genes in PCa cell lines with demethylating agents provides first hints for epigenetic alterations during spermatogenesis.

P20

The Influence of human ART Culture Media on Epigenetics in Mouse Preimplantation Embryos

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Introduction Assisted reproductive techniques (ART) are employed worldwide to help infertile couples reaching parenthood. Embryos are produced and cultured *in vitro* in culture media, which aim to mimic the natural intratubal and intrauterine environment. We have shown that *in vitro* culture (IVC) of murine zygotes in different human embryo culture media has an effect on blastocyst and fetal development [1]. What is more several ART are linked to changes of the epigenetic profile of imprinted genes in pre-implantation embryos. Changes in the methylation status of these genes may lead to epigenetic diseases like Beckwith-Wiedemann or Angelman Syndrome. ART children seem to be at an increased risk of these genomic imprinting disorders [2].

Study Question Do different human ART culture media have an effect on the methylation status of maternally and paternally imprinted genes in murine pre-implantation embryos?

Material and Methods 6 to 10 superovulated female mice (B6C3F1, 6–8 weeks) per group were mated with C57Bl/6 males. Zygotes from the oviduct or blastocysts from the uterus (*in vivo* control) were obtained on day 0.5 or 3.5 pc (*post coitum*) respectively. Zygotes were cultured in KSOM(aa) (provided by M. Boiani) or in the human sequential media ISM1/Blast Assist (BA; Origio, Berlin, Germany) and HTF/Multi Blast (MB; Irvine Scientific, Santa Ana, USA) up to day 4.5 pc. DNA from individual blastocysts was isolated and bisulfite treated. PCR and pyrosequencing were conducted for one paternally imprinted gene (*H19*) and two maternally imprinted genes (*Mest, Lit1*).

Results The methylation status of 86 individual blastocysts for three different imprinted genes was analysed. Blastocysts cultured in KSOM(aa) showed a mean methylation level of 31.59 ± 2.4% (n = 44) for *Mest*, 26.93 ± 4.5% (n = 30) for *H19* and 39.95 ± 4.2% (n = 7) for *Lit1*. Blastocysts cultured in HTF/MB showed a mean methylation level of 38.6 ± 3.2% (*Lit1*; n = 5). For *Lit1* there are no significant differences between the culture groups.

Conclusion We found methylation levels around 30–40% for *Mest* and *Lit1*, which is in line with previous findings. Methylation levels for *H19* are lower than expected, which could be due to low sample size. So far the use of different culture media seems to have no effect on the methylation status in *Lit1*. Analysing solely the inner cell mass, which gives rise to the fetus, would provide detailed insight into epigenetic mechanisms during pre-implantation development of murine embryos.

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P21

Traceability of a Sulfatase Pathway in the Human Testis

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Steroid synthesis in the testis might not only occur via *de novo* synthesis, but also by re-activation of sulfated steroid hormones. These precursor molecules, which can be synthesized in the Leydig cells of the testis itself as well as the adrenal cortex, may be de-sulfonated by steroid sulfatase (StS) activity, also known as sulfatase pathway. In a recent study, we detected the Sodium-dependent Organic Anion Transporter (SOAT), an effective membrane transporter for sulfated steroids in primary spermatocytes and early round spermatids. However, the central question how the hydrophilic sulfated steroids generally negotiate lipophilic cell membranes and in particular the blood-testis-barrier (BTB) is not completely understood yet. Tight junctions between neighbouring Sertoli cells near the basement membrane create an essential component of BTB. As sulfated steroids are not able to pass the cell membrane by diffusion, distinct uptake carrier and also efflux transporter have to be present in the different cell types of the testis especially in the Sertoli cells to transport them to the inside of seminiferous tubules. To evaluate possible components of a sulfatase pathway in the testis, we focused on two Organic Anion Transporting Peptides (OATP2B1 and OATP3A1), three active efflux transporter, i. e. Multidrug Resistance-related proteins (MRP1 and MRP4) and the Breast Cancer Resistance Protein (BCRP). Additionally we investigated the involved enzymes i.e. StS and sulfotransferases (SULT2A1, SULT1E1) and revealed the expression of all components on mRNA and protein level in human testis employing RT- and qPCR- techniques following laser assisted cell picking (LACP), *in situ* hybridyzation and immunohistochemistry.

So far, we sucessfully detected StS and uptake carrier OATP2B1 in Sertoli cells and pachytene spermatocytes and efflux transporter MRP1 in Sertoli cells on both mRNA and protein level. All examined elements were also present in interstitial Leydig cells.

Our preliminary data suggest a comprehensible route for sulfated steroid hormones (presumably synthesized in Leydig cells) to overcome BTB via Sertoli cells and enter germ cells. Taken together with previous data localizing androgen and estrogen receptors in Sertoli cells and the latter additionally in germ cells we assume a functional sulfatase pathway within the human seminiferous epithelium (DGF.FOR 1369, F/1927/1-2).

P22

Germ cells are Possible Targets of Oxytocin in the Human Testis

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Background and Study Question Oxytocin (OXT) and vasopressin (VP) are structurally closely related neurohypophyseal hormones. Both are able to target the genuine oxytocin receptor (OXTR). OXT is traditionally known as a "female" hormone, but its expression was also described in Leydig cells of the human testis and in the male gonad both OXT and VP were linked to direct inhibition of testicular androgen biosynthesis. Whether OXT and/or VP can target other testicular cells and what the cellular consequences may be in man, is not well known.

Materials and Methods We performed immunohistochemical studies of human testicular tissue, RT-PCR and functional studies of germ cell tumor cell line with stem cell-like characteristics, the seminoma cell line (TCam-2), including measurements of intracellular Ca^{2+} levels by FLUO-4 and live cell imaging.

Results The results showed that spermatogonia stained positive for the OXTR. RT-PCR studies, followed by sequence analysis, showed that TCam-2 cells express the genu-

ine OXTR. Its functionality was demonstrated in experiments measuring intracellular Ca^{2+} levels. OXT, within seconds, transiently elevated intracellular Ca^{2+} levels in most of the cells in a concentration-dependent manner. Live cell imaging of TCam-2 performed over a 24h period revealed that 100 nM OXT induced a form of cell death. OXT or VP did, however, not activate caspase 3/7, indicating a caspase 3/7-independent form of cell death in TCam-2. This contrasts to previous results that showed that OXT induces a caspase 3/7-dependent apoptotic cell death in human granulosa cells.

Conclusion Our preliminary results show that human germ cells, namely spermatogonia and TCam-2 cells, are targets of OXT and possibly VP. Thus OXT and VP may be involved directly in the regulation of the fate of germ cells in man. Whether they may target spermatogonial stem cells remains to be studied. Furthermore, whether the results obtained in TCam-2 might indicate a role in testicular cancers remains to be studied.

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P23

Short-term Exposure of Mature Oocytes to a Nitric Oxide Donor for Inducing Oxidative Stress Resistance on In Vitro Produced Bovine Embryos

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Introduction Recent studies have shown that short-term exposure of oocytes to stressors such as hydrostatic pressure, osmotic stress and oxidative stress might induce stress tolerance in embryos. In this research we studied the effect of short-term exposure of bovine *in vitro* matured cumulus-oocyte complexes (COCs) with a nitric oxide donor (SNP) on in vitro fertilization (IVF), embryo development and relative gene expression related with cell redox state regulation.

Methods Mature oocytes (COCs) were incubated during 1 hour with different concentration of sodium nitroprusside, SNP (Control without SNP, 10^{-6}M, 10^{-5}M and 10^{-4}M SNP) in maturation media at 38.5°C and 5% CO_2, 5% O_2 in humidified atmosphere. For IVF procedure, oocytes of each treatment and sperm of one bull were co-incubated for 18–20 hours at 38.5°C and 5% CO_2, 5% O_2. Presumptive zygotes were separately cultured in KSOM-FBS media until day 7 under mineral oil at 38.5°C and 5% CO_2, 5% O_2 and 90% N_2 in a humidified atmosphere. Relative gene expression analyzes of 3 pools of embryos for each treatment were evaluated after RNA extraction and cDNA synthesis in a stratagene MX 3000P real time equipment with the Agilent's qPCR software MX pro 4.1 version.

Results Cleavage percentage at 72 hours post-insemination were significantly different between control group and 10^{-4}M SNP (82 ± 8.4% vs. 77 ± 7.1%, respectively) and between 10^{-5}M and 10^{-4}M SNP (84.9 ± 4.1% vs. 77 ± 7.1%, respectively). Blastocyst percentage at 7day culture were significantly different between control group and 10^{-4}M SNP (34.1 ± 7.8 % vs. 26.2 ± 4.9%, respectively). Embryo development between control group and treatments were similar within early, expanded and hatched blastocyst percentage. Embryo relative gene expression shown that some genes were up-regulated (iNOS, PRDX5, HSP70) or down-regulated (iNOS, nNOS, HSP90, HIF1A, BCL2A) after SNP exposure.

Conclusion Oocytes incubated with high concentration of SNP shown lower cleavage percentage and lower blastocyst percentage, being deleterious for *in vitro* embryo production in bovine. SNP modulates gene expression pattern on 7 day cultured expanded blastocyst, and these changes are associated with stress tolerance induction process.

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P24

Stress Tolerance by Redox Modulation with Hydrogen Peroxide in Bovine Oocytes – Effect on *in vitro* Embryo Development

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Introduction The qualities of embryos generated by in vitro methods are lower than those embryos derived in *in vivo* procedures. The reduction-oxidation state (REDOX) affects not only energy production required for embryonic development, but also transcription factors that can alter the pattern of gene expression. Induction of oxidative stress with hydrogen peroxide (H_2O_2) in mature oocytes generates an increase in the blastocyst rate. This effect would be related to the selective expression of genes involved in the regulation of cellular redox. The aim of this study was to determine the rate of embryonic development after exposure of bovine oocytes at different concentrations of H_2O_2.

Methods Bovine oocytes from slaughterhouse ovaries were matured in TCM-199 medium supplemented for 22 hours at 38.5°C, 5% CO_2 and humidified atmosphere. At the end of 22 hours, the following treatments were applied for 1 hour: 0, 50, 100 and 200 µM H_2O_2. The *in vitro* fertilization was performed co-incubating oocytes 18 hours with a final concentration of 1×106 sperm/mL. The presumptive zygotes were denuded and cultured in KSOM-0.4% FAF-BSA medium at 38.5°C under low O_2 tension (5% O_2, 5% CO_2 and 90% N_2) and humidified atmosphere.

Results Induction of stress with H_2O_2 did not produce differences in cleavage rate at 72 hrs using 50 and 100 µMH_2O_2 compared to control, whereas 200 µMH_2O_2 generated a significant decrease in assessing the rate of blastocyst (7 days) no differences was observed when using 50 µMH_2O_2 compared to control. However, when using 100 and 200 µM, blastocyst rate decreases dramatically.

Conclusion The induction of oxidative stress with 50 µM H_2O_2 maintaining a proper embryonic development. It is possible that these embryos resistant to oxidative stress it can have a higher survival cryopreservation processes that generate high levels of reactive oxygen species in embryos.

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P25

Cellular Targets of Oxytocin in the Human Testis – Peritubular Cells

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Questions The nonapeptide hormone oxytocin (OXT) has established roles in the female reproductive tract and, for example, induces contraction of smooth muscle tissue of the myometrium upon binding to its specific receptor (OXTR). However, it also targets human ovarian granulosa cells and induces apoptosis, indicating a broader spectrum of actions. Smooth muscle cells are present in the human testis and several layers of these myoid, peritubular cells form the wall of the seminiferous tubules. Due to the contractile abilities of peritubular cells they are necessary for sperm transport and hence are important for male fertility. Whether these cells express OXTR and hence are possibly targeted by the corresponding neuropeptide ligand in man is not well known.

Methods To explore these topics we firstly performed immunohistochemical studies of human testicular tissue followed by qPCR/Western blotting studies as well as live cell imaging experiments of cultured human testicular peritubular cells (HTPCs).

Results The results indicate that peritubular cells but also spermatogonial cells were positively stained for OXTR. In order to verify expression and to further investigate OXT-mediated functions, we used cultured human testicular peritubular cells (HTPCs). We performed qPCR and Western blot studies, as well as in vitro live cell imaging experiments. We found that these cells express genuine OXTR mRNA and protein. Its functionality was demonstrated by transient elevations of intracellular Ca^{2+} levels upon addition of OXT within seconds. When HTPCs were incubated with OXT they contracted within minutes.

Conclusions In conclusion, our preliminary results show that testicular peritubular cells are targets of OXT. Thus OXT, derived from the circulation and local sources, may be involved directly in the regulation of contraction of smooth muscle-like cells of the wall of seminiferous tubules, sperm transport and possibly further functions of peritubular cells.

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P26

Comparison of Isolated Peritubular Cells of Seminiferous Tubules with Classical Smooth Muscle Cells

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Introduction Peritubular cells or so-called myofibroblasts represent the main cell type of the lamina propria (LP) surrounding human seminiferous tubules. Male infertility is predominantly associated with impaired spermatogenesis and considerably thickened LP. Excessive deposition of extracellular matrix proteins of the LP may fundamentally influence the contractile function of peritubular cells. In this study we characterized peritubular cells in a primary cell culture model according to Albrecht et al. [1] and compared these cells with classical smooth muscle cells.

Material and Methods To assign in detail the character of isolated cells from human testicular biopsies with different clinical background, we used immunofluorescence staining with specific markers to show contractile (SMA and calponin) and fibroblast (vimentin and CD90) characteristics *in vitro*. We found SMA, vimentin and CD90 in all isolated peritubular cells of diverse passages. In addition, we could demonstrate co-localization of calponin and CD90 indicating a myofibroblast phenotype of cultured cells. Surprisingly, comparable results were found, when investigating true smooth muscle cells from pulmonary artery (HPASMCs) and prostate (HPrSMCs) by immunofluorescence and FACS. Furthermore, we investigated gene expression pattern of contractile (SMA, cGMP-dependent proteinkinase I [PKGI], myosin-heavy chain 11 [MYH11], calponin and smoothelin) and fibrotic (CD90, vimentin, collagen I and fibronectin) markers with Real Time PCR. The expression pattern of fibroblast markers was regularly higher in comparison to contractile markers. These unexpected gene expression patterns of peritubular cells were also found when investigating true smooth muscle cells (HPASMCs, HPrSMCs).

Conclusion Our data show that cultured peritubular cells of diverse passages are not a mixed culture of smooth muscle cells/myofibroblasts and fibroblasts since we could show a co-localization of contractile (calponin) and fibroblast (CD90) markers. The mRNA data reveal that expression of genes characteristic for fibroblasts predominates under culture conditions, but that smooth muscle cell-specific genes are also expressed at all time points. Surprisingly, this myofibroblast phenotype of isolated human peritubular cells is also characteristic for classical smooth muscle cells during long term culture.

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P27

Spermatogenic Efficiency and Endocrine Function is Maintained in Ageing Non-Human Primates (*Macaca fascicularis*) although MAGE-A4 Positive Germ Cell Numbers are Decreasing

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Introduction Ageing effects on male reproduction have been suspected to result in decreasing fertility and an increased risk for the offspring to suffer from certain diseases which are associated with older paternal age. It remains widely unsolved what the underlying causes of this phenomenon are and whether the obviously worsening gamete quality is reflected by altered testicular function in older age. Using archived material from three Cynomolgous macaques, we investigated this issue in a non-human primate ought to be the most adequate model for human testicular function.

Methods Monkeys had been hemicastrated in adulthood and were fully castrated after a seven year period when they were all 23 years of age or older. Samples from both time points were analysed for testicular histology and morphometry and underwent a flow cytometric ploidy measurement. Testosterone, LH and FSH values were analysed from serum samples obtained during hemicastration and when animals were sacrificed. Additionally, a quantitative immunohistochemical detection of MAGE-A4 as a marker for spermatogonial stem cells (SSC) was performed to monitor the fate of the spermatogonial population during ageing.

Results The LH values account for 0.41 ± 0.06 nmol/l at the younger age and 0.55 ± 0.17 nmol/l at the older stage. The testosterone level varied negligibly from 57.6 ± 25.67 nmol/l to 42.2 ± 28.40 nmol/l after seven years. The testosterone to LH ratio indicated for normally working Leydig cells with a ratio of 150.6 ± 39.4 at the younger stage and at 105.2 ± 69.0 at the older stage. Concerning gonadal function, surprisingly, apart from a decrease in the labelling index for MAGE-A4 positive spermatogonia, no differences between the two time points were observed for any endpoint measured. The labelling index decreased from 82.17 ± 5.0% to 63.56 ± 8.8% after seven years of ageing. However, neither spermatogenic efficiency nor tissue composition or endocrine function was altered in the aged animals. The flow cytometric analysis of the ploidy shift in the testicular issue showed a similar ratio of 2C/1C cells at both time points.

Conclusion From our data – although limited to the small group size available – we conclude that in the non-human primate spermatogenic efficiency and endocrine function of the gonad is maintained during ageing but spermatogenesis is obviously driven by a reduced population of active spermatogonia in older age. Thus, the association found between age and reduced fertility and increased risk for offspring could be due either to post-testicular effects on the gametes or might be caused by a worsening quality control on a reduced spermatogonial population.

P28

Direct but no Major Transgenerational Effects of Epigenetic Drugs on Male Fertility

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Introduction Spermatozoa display specific DNA methylation patterns supposed to be important for male fertility and embryo development. In recent years, epigenetic drugs have been developed which influence epigenetic patterns and thereby inhibit tumour formation. Due to the described role of epigenetic patterns for male fertility, epigenetic changes induced by such drugs could influence the male reproductive system and fertility. In addition, artificially induced epigenetic aberrations could be transmitted to subsequent generations indicating a transgenerational epigenetic inheritance. We therefore aimed at investigating direct and transgenerational effects of two epigenetic drugs on the male reproductive system and fertility.

Methods For this purpose, we treated male mice for seven weeks (intraperitoneal, three times per week) with the DNMT inhibitor decitabine (0.1 µg/g) or the HDAC inhibitor vorinostat (50 µg/g) and analysed extensively the germ line of treated mice (P generation) as well as of subsequent generations (F1–F3 generations).

Results Treatment with the two epigenetic drugs affected following reproductive parameters: weight of testes (decitabine & vorinostat), weight of epididymides and accessory sex glands (vorinostat), size of seminiferous epithelium (decitabine) as well as sperm concentration and morphology (decitabine). In addition, spermatozoal DNA methylation of few genes was changed after decitabine treat-

ment. However, when analysing the fertility (fertilisation rate, litter size and sex ratio) of treated mice, only the fertilisation rate was minimally reduced after decitabine treatment (decitabine: 7/10, vorinostat: 9/10, vehicle control: 9/10) indicating no major effect of the selected epigenetic drugs on male fertility. In subsequent generations (F1–F3 generations) minor effects on reproductive organs, sperm parameters and DNA methylation but no effect on fertility could be detected.

Conclusion Consequently, we conclude that decitabine and vorinostat neither affect male fertility nor cause strong transgenerational effects. Thus, the use of these drugs in anticancer therapies may be safe for the patients in terms of male fertility and transgenerational epigenetic effects.

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P29

The Human *RHOX* Genes – Effect of Single Nucleotide Polymorphisms and a New Sequencing Approach

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Introduction Reproductive homeobox (*RHOX*) genes are clustered on the X chromosome sharing a unique 60 amino acid helix-turn-helix DNA binding domain. The human *RHOX* cluster is composed of three genes located on the X chromosome at position Xq24: *RHOXF1*, *RHOXF2* and *RHOXF2B*. *RHOXF2* variants share 99.8% sequence identity and are thus referred to as *RHOXF2/2B*. Previous data show that RHOX proteins are expressed exclusively by germ cells in human testis and that aberrant *RHOX* methylation is associated with several sperm parameters [1, 2]. The study aimed at examining the role of the *RHOX* gene cluster in human spermatogenesis and its involvement in male infertility.

Material and Methods Genomic DNA was extracted from blood samples of idiopathic infertile men and SNP genotyping was performed by using a customized TaqMan® Assay (n = 344). DNA samples were pooled (n = 10 or 20) and the entire coding region of *RHOX* genes was sequenced with the Ion PGM™ system (Life Technologies).

Results Patients having either SNP 467G > A or SNP 641C > T showed significant lower ejaculate volumes compared to patients lacking the SNPs (p-value ≤ 0.01). Furthermore, carriers of the 641C > T CT genotype had significantly higher numbers of total sperm count and an increased number of sperm with head defects than carriers of the CC genotype (p-value ≤ 0.05). By using the Ion PGM™ system we were able to detect a mosaic of known variations in *RHOXF2/2B* of 10 pooled DNA samples in a quantitative manner. Settings were optimized to get reproducible results in three independent runs and first experiments showed that the pooled sample size can be extended to 20 samples to get reliable data.

Conclusions As not only aberrant methylation of *RHOX* is associated with infertility but also polymorphisms affect distinct clinical parameters of infertile men we suggest that *RHOX* genes play an important role in human spermatogenesis. Both investigated SNPs are located within or close to the N-terminus of the homeodomain indicating that this region is essential for proper function of *RHOXF2/2B*. The X chromosome location of the *RHOX* gene cluster implicates that mutations in these genes will have a complete penetrance in males. For this, the Ion PGM™ system is a powerful tool to sequence the entire coding region of *RHOX* genes and detect variations up to 1 out of 20 samples in a high throughput sequencing approach. Afterwards, the impact of newly identified sequence variations in *RHOX* function and target gene expression can be analyzed by transcriptional profiling.

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P30

Separation of Somatic and Germ Cells is Required to Establish Primate Spermatogonial Cultures

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Introduction Spermatogonial cultures from rodents have become important tools for basic and applied research in reproductive biology. However, results from *in vitro* propagation of human spermatogonia are controversially debated. Since rodents and men exhibit remarkable differences regarding germ cell features, they might require different strategies for the isolation and culture of spermatogonia. Therefore, we used non-human primates, namely marmosets (*Callithrix jacchus*), which more closely resemble the situation in man, for germ cell culture experiments. We aimed at improved culture conditions for primate spermatogonia by analyzing testicular cell cultures systematically.

Methods & Results Testes from 9 adult marmosets were used for primary cell culture. After 24 hours floating cells, which represent 80–90% of the total cell population, were transferred into a new uncoated dish and cultured separately from the attached cells. Immunofluorescence and qPCR after 3, 6 and 11 days revealed low expression levels of somatic markers (*SMA, VIM*) and high expression levels of germ cell markers (*DDX-4, MAGE A-4*) in the separately cultured floating cells. Attached cells exhibited high and increasing levels of somatic marker expression and low levels of *DDX-4* expression. Expression levels of the premeiotic marker *MAGE A-4* were high after 3 days but substantially decreasing up to day 11 in the fraction of expanding attached cells. Analysis of DNA promotor methylation of germ cell marker genes (*DDX-4, MAGE A-4*) and imprinted genes (*H19, MEST*) revealed a germ cell-specific methylation profile for floating cells and a somatic methylation profile for attached cells after 11 days of culture. Germ cell transplantations into germ cell depleted nude mouse testes which were performed after 11 days of culture demonstrated that floating and attached cells contained similar proportions of spermatogonia being able to colonize host testes. However, since the number of floating cells was 8 times higher than the number of attached cells, the absolute number of spermatogonia being able to colonize host testes was higher in the floating fraction.

Conclusion This is the first report revealing an efficient isolation and culture of marmoset spermatogonia able to colonize depleted mouse testes. Our results indicate that separation of spermatogonia from testicular somatic cells is a crucial step during cell preparation. If suspension cultures of spermatogonia are superior to adherent culture systems needs to be evaluated in further studies. Our findings may be highly relevant to establish reliable human spermatogonial cultures which can be used for basic research and for spermatogonia based fertility preservation therapies.

P31

Protamines mRNA Ratio in Stallion Spermatozoa and its Impact on Fertility

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Objectives of the Study Highly compacted sperm DNA in protamine toroids and a minor fraction of nucleohistones are prerequisites for the efficient transmission of the paternal genome into the oocyte at fertilization. It is known that the protamine-1 to protamine-2 protein (P1/P2) ratio varies between different mammalian species, but is constant within a specific species. Aberrant protamine ratios have been demonstrated to be related to male infertility. The objective of the present study was to evaluate whether protamines might serve as a prognostic factor for stallion fertility.

Methods The study was performed on ejaculated sperm from 55 cross-bred stallions

aged 3–18 years. Testes samples were collected by castration from a total 34 horses. For the stage specific expression analysis of mRNA and proteins in testicular tissue, *in situ* hybridisation and immunohistochemistry (IHC) have been applied, respectively. mRNA of protamine 1 (P1) and two variants of protamine 2 (P2 ,P3) has been investigated using quantitative real-time polymerase chain reaction (qPCR). The flow cytometric version of the acridine orange test (FCAOT) has been performed in order to measure of the DNA defragmentation rate. The results were then correlated to ejaculated parameters and mares fecundity defined as None Return Rate 28% (NRR28%) – the percentage of mares which did not return within a period of 28 days after artificial insemination.

Results The specific expression of P1 mRNA has been detected in the cytoplasm of stage I–VII spermatids whilst comparable IHC-stainings showed protein expression was delayed till elongating spermatids in differentiation stages III–VIII. We identified mRNA transcripts of P1 and 2 variants of protamine- 2 (P2, P3) in ejaculated spermatozoa. Based on the NRR28% stallions could be divided into 3 groups: low fertility (20–50%; n = 14), reduced fertility (51–75%; n = 27) and high fertility (76–100%, n = 14). The P2/P1 mRNA ratio was found to be significantly reduced in the group with lower fertility ($p = 0.016$) and was negatively correlated with sperm concentration (correlation coefficient $r = 0.263$). Furthermore, morphologically abnormal sperm count negatively correlated with P2/P1 mRNA ratio, indicating that sperm carrying head defects display a diminished protamine ratio ($r = -0.348$). Conversely, the P2/P1 ratio was positively correlated with mare fertility or NRR28% ($r = 0.274$). Aberrant protamine transcripts content in equine sperm was not associated with DNA defragmentation rate.

Conclusions Based on these results, we suggest that, similar to human, equine protamine expression constituents a checkpoint of spermatogenesis and as a corollary the level of protamine mRNA may reflect the quality of spermatogenesis and sperm's fertilizing capacity.

P32

HMGB1 – A Novel Therapeutic Target in Experimental Autoimmune Orchitis

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High mobility group box protein 1 (HMGB1), the non-histone chromosomal protein, plays an important role in onset and progression of autoimmune diseases once released from the nuclei. In this study, we analyzed how HMGB1 can regulate inflammatory reactions in a rat model of experimental autoimmune orchitis (EAO). HMGB1 was translocated

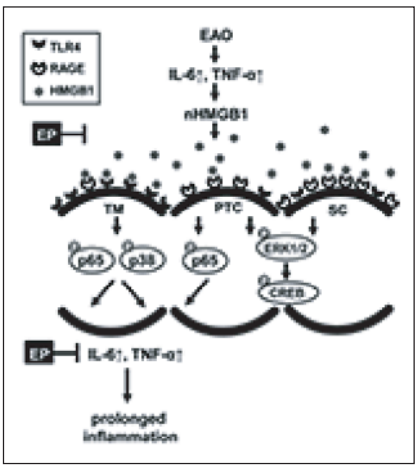

Figure 2. F. Aslani, et al. (P32)

from the nuclei in EAO testis and in the testis of infertile men with leukocytic infiltrates. HMGB1 levels in EAO testis were elevated at the chronic phase of disease compared to early proinflammatory cytokines such as IL-6 and TNF-α. Testicular somatic cells showed a cell-specific expression profile of HMGB1 receptors: Toll-like receptor 4 (TLR4) and receptor for advanced glycation end products (RAGE). HMGB1-TLR4 binding was dominant in testicular macrophages. HMGB1 induced TLR4-signaling in testicular macrophages which was evidenced by p38 MAPK and p65 NF-κB phosphorylation and resulted in an increased expression of TNF-α and IL-6. In contrast, Sertoli cells and peritubular cells showed higher levels of HMGB1-RAGE interaction. HMGB1 triggered RAGE-dependent ERK1/2 MAPK and CREB activation in Sertoli cells and peritubular cells. Treatment of immunized animals with ethyl pyruvate (inhibitor of HMGB1 release) starting 20 days after first immunization successfully reduced disease progression and rescued testicular weight loss and impairment of spermatogenesis. Animals treated with ethyl pyruvate demonstrated significantly lower number of testicular macrophages and lower levels of HMGB1 and IL-6 in the testis. For the first time our results show that inhibiting HMGB1's action even after onset of disease is a promising therapeutical approach to delay progression of the disease and rescue the remaining seminiferous tubules with normal spermatogenesis in experimental autoimmune orchitis (**Fig. 2**).

P33

Role of Activin and Follistatin in Chronic Testicular Inflammation in Mice

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Introduction Experimental autoimmune epididymo-orchitis (EAEO) is a model of chronic testicular inflammation induced in rodents by active immunization with testicular homogenates. It reproduces immunopathological changes seen in human testicular infertility. EAEO is characterized by production of auto-antibodies against testicular antigens, elevated levels of pro-inflammatory cytokines in the testis and infiltration of interstitium by immune cells followed by granuloma formation. The final stage of disease consists of germ cell apoptosis leading to aspermatogenesis.

Material and Methods Activin A is involved in control of inflammation, fibrosis and autoimmunity. In the testis, activin A together with follistatin and inhibin B regulates spermatogenesis and steroidogenesis. Immunoregulatory properties of activin A suggest that it may affect the maintenance of testicular immune privilege. However, the roles of activin A and its binding protein follistatin, in chronic testicular inflammation have been very poorly investigated. The aim of our study was to examine the involvement of activin A and follistatin in EAEO and associated spermatogenetic damage. EAEO was induced in mice by active immunization with testicular homogenates in complete Freund's adjuvant (CFA). Adjuvant control animals were treated with saline instead of testicular homogenate in CFA. Testes were collected 50 days after the first immunization.

Results As it has been shown that activin A and mast cells play a key role in inducing fibrosis, azan (azo-carmine and aniline blue) staining to stain the collagen fibers and mast cell staining by toluidine blue O was performed in EAEO, adjuvant control and untreated mice testes. We observed a strong fibrotic response in EAEO testis accompanied by an increase of mast cells numbers (especially in the severe form of the disease) compared to controls. Moreover, activin bA subunit staining on paraffin sections showed a clear downregulation of activin A in severe EAEO compared to controls. Quantitative RT-PCR analysis indicated a reduction in activin bA subunit expression. At the level of pro-inflammatory cytokines, TNFα mRNA expression showed a significant increase in severe EAEO. Inhibin a subunit mRNA was also decreased in severe EAEO, but no significant changes in activin bB subunit mRNA expression were observed. In contrast, total follistatin mRNA expression was significantly (3 times) upregulated in low grade EAEO testis compared to controls.

Conclusions Our preliminary results demonstrate a direct link between EAEO progression and activin A expression. Reduction in the activin A and inhibin expression in the severe phase of disease may be a result of germ cell and Sertoli cell loss and testicular damage. Blockade of activin A action by using follistatin during the early stage of testicular inflammation may serve as a therapeutic tool to reduce damage to spermatogenesis.

P34

Androgen- and Estrogen-Dependent Regulation of cGMP Pathway Components in Human Prostate Smooth Muscle Cells

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Background Our previous data showed that expression of cGMP pathway components is very low in rat prostate compared to other androgen-dependent organs [Müller et al., 2011]. This data is of special interest, since most recently inhibitors of the cGMP-hydrolyzing enzyme phospodiesterase 5 (PDE-5) were suggested for treatment of benign prostate hyperplasia. But the detailed localization of cGMP-related enzymes and the mechanisms of this medication in the prostate is still a matter of debate [Lin et al., 2013].

Methods Using Western blots, flow cytometry, and immunohistochemical staining, we characterized primary smooth muscle cells (SMCs) of the human prostate (HPrSMCs) as well as tissue samples, followed by treatment of HPrSMCs with androgen and estrogen in a dose dependent manner. Furthermore, we analyzed the prostate tissue of EDS-rat, aromatase-knockout (estrogen deprivation/androgen upregulation), and overexpressing (estrogen down-regulation/androgen up-regulation) mouse models [Ellem et al., 2009].

Results In human prostate tissue, PDE-5 was highly expressed in the interstitial tissue, particularly in smooth muscle cells, but not in epithelial cells of the gland. In androgen deprivation models, an increase of PDE-5, correlating with an increase of SMCs surrounding disturbed glands, was observed. Especially, in these glands, proliferative activity in epithelial glands was missing different to regular glands of the same section also observed. Preliminary results of treatment of HPrSMCs with hormones and analyses of corresponding AROM+/ARKO mouse prostate tissue showed androgen (5-DHT)- dependent down-regulation and estrogen- (β-estradiol-) dependent up-regulation of cGMP pathway components (PDE-5 and PKG-I) in a dose-dependent manner.

Conclusion This work demonstrates for the first time a direct androgen-dependent down-regulation and estrogen-dependent up-regulation of PDE-5 in human HPrSMCs as well as in corresponding animal models. These should be important results for use of PDE-5 inhibitors as a possible block buster for therapy of benign brostate hyperplasia (BPH)

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P35

Influence of Testosterone on LPS Induced acute Inflammatory Response in Sertoli Cells

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Introduction Androgens in addition to their spermatogenic function also have a role in the modulation of autoimmune disease and contribute to suppression of inflammatory/autoimmune responses. Previously, we have shown that substitution of reduced testosterone levels in a rat model of chronic testicular inflammation has led to a significant amelioration of disease characteristics by inhibition of inflammatory responses in the testes. Subsequent *in vitro* studies demonstrated a direct influence of testosterone on lipopolysaccharide (LPS) induced inflammatory response in Sertoli cells (SC), which are important contributors to immunological balance in the testis. Pre-incubation of SC with increasing concentrations of testosterone led to inhibition of LPS-induced TNF-α mRNA expression. These findings prompted us to perform a broad transcriptomic analysis of LPS stimulated SC pretreated for 24h with testosterone using microarray analysis.

Material and Methods Overall 1300 genes were found to show a differential expression pattern in LPS challenged SC with/without testosterone pretreatment. Several putative candidate genes showing an effect of testosterone on the LPS response were identified and selected for further functional analysis. One promising example is CD83, a dendritic cell marker, previously unrecognized to be expressed in SC. Preliminary results show a time-dependent significant induction of CD83 following LPS stimulation, which was diminished after testosterone pretreatment. Addition of the androgen antagonist flutamid abolished the testosterone effect. Interestingly, the soluble form of CD83 is known to suppress T-cell immune responses [1].

Conclusion Taken together, our study identified a new gene in SC that could be involved in maintaining the delicate balance between immune privilege and ability to mount inflammatory responses in the testis.

Reference:

1. Chen L, et al. CD83-stimulated monocytes suppress T-cell immune responses through production of prostaglandin E2. Proc Natl Acad Sci U. S. A. 2011; 108: 18778–83.

Endokrinologie und Hypogonadismus

P36

Ein miR-375-Knockdown inhibiert die adipogene Differenzierung humaner SGBS-Präadipozyten

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Fragestellung Late-onset-Hypogonadismus (LOH) geht häufig mit einer Vermehrung des viszeralen Fettgewebes und eine Verminderung der Insulinsensitivität einher. Molekulare Studien unserer Arbeitsgruppe konnten in humanen SGBS-Präadipozyten zeigen, dass die miR-375 verstärkt und ADIPOR2 (Adiponektinrezeptor 2) als Zielgen der miR-375 vermindert während der Adipogenese exprimiert werden. Eine niedrige ADIPOR2-Expression wird in der Literatur als mit einer Insulinresistenz bzw. Übergewicht assoziiert beschrieben. Durch Zugabe von Testosteron bzw. Dihydrotestosteron wurde eine Inhibierung der Adipogenese und eine Verminderung der miR-375-Expression bzw. Erhöhung der ADIPOR2-Expression erzielt. Daher wurden miR-375-Knockdown-Versuche durchgeführt um zu eruieren, ob die durch Androgene verursachte Reduktion der miR-375-Expression Ursache oder Nebenerscheinung der inhibierenden Wirkung von Androgenen auf die Adipogenese ist und dies mit einer Erhöhung der ADIPOR2-Expression einhergeht.

Methodik Humane SGBS-Präadipozyten wurden kultiviert und der adipogenen Differenzierung über 14 Tage ausgesetzt. In den Zellen wurde ein miR-375-Knockdown mittels Transfektion von miR-375-Inhibitoren (100 nM) induziert. qRT-PCR- bzw. Western-Blot-Analysen dienten der Darstellung der ADIPOR2 mRNA- bzw. Proteinexpressionsprofile. Parallel dazu wurden die differenzierten reifen Adipozyten ± Transfektion mit miR-Inhibitoren mit dem Farbstoff Oil Red O gefärbt.

Ergebnisse Knockdown-Versuche mittels miR-375-Inhibitoren zeigten nach Oil-Red-O-Färbung eine Inhibierung der adipogenen Differenzierung, fotographisch dargestellt durch eine stark verminderte Anzahl an reifen Adipozyten im Vergleich zu rein adipogen differenzierten Zellen. qRT-PCR-Analysen zeigten nach miR-375-Knockdown im Verlauf der adipogenen Differenzierung ein deutlich erhöhtes ADIPOR2 mRNA-Expressionsprofil im Vergleich zu den differenzierten Adipozyten (miR-375-Knockdown). Western-Blot-Analysen bestätigten diese Effekte auf Proteinebene.

Schlussfolgerung Die erzielten Daten zeigen eine Inhibierung der Adipogenese, einhergehend mit einer erhöhten ADIPOR2 mRNA- bzw. Proteinexpression durch einen induzierten Knockdown der miR-375 mittels spezifischer miR-Inhibitoren, vergleichbar mit der von uns früher nachgewiesenen Reduktion der miR-375-Expression unter Testo-

steron-Einfluss. Der postulierte Testosteron-supprimierte miR-375-ADIPOR2-Regelkreis im Rahmen der Adipogenese ist im Zusammenhang mit der Zunahme der viszeralen Fettmasse und Insulinresistenz beim LOH ein interessanter Ansatzpunkt für die weitere Analyse von molekularen Mechanismen in Bezug auf die verstärkte Adipogenese im Krankheitsbild des Late-onset-Hypogonadismus.

P37

Temperatures in the Devitrification Process are Essential for Preserved Human Sperm Function

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Introduction Sperm vitrification is a method of cryopreservation based on high speed freezing by direct exposure of the cell in liquid N_2, which allows avoiding the traditional cooling curves of freezing. The objective of this work was to determine the optimal warming temperature for vitrified human spermatozoa in order to maintain their fertilization potential.

Material and Methods Donors spermatozoa were cryopreserved by direct plunging into N_2L and warmed at different temperatures per 5 seconds at 38°, 40° and 42°C. The following parameters as: progressive motility (CASA), plasma membrane integrity (SYBR-14/PI; flow cytometry), an function (HOST test), oxidation of thiol groups (monobromobimane, confocal laser microscopy), lipid peroxidation (TBARS, spectrophotometer), HOST Test, sperm morphology (electron microscopy) were evaluated. For statistical analysis, the nonparametric ANOVA (Kruskal-Wallis), with a significance level of 0.05 and the multiple comparison test of Dunn to establish differences between groups were used.

Results It was detected that progressive motility of warmed at 38°C, 40°C and 42°C spermatozoa was: 26.4 ± 8.4; 56.6 ± 16.3 and 65.4 ± 15, respectively, with statistical difference between the temperatures of 38° and 40°C and 38° and 42°C ($p < 0.05$). It was shown that the warming temperatures 40°C (49.8 ± 14.8) and 42°C (45.2 ± 12.6) significantly ($p < 0.05$) protected of the spermatozoa plasma membrane compare to warming at 38°C (19 ± 13.9). The plasma membrane function evaluated by HOST test was better preserved at 42ºC (76.3 ± 2.0) compared to 40ºC (43 ± 2) and 38ºC (65.6 ± 1.5). It was not established effect of all warming temperature on the protection of cell membrane against of oxidation of thiol groups (38°C [50.6 ± 9.7] 40°C [48.7 ± 15.3]; 42°C [50.3 ± 12.4]). It was shown that warming temperature significantly protect the cytoplasmic membranes against of lipid peroxidation: immediately after warming the present lipid absorbance was significantly higher at 40°C (0.018 A) and 42°C (0.022 A) compare to warming at 38°C (0.082 A) and after 2 hours of in vitro culture the lowest significant sign of present lipid absorbance had the spermatozoa warmed at 42°C (0.011 A) compare to warming at 38°C (0.076 A) and at 40°C (0.068 A). Sperm morphology showed after warming at 42°C showed more stable and regular ($p < 0.05$) compare to warming at 38°C.

Conclusion The results demonstrate that the warming temperature can generate the morphological and biochemical changes which can affect the motility, plasma membrane integrity and function, lipid peroxidation (TBARS) and sperm morphology.The warming of the vitrified sperm at 42 ° C is the optimum temperature for preservation the sperm physiological parameters.

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P38

Prävalenz von Hypogonadismus bei Männern mit lokalisiertem und metastasiertem Nierenzellkarzinom

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Einleitung Der gonadale Hypogonadismus (GHG) bei Männern > 60 Jahren wird mit einer Prävalenz von etwa 20 % angegeben. Die klinischen Symptome des GHG sind denen sehr ähnlich, die bei Patienten mit lokalisiertem (loRCC) und vor allem metastasiertem (mRCC) Nierenzellkarzinom (RCC) gefunden werden. Patienten mit klinisch manifestem GHG bei RCC könnten von einer Testosteron-Substitutionstherapie (TST) profitieren. Ziel dieser Studie war es, die Prävalenz des Hypogonadismus bei Patienten mit loRCC und mRCC in unserem Zentrum zu untersuchen.

Material und Methoden GHG wurde durch Messung der morgendlichen Gesamt-Testosteron-Konzentrationen im Serum an zwei verschiedenen Tagen festgestellt. Für die Berechnung des freien Testosterons im Serum wurde die Vermeulen-Formel verwendet. Zusätzlich wurden die klinischen Symptome des GHG unter Verwendung von standardisierten Fragebögen zur Beurteilung der sexuellen Funktion (IIEF-5, ADAM) und der Lebensqualität (QLQ, SF-36) dokumentiert sowie das Hodenvolumen, BMI, Hip-to-waist-Ratio und verschiedene Hormon- und Stoffwechsel-Serumwerte untersucht. Insgesamt wurden 51 mRCC-Patienten unter „targeted therapy“ und 30 loRCC-Patienten in diese Studie eingeschlossen.

Ergebnisse Die Prävalenz eines biochemischen Hypogonadismus betrug 19 % und 18 % für die loRCC- und mRCC-Kohorte (p = 0,84), wohingegen sich klinische Symptome in 63 % und 93 % zeigten (p = 0,01). Die morgendlichen Gesamt-Testosteronkonzentrationen im Serum wie auch die berechneten freien Testosteronkonzentrationen zeigten keinen signifikanten Unterschied bei Patienten mit loRCC und mRCC (T [79] = 1,52; p = 0,13 und T [42] = 1,13; p = 0,26). Jedoch wurde ein signifikanter Unterschied für die klinischen Symptome, einschließlich ADAM-Score (p = 0,02), IIEF-5 (p = 0,02) und Hodenvolumen (p = 0,01) gefunden, bei BMI und Hip-to-waist-Ratio fand sich dagegen kein signifikanter Unterschied.

Diskussion Klinische Symptome des Hypogonadismus finden sich in einer signifikanten Zahl von RCC-Patienten. Sie treten signifikant häufiger bei mRCC-Patienten auf. Diese Symptome korrelieren jedoch nicht mit den klinisch verwendeten Serumtestosteron „Cut-off“-Werten. Weitere Studien mit größeren mRCC-Kohorten sind zur Beurteilung der Wirksamkeit und Lebensqualitätverbesserung einer TST notwendig.

P39

Inzidenz von Prostata-Karzinom (PCa) bei 340 hypogonadalen Männern unter Langzeitbehandlung mit Testosteron-Undecanoat-Injektionen (TU) bis zu 7 Jahren – Beobachtungsdaten einer Register-Studie

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Hintergrund und Fragestellung Es gibt nur wenige Langzeitdaten zur Sicherheit der Testosteronersatztherapie für die Prostata. Hier untersuchen wir Prostata-Parameter inkl. der Inzidenz von PCa bei hypogonadalen Patienten unter Langzeitbehandlung mit Testosteron.

Material und Methoden In einer prospektiven, kumulativen Registerstudie erhielten 340 Männer (Alter: 57,37 ± 7,03 Jahre) mit Testosteron ≤ 12,1 nmol/l bis zu 7 Jahre lang TU 1000 mg alle 12 Wochen nach einem Anfangsintervall von 6 Wochen. Prostata-Volumen (PV), PSA und DRE/TRUS wurden vor Behandlungsbeginn und danach regelmäßig alle 3–6 Monate durchgeführt. Bei Verdacht auf PCa wurden Biopsien durchgeführt.

Ergebnisse PV stieg von 28,96 ± 10,41 auf 29,88 ±1 3,85 ml um Modell-bereinigte 2,59 ± 0,2 ml ($p < 0,0001$). Der Anstieg war während der ersten vier Jahre jeweils signifikant zum Vorjahr. PSA stieg von 1,74 ± 0,94 auf 1,96 ± 1,03 ng/ml um Modell-bereinigte 0,23 ± 0,52 ng/ml ($p < 0,0001$). 53-mal wurden bei Testosteron-behandelten Patienten Biopsien durchgeführt. Davon waren 5 (9,4 %) positiv und 48 (90,6 %) negativ. Der Anteil von PCa bei den Testosteron-behandelten Patienten in der Registerstudie betrug 1,5 % mit einer Inzidenz von 30,7 pro 10.000 Patientenjahre.

Bei hypogonadalen Patienten ohne Testosteron-Therapie wurden 314 Biopsien durchgeführt, von denen 111 (35,4 %) positiv und 203 (64,6 %) negativ waren. Bei eugonadalen Patienten wurden 584 Biopsien durchgeführt, von denen 263 (40,4 %) positiv und 321 (55 %) negativ waren. Insgesamt wurden in der Praxis von 2004–2013 898 Prostata-Biopsien durchgeführt, von denen 379 (42,2 %) positiv und 519 (57,8 %) negativ waren.

Schlussfolgerungen Die Langzeit-Therapie mit Testosteron bei hypogonadalen Männern bei regelmäßigem Monitoring erhöht nicht die Inzidenz von PCa.

P40

Veränderungen von Gewicht und Prostata-Parametern bei 561 hypogonadalen Männern unter Langzeitbehandlung mit Testosteron-Undecanoat-Injektionen (TU) bis zu sechs Jahren sind unabhängig vom Alter – Beobachtungsdaten von zwei Registerstudien

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Hintergrund und Fragestellung Es gibt wenige Daten zur Langzeitbehandlung von hypogonadalen Männern mittleren und höheren Alters mit Testosteron (T). Wir untersuchen die Effekte der T-Behandlung auf Gewicht und Prostata-Parameter bei hypogonadalen Männern bis zu 65 Jahren und älter.

Material und Methoden 561 hypogonadale Männer zweier Registerstudien wurden in Altersgruppen unterteilt: Gruppe A (n = 450, Alter ≤ 65 Jahre; Minimum: 32, Maximum: 65 Jahre) und Gruppe B (n = 111, Alter > 65 Jahre; Mimimum: 66, Maximum: 84 Jahre). Nach einem initialen 6-Wochen-Intervall wurden alle Männer mit TU in dreimonatlichen Abständen bis zu sechs Jahre lang behandelt.

Ergebnisse Gruppe A: Das Gewicht fiel um Modell-bereinigte 14,78 kg, der Bauchumfang um 9,34 cm. Das Prostatavolumen (PV) stieg von 26,77 auf 31,58 ml ($p < 0,0001$) mit einer Modell-bereinigten Vergrößerung im Vergleich zum Ausgangswert um 4,02 ml. Prostata-spezifisches Antigen (PSA) stieg von 1,33 auf 1,65 ng/ml (Modell-bereinigt +0,36). Das Restharnvolumen fiel von 34,87 auf 16,72 ml (Modell-bereinigt –22,17). Der International-Prostate-Symptom-Score (IPSS) fiel von 7,74 auf 3,89 (Modell-bereinigt –4,4). Bei sechs Patienten wurde ein Prostata-Ca diagnostiziert, der jüngste mit 51 Jahren bei Therapiebeginn hatte ein Klinefelter-Syndrom.

Gruppe B: Das Gewicht fiel um Modell-bereinigte 15,14 kg, der Bauchumfang um 10,45 cm. PV stieg von 33,85 auf 39,95 ml ($p < 0,0001$) mit einer Vergrößerung im Vergleich zum Ausgangswert um 3,37 ml. PSA stieg von 1,44 auf 1,88 ng/ml (+0,38). Das Restharnvolumen fiel von 41,46 auf 18,84 ml (–27,25). Der IPSS fiel von 10,7 auf 4,63 (–5,89). Bei 5 Patienten wurde ein Prostata-Ca diagnostiziert.

Schlussfolgerungen T-Therapie bei hypogonadalen Männern zeigte vergleichbare Effekte auf Prostata-Parameter bei Männern bis zu 65 Jahren und älter als 65 Jahre.

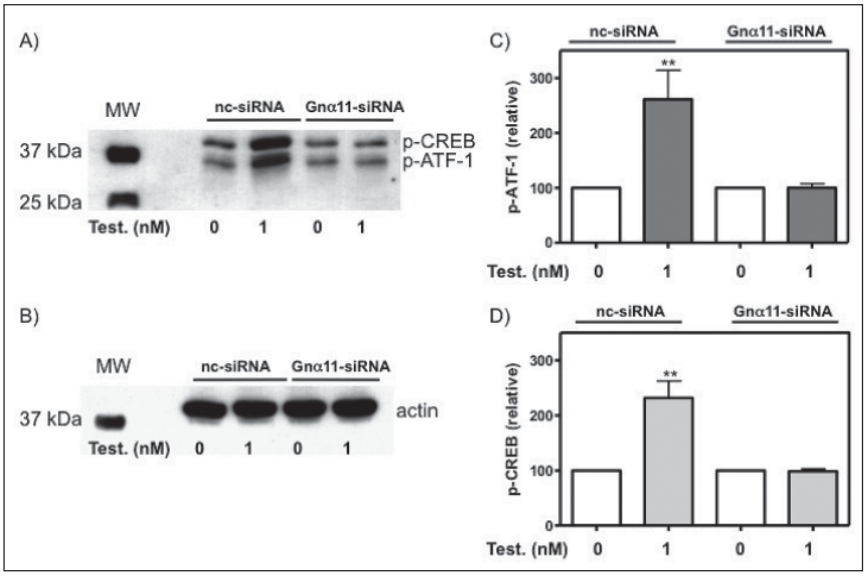

Figure 3. M. Shihan, et al (P41). Western blot analysis of p-ATF-1 and p-CREB after silencing Gnα11 expression by siRNA. **(a):** Testosterone stimulates p-CREB and p-ATF-1 formation in cells treated with negative control (nc)-siRNA but not in cells that had received Gnα11-siRNA. **(b):** Expression of total actin was not affected by either type of siRNA or testosterone. (C and D). Data in **(c)** and **(d)** were corrected for the amount of total actin (n = 3; means ± SEM; ** = $p \leq 0.01$)

P41

Testosterone-induced Signaling Cascades Mediated through a G-Protein Coupled Receptor

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The androgen testosterone mediates its effects by two different ways of action: In the so-called "classical" pathway testosterone binds to cytosolic androgen receptors (AR), which essentially function as ligand-activated transcription factors. Once activated, these receptors bind to DNA and activate the expression of target genes. In the so-called "non-classical" pathway, the steroid hormone bind to receptors associated with the plasma membrane and induces signaling cascades mediated through activation of Erk1/2. The nature of the membrane associated AR remains, however, controversial. Next to the assumption that the membrane and cytosolic AR are identical, there is strong evidence that the AR of the membrane is a G-protein coupled receptor (GPCR). To evaluate either of the two possibilities we first searched for testosterone-induced signaling cascades in the spermatogenic cell line GC-2. We identified a testosterone-induced stimulation of Erk1/2, CREB and ATF-1 phosphorylation, equivalent to the already described non-classical action of testosterone. Silencing of AR expression by means of siRNA did not influence at all the androgen-induced activation of Erk1/2, CREB or ATF-1. In contrast, suppression of Gnα11 expression by siRNA abolished the testosterone-induced activation of Erk1/2, CREB and ATF-1 (**Fig. 3**), suggesting that the non-classical testosterone-induced signaling is not due to the interaction of the steroid with AR but rather with GPCR. Taking into consideration data obtained with various steroid hormones by others, one might assume that non-classical signaling pathways of steroid hormones are in general mediated through GPCRs.

Grants: Supported through DFG, SCHE 307/7-1

P42

Dehydroepiandrosterone Sulphate-induced Signaling events in Spermatogenic cell line GC-2 are mediated via a G-Protein Coupled Receptor interacting with Gnα11

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Dehydroepiandrosterone sulfate (DHEAS) is a circulating steroid produced in the adrenal cortex, brain, and gonads. Whereas a series of investigations attest to neuroprotective effects of the steroid in the brain, surprisingly little is known about the physiological effects of DHEAS on cells of the reproductive system. Here we demonstrate that DHEAS acting on the spermatogenic cell line GC-2 induces a time- and concentration-dependent

phosphorylation of c-Src and Erk1/2 and activates the transcription factors ATF-1 and CREB. These actions are consistent with the non-classical signaling pathway of testosterone and suggest that DHEAS is a pro-androgen that is converted into testosterone in order to exert its biological activity. The fact, however, that steroid sulfatase mRNA was not detected in the GC-2 cells and the clear demonstration of DHEAS-induced activation of Erk1/2, ATF-1 and CREB after silencing the androgen receptor by siRNA clearly contradict this assumption and make it appear unlikely that DHEAS has to be converted in the cytosol into a different steroid in order to activate the kinases and transcription factors mentioned. Instead, it is likely that the DHEAS-induced signaling is mediated through the interaction of the steroid with a membrane-bound G-protein-coupled receptor, since silencing of Gnα11 leads to the abolition of the DHEAS-induced stimulation of Erk1/2, ATF-1, and CREB. The investigation presented here shows a hormone-like activity of DHEAS on a spermatogenic cell line. Since DHEAS is produced in male and female reproductive organs, these findings might help to define new roles for DHEAS in the physiology of reproduction (**Fig. 4**).

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P43

Dehydroepiandrosterone Sulfate Stimulates Activation of Transcription Factors CREB and ATF-1 in Sertoli Cells

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Introdution Dehydroepiandrosterone (DHEA) is produced mainly by the adrenal zona reticularis and is almost entirely converted by the enzyme sulfotransferase to dehydroepiandrosterone sulfate (DHEAS), which is then secreted into the serum. DHEAS is the most abundant circulating steroid: its concentration in plasma is between 1.3 and 6.8 µM, which is approximately 200-fold higher than the plasma concentrations of DHEA (7–31 nM).

Material and Methods While sulfated steroids have long been considered to be biologically inactive waste products of steroid hormone metabolism, the discovery of cytosolic steroid sulfatase prompted the new idea that the sulfates constitute a reservoir that upon desulfation can deliver precursors for steroid hormone synthesis. Thus, DHEAS has been viewed as a pro-androgen that, after being transported into cells, becomes desulfated by steroid sulfatase to DHEA and further converted into testosterone or other steroid hormones in order to exert its biological activity. DHEA and DHEAS are also produced in brain and gonads. With respect to the brain their biological function is considered to be neuroprotection. Little is known about the action of these steroids on cells of the reproductive system.

Conclusion Recent results from our laboratory attest to DHEAS itself having a hormone-like function on the spermatogenic cell line GC-2. By interacting with a G-protein-coupled receptor, DHEAS induces signaling events that may be of physiological significance [1]. In order to broaden our knowledge regarding DHEAS-specific effects on other cells of the reproductive system we investigated DHEAS-specific signaling in the Sertoli cell line 93RS2. Incubation of these cells with nanomolar concentrations of the steroid leads to Erk1/2 activation and stimulation of the transcription factors CREB and ATF-1 within the nucleus. The physiological significance of the DHEAS-induced activation of the transcription factors, demonstrated for the first time in a Sertoli cell line, is currently under investigation.

Reference:

1. Shihan M, et al. Dehydroepiandrosterone sulfate mediates activation of transcription factors CREB and ATF-1 via a Ga11-coupled receptor in the spermatogenic cell line GC-2. Biochim Biophys Acta 2013; 1833: 3064–75.

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Erektile Dysfunktion und Sexualstörungen, Onkologische Andrologie und Fertilitätsprotektion

P44

Die langfristigen Ergebnisse für Patienten, die nach einer radikalen Prostatektomie mittels Schwellkörperautoinjektionstherapie behandelt wurden

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Einleitung Oftmals wird Patienten nach radikaler Prostatektomie (RP) ein sexuelles Rehabilitationsprogramm (SR) vorgeschrieben. Die langfristigen Ergebnisse sind jedoch selten bekannt.

Fragestellung Ziel dieser Arbeit ist es, die langfristigen Ergebnisse von Patienten in einem SR zu untersuchen und festzustellen, welche Patienten- und Therapiefaktoren dazu führen, dass Patienten nach sechs Jahren noch Geschlechtsverkehr ausüben.

Methoden 62 Patienten mit normaler präoperativer erektiler Funktion, die nach RP in einem SR waren, wurden nach einem Jahr und nochmals nach sechs Jahren mittels Fragebögen untersucht. Die anfängliche Therapie nach RP war immer mittels Schwellkörperautoinjektionstherapie (SKAT). Nach sechs Jahren wurde festgestellt, ob Patienten noch Geschlechtsverkehr ausübten und welche Behandlung sie derzeitig für Erektile Dysfunktion bekamen. Nach sechs Jahren wurden auch der International Index of Erectile Function (IIEF), der Erection Hardness Scale (EHS), der Index of Sexual Life (ISL) und die Schmerzen bei SKAT nach einem Jahr zwischen noch sexuell aktiven Patienten und nicht mehr sexuell aktiven Patienten verglichen. Es wurde auch festgestellt, ob IIEF und EHS bei noch sexuell aktiven Patienten zwischen dem ersten und sechsten Jahr abnahmen.

Ergebnisse Die 34 Patienten, die nach sechs Jahren noch Geschlechtsverkehr ausübten, hatten keine Reduzierung im EHS (mit und ohne Therapie) und auch keine Reduzierung im IIEF nach sechs Jahren (17,3 im ersten Jahr und 22 im sechsten Jahr). Patienten, die sechs Jahre lang exklusiv SKAT angewendet hatten, zeigten jedoch eine statistisch signifikante Abnahme im EHS ohne Therapie. Faktoren, die dazu führten, dass ein Patient nach sechs Jahren noch sexuell aktiv war, waren sowohl ein jüngeres Alter als auch ein höherer Wert im IIEF-Sexuelles-Verlangen-Score ein Jahr nach RP.

Schlussfolgerung Jüngere Patienten, die ein Jahr nach RP auch einen höheren IIEF-Sexuelles-Verlangen-Score haben, neigen eher dazu, nach sechs Jahren noch Geschlechtsverkehr auszuüben. Bei Patienten, die nach sechs Jahren noch sexuell aktiv sind, ist keine Reduzierung im IIEF und EHS zu sehen, unabhängig von der aktuellen Therapie.

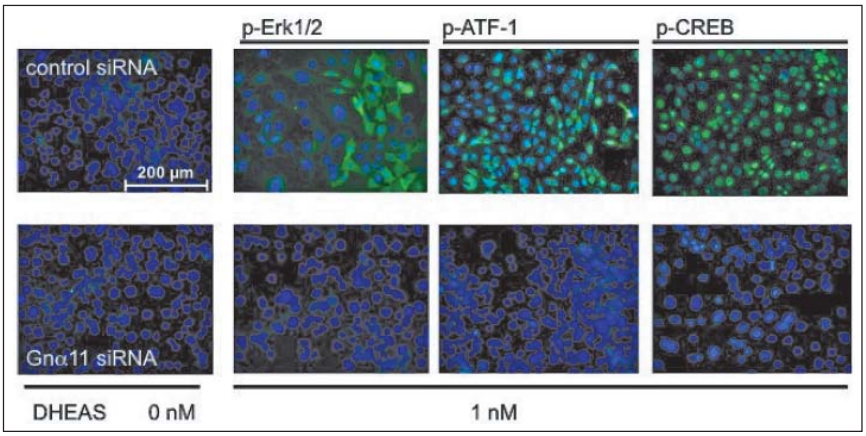

Figure 4. M. Shihan, et al (P42). Activation of Erk1/2, ATF-1 and CREB by DHEAS in the presence or absence of Gna11. While treatment of the cells with control siRNA is without any effect on the DHEAS-induced activation of Erk1/2, ATF-1 or CREB, silencing Gnα11 expression by siRNA completely abolishes the activation of all three factors by the steroid.

P45

Sollte Schwellkörperautoinjektionstherapie als sexuelle Rehabilitation länger als ein Jahr nach radikaler Prostatektomie fortgeführt werden? Eine französische Erfahrung

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Fragestellung Ziel dieser Arbeit ist es, festzustellen, ob die Fortführung einer Schwellkörperautoinjektionstherapie (SKAT) als Teil der sexuellen Rehabilitation für Patienten, bei denen PDE5-Inhibitoren nach einer radikalen Prostatektomie erfolglos waren, wirksam ist.

Methoden Bei 40 Männer mit normaler präoperativer erektiler Funktion wurde eine nerverhaltende, laparoskopische, radikale Prostatektomie durchgeführt, danach wurden sie als Teil ihrer sexuellen Rehabilitation 12 Monate lang mit SKAT behandelt. PDE5-Inhibitoren hatten 12 Monate postoperativ keine Wirkung. SKAT wurde für weitere 12 Monate fortgesetzt und die sexuelle Funktion wurde mittels des IIEF-15 und des „Erection Hardness Score (EHS)" festgestellt. Weiterhin wurde der Patienteneindruck des Schmerzes während der Behandlung ausgewertet. Die statistische Analyse wurde nach 12 und 24 Monaten durchgeführt.

Ergebnisse Kein statistisch signifikanter Unterschied konnte nach 12 und 24 Monaten mittels des IIEF-15 festgestellt werden – dieses galt sowie mit (19,9 ± 10,06 vs. 18,65 ± 10,73) als auch ohne Therapie (4,53 ± 2,93 vs. 4,98 ± 4,35). Die Geschlechtsverkehrsbefriedigung (7,33 ± 4,23 vs. 6,88 ± 4,23), das sexuelle Verlangen (6,10 ± 2,28 vs. 6,20 ± 2,04) und der EHS-Score mit (3,05 ± 1,22 vs. 2,86 ± 1,09) und ohne Therapie (0,58 ± 1,05 vs. 0,83 ± 0,88) waren unverändert. Patientenzufriedenheit nahm mit der Zeit ab (p = 0,03). Patienten berichteten eine subjektive Verbesserung der erektilen Funktion in 42,5 % der Fälle, eine Abnahme der Funktion in 32,5 % der Fälle und in 25 % der Fälle war die Funktion zwischen 12 und 24 Monaten unverändert. Patienten mit einem höheren IIEF-15 nach 12 Monaten zeigten keine Zunahme in spontanen Erektionen nach 24 Monaten.

Schlussfolgerung Der Effekt der SKAT als Teil des sexuellen Rehabilitationsprogramms ist zwischen 12 und 24 Monaten unverändert, es kann keine Zunahme in spontanen Erektionen mit einer Verlängerung der Therapie festgestellt werden. Wenn SKAT nach 12 Monaten erfolglos ist oder Patientenunzufriedenheit besteht, sollte daher eine andere Therapie angeboten werden.

P46

Ändert sich die operative Vorgehensweise zur Therapie des Peniskarzinoms durch die neuen EAU-Leitlinien?

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Einleitung Während bis 2009 topische Therapien für oberflächliche Karzinome und Penisteilamputationen für invasive Karzinome die gängigen Therapien des Primärtumors darstellten, sind entsprechend der neuen EAU-Leitlinien die Indikationen zur Glansrekonstruktion bis auf pT2-Tumoren ausgedehnt worden. Bei steigender Inzidenz des Peniskarzinoms sollen die Auswirkungen auf die eigene Vorgehensweise dargestellt werden.

Material und Methode Von 2009–2013 wurden 31 Patienten mit einem Peniskarzinom zur operativen Therapie eingewiesen. Bei 12 Patienten mit pTis-pT2-Tumoren wurde, unterstützt von Schnellschnittuntersuchungen, eine Glansresektion und Glansrekonstruktion mittels Spalthautplastik durchgeführt. Die Empfehlungen der EAU-Leitlinie zur Reduzierung der Sicherheitsabstände wurden umgesetzt. Bei vier Patienten wurde nach tiefer Penisteil- oder -totalamputation eine Rekonstruktion des Penis mit mikrochirurgisch transplantiertem Radialislappen und späterer Prothesenimplantation vorgenommen. Die Indikation zur inguinalen Lymphadenektomie wurde entsprechend der EAU-Leitlinien gestellt.

Ergebnisse Alle Glansrekonstruktionen mit Spalthaut führten ohne Revisionseingriffe zu einem guten funktionellen und ästhetischen Ergebnis. Drei von vier penilen Rekonstruktionen aus Radialislappen konnten erfolgreich mit hydraulisch peniler Prothetik versorgt werden. Ein Rezidiv konnte sieben Jahre nach Glansresektion registriert werden. Ein Patient entwickelte eine postoperative Meatusstenose.

Schlussfolgerungen Der steigenden Inzidenz des Peniskarzinoms und der zunehmenden Lebenserwartung kommt der Trend zu organerhaltenden und rekonstruktiven Operationstechniken entgegen. Jedoch ist bei gleichzeitiger Reduzierung der Sicherheitsabstände mit höheren Rezidivraten aus der aktuellen Literatur zu rechnen. Dem soll mit einem engmaschigen Follow-up-Programm und der Registrierung in regionalen Krebsregistern Rechnung getragen werden.

P47

Sexualität in Partnerschaften fünf Jahre nach offener „nerve-sparing"-radikaler Prostatektomie

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Fragestellung Es wurde die sexuelle Aktivität und Funktion sowie die Zufriedenheit mit dem Sexualleben sowohl der Patienten als auch deren Partnerinnen fünf Jahre nach Prostatektomie erfasst.

Methoden 36 Paare, die ein Jahr nach offener „nerve-sparing"-radikaler Prostatektomie Geschlechtsverkehr (GV) hatten, wurden mittels IIEF und Female Sexual Function Score (FSFI) zu deren sexueller Funktion und GV-Häufigkeit präoperativ, ein und fünf Jahre nach der OP befragt. Zusätzlich wurde die Zufriedenheit mit dem Sexualleben mittels 10-Punkte-Skala und die verwendeten Hilfsmittel (HM: PDE-5-Inhibitoren, SKAT, MUSE®, Vakuumerektionshilfe) evaluiert.

Ergebnisse Die Rücklaufquote lag bei 83,3 %. Zum Zeitpunkt der Befragung waren die Patienten im Median 67,7 (Range: 51,6–78,3) und deren Partnerinnen 60,5 (45–76) Jahre alt. Fünf Jahre postoperativ hatten 70,0 % der Paare GV, im Durchschnitt 4,6 ×/Monat (1–12). 9/21 Paaren hatten GV ohne HM, 10 Paare mit PDE-5-Inhibitoren (davon jeweils eines in Kombination mit SKAT bzw. Vakuumerektionshilfe), eines mit MUSE® und eines nur mittels Vakuumerektionshilfe. Paare, die nicht auf HM angewiesen waren, hatten seltener GV als Paare, die HM verwendeten (3,8 ×/Monat [2–7] vs. 5,3 ×/Monat [2–12]). Der IIEF-6-Score lag bei den Patienten, welche GV hatten, durchschnittlich bei 21,6 (7–30) und bei den Patienten, welche keinen GV hatten, bei 5,0 (1–8). In der Gruppe der Paare, die keine HM verwendeten, lag der IIEF-6-Score etwas niedriger (20,4 [7–28]) als bei den Paaren, die HM verwendeten (22,3 [9–30]). Die durchschnittlichen FSFI-Scores für sexuelle Erregung, Orgasmus und Befriedigung lagen bei den Frauen, die ohne HM mit ihrem Partner GV hatten, höher (5,3/5,4/5,5) als bei den Frauen, deren Partner HM verwendeten (5,2/4,7/4,5). Die Zufriedenheit mit dem Sexualleben im Allgemeinen lag bei den Paaren mit GV bei den Männern (6,2 von 10) und Frauen (7,1) höher als bei den Paaren ohne GV (Mann 3,4/Frau 6,1).

Schlussfolgerung Auch fünf Jahre nach „nerve-sparing"-Prostatektomie haben mehr als die Hälfte der Paare Geschlechtsverkehr, wiederum die Hälfte dieser Paare auch ohne Hilfsmittel. Insgesamt ist die Zufriedenheit mit dem Sexualleben bei diesen Paaren hoch, die Partnerinnen zeigen sich dabei zufriedener als die Patienten.

P48

Die ärztliche Aufklärung vor andrologischen Eingriffen

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Einleitung Das Selbstbestimmungsrecht des Patienten erfordert eine ordnungsgemäße ärztliche Aufklärung vor einer operativen Intervention. Da Eingriffe aus dem andrologischen Fachgebiet häufig Wahleingriffe sind, ist eine rechtzeitige Aufklärung nach Erhebung der Diagnose und vor Festlegung des Operationstermins wesentlich. Die Aufklärungspflicht über die konkrete Behandlung wird im neuen „Patientenrechtegesetz" § 630e BGB geregelt, wobei die Aufklärung Voraussetzung für die Einwilligung des Patienten vor Durchführung einer medizinischen Maßnahme ist. Die Nichteinholung der erforderlichen Einwilligung stellt eine vertragliche Pflichtverletzung dar.

Methoden Im Rahmen der Aufklärung soll der Patient insbesondere über die Art, den Umfang, die Durchführung, über die zu erwartenden Risiken des Eingriffes, sowie über die Notwendigkeit, Dringlichkeit und über die Eignung und Erfolgsaussichten bezüglich der Diagnose und Therapie aufgeklärt werden. Formelle Aspekte einer ordnungsgemäßen Aufklärung beinhalten eine mündliche und rechtzeitige sowie eine verständliche Durchführung (ggf. mit Dolmetscher), welches insbesondere dazu dienen soll, dass der Patient in einem persönlichen Gespräch die Möglichkeit hat, Fragen an den Behandelnden zu stellen, sodass die Entscheidungskompetenz des Patienten letztlich verbessert wird („informed consent").

Zusammenfassung Die Präsentation soll einen orientierenden Überblick über die Aufklärungspflichten und die Einwilligung im Rahmen geplanter andrologischer Operationen vor dem Hintergrund des im Jahr 2013 in Kraft getretenen Patientenrechtegesetzes (PatRG) geben.

P49

Die Geschichte der Onanie

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Aufgrund einer religiös und medizinisch geprägten Sozialmoral gilt in vielen Gesellschaften die sexuelle Selbstbefriedigung auch heute noch als Tabu oder Perversion. Von der Antike bis in unsere aktuelle Gesellschaft zieht sich diese Verleugnung des Körperempfindens wie ein roter Faden. So war z. B. nach der Entdeckung des Mechanismus der Befruchtung der Eizellen durch den Belgier Eduard Van Beneden 1875 die weibliche Klitoris zum überflüssigen Organ erklärt worden. Sie wurde verdächtigt, Hysterie, Epilepsie und andere Formen des Wahnsinns zu verursachen. Im 18. Jahrhundert wurde die Masturbation bei jungen Männern als auszehrende Erkrankung eingestuft, die fast unweigerlich zum Tode führte. Ein historischer Überblick über die Geschichte der Onanie von künstlerischer, philosophischer und medizinischer Seite soll nun dieses Kapitel der menschlichen Sexualität vorurteilslos erörtern.

P50

Die laparoskopische Ligatur des Plexus santorinii bei Patienten mit erektiler Dysfunktion – ein neues Verfahren

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Einleitung Seit der Veröffentlichung der Langzeitergebnisse der Penisvenenligaturen zur Behandlung der ED 1994 ist die Methode nahezu obsolet. Sie weist zwar primär eine ausgezeichnete Erfolgsrate auf, führt jedoch bereits meist nach wenigen Monaten zu einer erneuten *Impotentia coeundi*. Es stellt sich nun die Frage, ob sich dieses schlechte postoperative Ergebnis durch die Höhe der durchgeführten Venenligatur beeinflussen lässt.

Methoden Ein 40-jähriger Mann mit seit Jahren bestehender erektiler Dysfunktion, SKAT-Non-Responder und einer diffusen kavernösen Insuffizienz stellt sich zur weiteren Therapie vor. Die Implantation einer Penisprothese lehnt der Patient strikt ab, daher erfolgt der Versuch einer laparoskopischen Ligatur des Plexus santorinii. Wir führten den Eingriff laparoskopisch extraperitoneal durch. Die OP-Zeit betrug 60 Minuten. Der stationäre Aufenthalt betrug vier Tage. Postoperativ zeigte der Patient unter oralen PDE-5-Hemmern eine suffiziente Erektion auch zwei Jahre postoperativ.

Zusammenfassung Die laparoskopische Ligatur des Plexus santorinii ist ein minimal-invasives Verfahren, das eine therapeutische Alternative besonders bei jungen Patienten zur Implantation der Penisprothese darstellen kann. Über den Langzeitverlauf der kavernösen Insuffizienz und die Rezidivierung der erektilen Dysfunktion kann infolge der Kürze des Beobachtungszeitraums noch keine Aussage getroffen werden. Wenn jedoch durch dieses Verfahren die Implantation einer Penisprothese bei jungen Patienten noch hinausgezögert werden kann, stellt es unseres Erachtens in Einzelfällen eine echte Alternative dar.

P51

Raman Microspectroscopy offers a possible Means of Identifying Metastatic Seminomas and gives a Clue as to how they grow

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Introduction Germ cell tumours (GCT) constitute over 60% of all malignancies diagnosed in men between the ages of 17 and 45 years with the most frequent being seminomas and embryonal carcinomas. Although GCTs have excellent cure rates, the success of the therapy is dependant upon the accuracy of diagnosis and choice of treatment. A means capable of recognising which tumours are likely to metastasize would greatly aid in the speedy provision of the appropriate therapy and limit the more severe treatment options to those patients who truly require them. To date no such method exists.

Materials and Methods Fixed and paraffin embedded seminoma tissue that did and did not metastasise was evaluated microscopically and characterised immunohistochemically using a routine panel of marker antibodies. 7 µm sections of each tissue were placed onto suprasil slides, de paraffinised then rehydrated. Raman spectra were obtained using the Horiba LabRAM ARAMIS. After determination of the optimal analytical settings, mappings consisting of 200 spectral arrays (50 nm separation) were acquired through out the entire depth of the sample (1 µm intervals). Beyond broad peak assignments, three dimensional hyperspectral representations were obtained using LabSpec 6 software.

Results Morphological and immunohistochemical assessments showed uniformity through out the tissue and could not differentiate between tumors that did or did not metastasize. Overall Raman spectral profiles confirmed these findings and corroborated that the sections probably comprised one cell type. However, 3D hyperspectral representations showed that within tissues prone to metastasis there were discrete regions of enhanced protein production and secretion, the direction of which coincided with the route of new tumor growth.

Conclusions Raman microspectroscopy identified unique foci of apparently hyper activated cells only in seminomas that subsequently metastasized. If verified and validated, the presence of these regions could provide a means of diagnosing, studying and perhaps even undermining the root cause of metastasis in GCTs.

P52

Ejaculatio retarda – Gibt es einen therapeutischen Fortschritt?

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Hintergrund Hat man lange die primäre Ejaculatio praecox des jungen Mannes als eine psychische Erkrankung angesehen, auf die Unerfahrenheit und sympathikusbetonte Aufgeregtheit zurückgeführt, ist mit Verständnis der serotonergen Neurotransmitter-Signalwege ein therapeutisches Tor aufgestoßen worden. Nichtsdestotrotz wird es schwierig, dieses Ziel auch für die Ejakulationsverspätung zu erlangen. So plausibel die Ejakulationsverzögerung des älteren Mannes im Einklang mit Erektionsstörungen, diabetischer Neuropathie, Prostataerkrankungen etc. erscheint, so unlogisch ist es, degenerativen Entwicklungen bereits in jungen Jahren anzuschuldigen.

Methoden Bisherige Konzepte wie medikamentöse Therapieansätze mit Alpha-1-Sympathomimetika (Midodrin, Imipramin) haben Placebofunktion. Auch Yohimbin ist wenig evidenzbasiert. Neuere Konzepte mit dopaminerger Wirkung (dem Serotonin-Gegenspieler) werden eingesetzt. Das Apomorhin war zwar als Therapeutikum partiell wirksam, allerdings mit deutlicher Übelkeit verbunden und vom Markt genommen. Amantadin 100 (ca. 5–6 h vor dem Geschlechtsverkehr eingenommen) erfordert eine gute Planung. Bupropion, ein anregender Dopamin-Noradrenalin-Wiederaufnahmehemmer (NW: Schlaflosigkeit) kann Priapismus auslösen. Cabergolin ist ein D2-Agonist, welcher u. a. beim Prolaktinom Einsatz findet. Eine Kombination mit der Modedroge Oxytocin, dem „Kuschelhormon“ (emotionelle Bindung) aus dem Hypothalamus ist Gegenstand unserer Betrachtung. Das Peptidhormon ist wichtig für die sexuelle Erregung und Orgasmus (Muskelkontraktion des Nebenhodens beim Orgasmus, postorgasmische penile Detumeszenz).

Ergebnisse Die diagnostische Abklärung mit Abgrenzung zu anderen Formen der Ejakulationsstörung (retrograde Ejaculatio, Anejakulation, Ejakulation ohne Orgasmus) wird demonstriert und das praktische Vorgehen anhand von Kasuistiken aufgezeigt. Anejakulation stellt zudem ein Problem in der Fertilitätsmedizin, welches mit Vibrations- bzw. Elektostimulation, bei Versagen mit TESE behandelt wird, dar.

Schlussfolgerung Die psychische Orgasmusstörung ist eine Ausschlussdiagnose.

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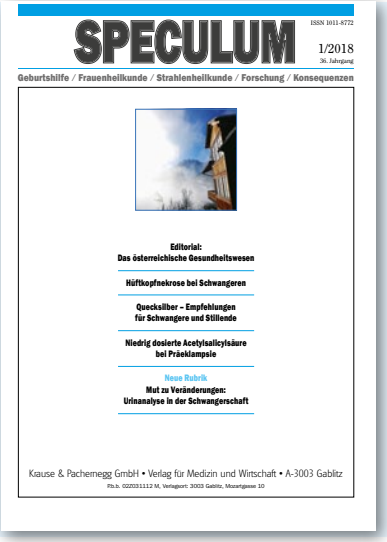

Speculum

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