Introduction: Mammalian spermatogenesis is regulated through paracrine and endocrine activity, cell signaling, and local control mechanisms. These highly specific signaling interactions are effectively absent upon placing testicular cells into two-dimensional primary culture. The specific changes that occur between key cell types and involved spermatogenesis signaling pathways during primary culture remain to be elucidated. However, current protocols to produce mature germ cells in vitro are inefficient and are limited in supporting post-meiotic cells. In order to address these limitations we have developed a 3-dimensional (3D) testis organoid in vitro by combining stem cell and tissue engineering approaches. This model can be utilized as a means to evaluate gonadotopic agents, and as a means to address critical deficiencies in our understanding of basic human spermatogenesis. The overall goal of this study is to establish, characterize, and culture a multicellular, 3D, human testis organoid and to assess its functionality and spermatogenic capacity over time.

Methods: Development of our model system consisted of (1) Identification and analysis of specific cellular components necessary for use in our 3D culture method, (2) Establishment of basic design parameters, culture conditions, and (3) Characterization of human testicular organoids using live cell imaging, immunofluorescence, immunohistochemistry, cell type and stage-specific gene expression, and viability assays.

Results: Human spermatogenial stem cells (SSCs), Sertoli, and Leydig cells were isolated, characterized, and expanded from tissue obtained through the National Disease Research Interchange (Philadelphia, PA, USA). These cell types were integrated successfully into 3D organoids and maintained viability as determined by ATP and Live/Dead assays for over 4 weeks in culture. Gene expression within these multicellular human testis organoids was measured over time for cell and stage-specific markers including UCHL1, DAZL, VASA, SYCP3, SPO11, PRM1, ACROSIN, SOX9, GATA4, INSL3, and HSD3B.

Conclusion: Testicular in vitro organoids were successfully generated using isolated human SSC, Sertoli, and Leydig cells and maintained long term. Future directions include optimizing the spermatogenic capacity of the organoids and evaluating their use as a novel testicular toxicity model.

Funding: AFIRM II, Award No. W81XWH-13-2-0052, NIH grant 5U42RR006042 and Erret-Fisher Foundation grant GTS 3679.

2 (Oral/Poster)
CONDITIONAL STEROIDOGENIC CELL-TARGETED DELETION OF THE TRANSLOCATOR PROTEIN (TSPO) UNVEILS ITS CRUCIAL ROLE IN VIABILITY AND HORMONE-DEPENDENT STEROID FORMATION

Andrew Midzak, PhD¹, Jinhjiang Fan, PhD², Enrico Campioli, PhD, PharmD², Martine Culty, PhD² and Vassilios Papadopoulos, PhD, PharmD²
¹Res; ²Research Institute of the McGill University Health Centre (Presented By: Andrew Midzak, PhD)

Introduction: Translocator protein (18-kDa; TSPO) is a high-affinity drug- and cholesterol binding protein that is abundantly expressed in steroid synthesizing cells. Multiple lines of evidence have demonstrated that TSPO is involved in adrenal, gonadal and brain steroid biosynthesis.

Methods: To further understand the role of TSPO in steroidogenesis, we generated two lines of Cre-mediated Tspo conditional knockout (cKO) mice. Sertoli/Leydig cell-targeting Amhr2-Cre mice were crossed with Tspo-floxed mice to obtain F1 Amhr2-driven Tspo cKO mice (Tspo /l/l; Amhr2-Cre /+). An unexpected Mendelian ratio of 4.4% cKO mice, instead of 25%, was observed. To confirm this finding, dpc 12.5 embryos were genotyped, the age at which Amhr2-Cre is gonadally expressed. The same 4% ratio of Tspo cKO was observed, suggesting preimplantation selection. Tspo expression profile analysis across several microarray datasets, ranging developmentally from oocyte to early embryo, showed that Tspo expression increases after the morula stage. Amhr2 levels, however, briefly spike at the two and eight cell stages, suggesting that Tspo gene modification occurred very early in development, supporting the observed abnormal Mendelian ratio. These confounding findings led us to establish steroidogenic cell-specific Tspo cKO mice, generated by crossing Nr5a1-Cre mice with Tspo-floxed mice.

Results: The resulting F1 population showed the expected Mendelian ratio. Nr5a1-driven Tspo cKO mice exhibited reduced Tspo expression in their gonads and adrenals, though no significant changes in gonadal or adrenal morphology were observed. Interestingly, basal steroid production by the gonads and adrenals was unchanged in Tspo cKO mice. However, although the response of these genetically modified animals to hCG treatment resembled that of their wild-type littermates, the Tspo cKO mice lost their ability to respond to ACTH and form corticosterone. Changes in the mRNA expression of Star and Cyp11a1 steroidogenic genes were observed to be increased in testes and adrenals of these mice, suggestive of adaptive changes. Moreover, expression of steroidogenic signaling receptors were divergent in the tissue of the Tspo cKO mice, with Lhcgr levels increased in testis, whereas adrenal Mc2r levels were unaffected.

Conclusion: The results of these genetic engineering experiments provide evidence that, in an in vivo setting, TSPO is required for preimplantation embryo development as well as hormone-stimulated adrenal steroid biosynthesis.
Conclusion: Organochlorines (OC) are a family of persistent hydrocarbon compounds that were used for industrial and agricultural purposes in North American and Europe between 1930 and the mid-1980s. Due to their high lipophilicity and resistance to biodegradation, OC bioaccumulate in fatty tissues, are biomagnified through the food chain and have accumulated in Arctic populations. OC exposure is associated with decreased sperm quality in men and prenatal exposure to an environmentally-relevant OC mixture impairs reproductive development in male rats. We hypothesized that early-life paternal exposure to an environmentally-relevant OC mixture reduces fertility and changes the protein profile of sperm across multiple generations.

Methods: We compared sperm proteins from paternally non-exposed and exposed rat lineages to an OC mixture over three generations (F1, F2, F3). We used isobaric tags for relative and absolute quantitation (iTRAQ) labelling and 2D-LC-MS/MS analysis to identify proteins that were differentially expressed. One differently expressed protein per generation was confirmed by immunoblotting.

Results: F1 males exposed to OC during early development had decreased sperm motility (P=0.04), lower daily sperm production per testis (P=0.006), and decreased epididymal sperm resistance (P=0.0001). Their F2 OC sons were subfertile (P=0.02) and their F3 OC grandsons had fewer pups per litter (P=0.006). In generations F1, F2 and F3, respectively 7, 19 and 37 differentially-expressed sperm proteins were identified due to OC exposure of the F1 fathers. Cytochrome C, Superoxide Dismutase 1 (SOD1) and Glutathione Peroxidase 4 (GPX4) were reduced in F1 OC males. In their F2 OC sons, Citrate Synthase, Solute Carrier Family 2 member 3 (SLC2A3) and Calcin were decreased. In F3, IZUMO and Zona Pellucida Binding Protein (ZPB) were reduced in OC-exposed males. By western-blot, we confirmed that SOD1, Citrate Synthase and ZPBP are significantly reduced in F1, F2 and F3 OC males, respectively.

Conclusion: This is the first study to compare sperm protein levels due to paternal toxicant exposure across multiple generations using iTRAQ technology. OC exposure induced a decrease in key proteins implicated in sperm motility and cell death (SOD1 and GPX4) in F1 fathers, a reduction in proteins involved in gamete fusion and sperm head cytoskeleton (Citrate Synthase, SLC2A3 and Calcin) in their F2 sons, and finally, a decrease in proteins playing a role in fertilization (IZUMO and ZPB) in their F3 grandsons.
**ABSTRACTS**

**5 (Oral/Poster)**

**THE SPlicing FACTOR RBM5 IS REQUIRED FOR SPERMATOGONIA DIFFERENTIATION**

Duangporn Jamsai, PhD, Morgan Oatley, BSc, Anne O’Connor, BSc (Hons), Jo Merriner, BSc, Robin Hobbs, PhD and Moira O’Bryan, PhD
Monash University
(Presented By: Duangporn Jamsai, PhD)

**Introduction:** Balance of spermatogonial stem and progenitor cell (SSPC) self-renewal and differentiation is essential for the homeostasis of spermatogenesis and the maintenance of male fertility. Regulation of SSPC function requires a complex interplay of intrinsic and extrinsic niche-derived factors. In this study, we identified the splicing factor RBM5 as a novel regulator of spermatogonia differentiation. Male mice carrying an ENU-induced missense mutation (R263P) in the second RNA recognition motif (RRM2) of RBM5 were sterile due to a round spermatid arrest, which ultimately led to azoosperma. We have shown that RBM5 is an essential splicing factor in round spermatids and the R263P mutation resulted in aberrant splicing in several target pre-mRNAs that are required for spermatid differentiation. Within the adult mouse testis, RBM5 localises to the nucleus of somatic and germ cells including spermatogonia, spermatocytes and round spermatids. Further, a stereological analysis revealed that in addition to the spermatid arrest phenotype Rbm5 mutant mice have a decreased conversion of spermatogonia into spermatocytes and significant loss of late spermatocytes.

**Methods:** In order to investigate the loss of spermatocytes, Rmb5 mutant versus wild type testes were stained for MVH as a marker of total germ cell content.

**Results:** The number of germ cells observed in postnatal day 0 and day 3 testes in the Rbm5 mutant testes was normal; however, a significant reduction compared to that in wild type animals was seen at postnatal day 7, suggesting a failure of spermatogonial commitment. Further, FACS analyses of the adult testes showed a significant increase in number of undifferentiated spermatogonia (PLZF positive, c−Kit negative, Ki67 positive) in the mutant compared to wild−type increase in number of undifferentiated spermatogonia (PLZF positive, c−Kit negative, Ki67 positive) in the mutant compared to wild−type. This result was confirmed by PLZF immunostaining showed that mutant testes contained a significant increase in number of undifferentiated spermatogonia per tubule.

**Conclusion:** Taken together, our findings define for the first time a critical role for RBM5 in spermatogonia differentiation.

**6 (Oral/Poster)**

**MECHANISM OF HYPOGONADISM IN THE TRANSGENIC SICKLE CELL MOUSE**

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Johns Hopkins University
(Presented By: Biljana Musicki, PhD)

**Introduction:** Hypogonadism is associated with sickle cell disease (SCD), but its underlying mechanism is not known. We investigated the mechanism of testosterone (T) deficiency in a mouse model of human SCD.

**Methods:** 7 month old homozygote SCD (Sickle) mice were used. Age-matched wild type (WT) and heterozygote SCD (Hemi) mice served as controls. Blood was obtained for measurements of T and luteinizing hormone (LH) by radioimmunoassay (RIA). Testes were collected for Leydig cell isolation, measurements of intratesticular T by RIA, and protein expressions of steroidogenic acute regulatory protein (StAR), cholesterol side-chain cleavage enzyme (P450scc), gp91phox subunit of the reactive oxygen species-generating enzyme NADPH oxidase, oxidative stress (4−hydroxy−2−nonenal, 4−HNE), and an antioxidant glutathione peroxidase−1 (GPx1) by Western blot. Leydig cells were treated with LH (0.5 and 10 ng/ml), dibutyryl cAMP (dbcAMP, 1 mM), 22−hydroxycholesterol (22HC, 25 µM), and pregnenolone (P5, 25 µM), and T produced into the media was measured by RIA.

**Results:** Plasma T levels were significantly (P<0.05) decreased in Sickle compared to WT and Hemi mice, while intratesticular T levels were significantly (P<0.05) decreased in Sickle compared to WT mice. Serum LH levels were significantly (P<0.05) increased in Sickle and Hemi compared to WT mice. LH−, dbcAMP−, and P5− (but not 22HC) stimulated testosterone production from Leydig cells isolated from Sickle and Hemi mouse testis was significantly (P<0.05) decreased compared to that of WT mice. Protein expression of StAR (but not P450scc) was significantly (P<0.05) reduced in the testis of Sickle and Hemi compared to that of WT mice. Protein expression of gp91phox was significantly (P<0.05) increased in the testis of Sickle compared to that of WT mice, while 4−HNE was significantly (P<0.05) increased in the testis of Sickle and Hemi compared to that of WT mice. Protein expression of GPx1 did not differ between WT, Hemi, and Sickle mouse testis.

**Conclusion:** Hypogonadism is present in Sickle mice, mimicking the human condition. The defects in Leydig cell steroidogenic pathway, mainly due to reduced availability of cholesterol for T production, may be related to NADPH oxidase−derived oxidative stress. Mice heterozygous for the human sickle globin exhibit intermediate hypogonadal changes between those of control and Sickle mice.
Introduction and Objectives: Approximately 15% of couples have fertility problems, with a 50% male factor contribution. While assisted reproductive technologies (ART) have greatly enhanced the ability of couples with fertility difficulties to conceive, evidence suggests an increased risk for congenital defects in children conceived using ART. Both the technique of in vitro fertilization (IVF) as well as infertility itself are possible explanations. We sought to determine if the severity of male factor infertility, as assessed by sperm quality and mode of conception, was associated with birth defect rates.

Methods: Fathers with semen analysis data in the Baylor College of Medicine Semen Database (BCMSD) were linked with offspring in the Texas Birth Defects Registry (TBDFR) using data from 1999–2009. To determine the association between birth defects and semen parameters, we identified the subset of men with complete semen parameters. Hierarchical linear modeling was used to determine odds ratios between birth defect rates, semen parameters, and mode of conception before and after adjustment for paternal, maternal, and birth covariates. Semen parameters were stratified based on subfertile cutoffs defined by the WHO 5th edition.

Results: Initial linkage between the BCMSD and TBDFR yielded 6,087 men with linked data. No association between semen parameters and birth defects was observed. As a sensitivity analysis, a subset of 1,382 men who had been evaluated for infertility was identified. No statistically significant association was observed between birth defect rates and semen parameters, before or after adjustment for covariates (Table 1). Likewise, mode of conception, including infertility treatment and ART, did not affect birth defect rates.

Conclusion: Birth defect rates do not appear to be associated with semen quality or mode of conception. The current study suggests that the severity of male factor infertility does not impact the rate of congenital anomalies. This information is important when counseling couples concerned about the relationship between impaired semen quality and birth defects.
ABSTRACTS

Results: LPCN 1021 restored and maintained T levels in the eugonadal range (300–1140 ng/dL) in 89.0% of non-obese hypogonadal men (lower bound 95% CI = 80.3%) and 86.5% of obese hypogonadal men (lower bound 95% CI = 78.8%). Mean T Cave, 24h value was 498±200 ng/dL and 467±194 ng/dL, mean T Cmax value was 1288±557 ng/dL and 1224±625 ng/dL for non-obese and obese men, respectively. No significant differences were observed between obese and non-obese hypogonadal men in terms of percent of subjects restored in the eugonadal range, mean T Cave,24h and mean T Cmax (p>0.1) suggesting LPCN 1021 is effective in treating both non-obese and obese hypogonadal men.

Conclusion: LPCN 1021 is an orally administered TRT product with acceptable serum T levels for both non-obese and obese hypogonadal men. LPCN 1021 may improve patient adherence as a generally safe, effective, and convenient option compared to presently used T products.

9 (Oral/Poster)
CIGARETTE SMOKING AND THE SPERM EPIGENOME
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(Presented By: Timothy Jenkins, PhD)

Introduction: Objective: To evaluate the negative impacts of smoking on the sperm epigenome.

Methods: Illumina 450k human methylation array was used to assess sperm DNA methylation patterns across the entire genome in general population subjects attending University of Utah Andrology and IVF Laboratories for an Institutional Review Board approved study. We analyzed regional and single CpG DNA methylation patterns by two different approaches. First, we analyzed the differences in methylation patterns between smokers and age matched individuals who do not smoke. We then analyzed the effects of both length of time smoking and the volume of cigarettes consumed by analyzing the effect of “pack years”on sperm methylation patterns with a pack years value of <10 (n=11) being considered low and >10 (n=7) being considered high.

Results: Our findings indicate that there are some regions of the sperm genome that are consistently affected by cigarette smoke. Two genes displayed significant alterations to their methylation profile in smokers, namely GPCR133 and SDK1. Additionally, we identified increased methylation variability in smokers across all CpGs probed in our analyses with an average coefficient of variance of 18.67 in our non-smoking group and 26.23 in our smoking group. This difference was significant (p=0.022).

Conclusion: Our data demonstrate that there are alterations that occur to the sperm epigenome as a result of cigarette smoke exposure. Of particular interest in this study are changes seen to general methylation variability in the sperm suggesting that smoke exposure has a destabilizing effect on the sperm epigenome which may affect an individual’s fertility or possibly their ability to produce healthy offspring. More targeted studies are required to fully address this hypothesis and the potential impact these alterations may have on general fertility, fertilization capacity, embryogenesis, and offspring health.

10 (Oral/Poster)
WHOLE-EXOME SEQUENCING IDENTIFIES NOVEL HOMOZYGOUS MUTATION IN NPAS2 IN FAMILY WITH NONOBTUCUTIVE AZOOSPERMIA
Ranjith Ramasamy¹, M. Emre Bakircioglu, MD², Cenk Cengiz, BS¹, Ender Karaca, MD¹, Jason Scovell, BS¹, Matthew Bainbridge, PhD², James Lupski, PhD¹ and Dolores Lamb, PhD¹
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(Presented By: Ranjith Ramasamy)

Introduction: Nonobstructive azoospermia (NOA) is characterized by lack of sperm in the ejaculate due to severe testicular failure. Due to genetic and clinical heterogeneity, the diagnosis is not straightforward. Current clinical practices have focused on karyotype and microdeletions. In the present study, we investigated the genetic cause of NOA in a consanguineous Turkish family through homozygosity mapping followed by targeted exon/whole–exome sequencing to identify genetic variations.

Methods: We designed a whole-exome sequencing (WES)-based approach using an in-house designed capture reagent followed by high-throughput sequencing on the Illumina platform. We sequenced the exomes of two affected siblings. Exome analysis resulted in the identification of 442 variants in the index patients. All variants passing filter criteria were validated with Sanger sequencing to confirm familial segregation and absence in the control population.

Results: A novel non-synonymous mutation in neuronal PAS 2 domain (NPAS2) was identified in a consanguineous family from Turkey. This mutation in exon 14 (chr2: 101592000 C>G) of NPAS2 is likely a disease−causing mutation as it segregates with the disease. Family segregation of the variants showed the presence of homozygous mutation in the three brothers with NOA and heterozygous mutation in one brother and one sister who were both fertile. The mutation is not found in the single nucleotide polymorphism (SNP) database, the 1000 Genomes Project, Baylor College of Medicine cohort of 500 Turkish patients (not a founder mutation) or matching 50 fertile controls.

Conclusion: Using WES, we identified a novel homozygous mutation in NPAS2 as a likely disease-causing variant in a Turkish family diagnosed with NOA. Our data reinforce the clinical role of WES in the molecular diagnosis of highly heterogeneous genetic diseases where conventional genetic approaches have previously failed in achieving a proper diagnosis.

11 (Oral/Poster)
ENCLOMID AND TOPICAL TESTOSTERONE ELEVATE TESTOSTERONE IN HYPOGONADAL MEN BUT ENCLOMID DOES NOT DECREASE TESTES SIZE
Ronald Wiehle, PhD, Gregory Fontenot, PhD, Martin Sandel, BS and Jaye Thompson, PhD
Repos Therapeutics
(Presented By: Ronald Wiehle, PhD)

Introduction: Men with secondary hypogonadism have low normal LH and low testosterone and are often treated with exogenous or topical testosterone.

Objective: Our aim was to evaluate oral enclomiphene citrate or Androxal as an alternative to topical testosterone replacement therapy for men with secondary hypogonadism.

Methods: Two trials (ZA−304 and ZA−305) were randomized, double
blind, placebo- and active-control, multi-center phase III studies in 224 men with secondary hypogonadism between 25 and 60 years of age. Men received 12.5mg or 25mg of enclomiphene as a daily capsule and were provided with a placebo gel. Other men received AndroGel 1.6% and placebo capsules. Other men received placebo capsules and gels. **Results:** To be enrolled subjects needed to have two baseline testosterone (T) values below 300ng/dL. The End of Study (EOS) was after 16 weeks of treatment. There was a statistically significant rise in T in men receiving either enclomiphene citrate or topical testosterone into the normal range (see table). Placebo subjects did not change. Enclomiphene did not decrease sperm counts unlike the topical gel. As we have seen before, enclomiphene citrate increased LH and FSH while men in the topical arm showed decreases (not shown). All men were similar at baseline in testes volume (p = 0.94, ANOVA) by orchidometry. In both studies, men on topico testosterone demonstrated decreases in mean testicular volume (−0.86 cm³) and a significant decrease overall compared to the enclomiphene (p < 0.05) or placebo (p < 0.05).

**Conclusion:** Enclomiphene citrate significantly increased total serum testosterone, LH and FSH which suggests that the drug normalized endogenous testosterone production through the hypothalamic–pituitary–testicular axis and supported the natural continuation of sperm number and testes volume.

![](image)

**12 (Oral/Poster)**

**CONCENTRATIONS OF THE RETINOIC ACID SYNTHESIZING ENZYME ALDH1A2 ARE SIGNIFICANTLY REDUCED IN MEN WITH OLIGOZOOSPERMIA**

John Amory, MD, MPH, Margarett Shnorhavorian, MD, MPH, Samuel Arnold, MD, Faith Stevison, BS, Nina Isoherranen, PhD, Thomas Walsh, MD, MPH and Charles Muller, PhD University of Washington

(Presented By: John Amory, MD, MPH)

**Introduction:** Vitamin A, and its active metabolite, retinoic acid, are known to be necessary for spermatogenesis in many species including man. Retinoic acid is synthesized in tissues from Vitamin A by one of three aldehyde dehydrogenases, ALDH1A1, 1A2 or 1A3. We have shown that testicular ALDH1A2 levels are reduced in men with infertility in proportion to germ-cell number on testicular biopsy; however, the relationships between testicular ALDH1A2 and semen parameters, as well as the cellular localization of ALDH1A2 within the testes have not been reported.

**Methods:** We conducted an observational analysis of testicular ALDH1A2 on human testis samples from 5 men with normal sperm parameters and 5 men with infertility due to oligozoospermia. Testicular tissue was analyzed by immunohistochemistry for ALDH1A2 and ALDH1A2 protein levels were quantified by a LC/MS/MS peptide assay.

**Results:** Men with oligozoospermia had significantly reduced levels of ALDH1A2 in their testicular tissue compared to men with normozoospermia (p<0.03). Immunohistochemistry revealed that ALDH1A2 was localized primarily in spermatogonia, and absent from Sertoli cells.

**Conclusion:** The finding that ALDH1A2 co-localizes with early germ cells in the human testis suggests that i) early germ cells are a site of retinoic acid biosynthesis within the seminiferous epithelium, ii) reduced ALDH1A2 may be associated with male infertility, iii) inhibition of ALDH1A2 may be a reasonable strategy for the development of novel male contraceptives.

This work was funded by The Eunice Kennedy Shriver National Institute of Child Health and Human Development supported this work through cooperative agreement U54 HD42454 as part of the Cooperative Contraceptive Research Centers Program.

**13**

**INVESTIGATIONS ON THE EFFECTS OF TYPHA CAPENSIS ON TM3 LEYDIG CELLS**

Abdulkarem Ilfargane, MSc, Nicole Haines-Arries, MSc, Leonardo van Zyl, MSc and Ralf Henkel, PhD University of the Western Cape

(Presented By: Ralf Henkel, PhD)

**Introduction:** Typha capensis (bulrush) is one of South Africa’s indigenous medicinal plants used to treat male fertility problems. Anecdotal data claim that T. capensis has beneficial effects on male reproductive functions and aging male symptoms. The aim of the study was to investigate these effects of T. capensis and to identify active fractions in an in vitro system using TM3-Leydig cells in respect to the induction of testosterone production.

**Methods:** Rhizomes of the plant were harvested in the four seasons (spring, summer, autumn and winter), extracted with water and fractionated using HPLC. TM3 Leydig cells were then cultured in DMEM medium and incubated with the extract under standard conditions at different concentrations (0.01, 0.02, 0.1, 1, 10, 100 µg/ml) for 24 and 96 hours, respectively. Viability (MTT test), cell morphology, early apoptotic events by means of Annexin V-Cy3 binding, DNA fragmentation by means of the TUNEL assay, and testosterone production (ELISA) were tested. HPLC fingerprinting was carried out.

**Results:** At concentrations less than 10 µg/ml, the extracts showed no effect on cell viability. At higher concentrations, viability increased, indicating to cellular stress. Testosterone production of TM3 cells increased significantly after exposure to concentrations of T. capensis higher than 0.1 µg/ml. Exposure at low concentrations (0.01, 0.02, 0.1, 1, 100 µg/ml) for 24 – 96 hours showed no increase in early apoptosis and DNA damage when compared to the control. Higher concentrations (10, 1000µg/ml) revealed an increase in the percentage of cells with signs of early apoptosis and DNA damage. HPLC data showed that the most effective fraction was the F1 fraction from the summer harvest.

**Conclusion:** Typha capensis enhanced the production of testosterone and might be useful to treat male infertility and aging male problems. Results further reveal that the F1 fraction from the summer harvest had highest biological activity.
Objectives: Controversies abound on whether testosterone causes myocardial infarctions (MI). Some studies show an association of testosterone therapy with MI, while others show a protective or neutral effect. The association of MI with testosterone treatment seems to be linked to age or underlying medical conditions.

Methods: After IRB approval, cases of MI were identified by ICD-9 coding, using the electronic medical record. Conference calls were held with centers to ensure that each patient was asked specifically for MI and that coding was accurate. 40 Centers were examined. Interviews were also performed on patients & families of patients with MI, and cardiac risks factors were identified. The data was entered into a spreadsheet and descriptive as well as comparative statistics performed.

Results: 39,937 charts were reviewed and about 19,968 patients received testosterone treatment. Of these, there were 9 cases of new MI and 46 patients with pre−existing MI. Of the 9 patients, all had risk factors except one. Our MI rates at 45 per 100,000 are very low in comparison to managed care (Kaiser Permanente) rates, which were 208 per 100,000. Of those who were on testosterone and had MI, 44% were smokers or had hypertension (HTN), 22% had Diabetes (DM). In comparison the prevalence of smoking was 3.5%, HTN 15%, DM was 4% in the overall testosterone treated group. When chi square was applied for differences between the 2 groups (smoking, HTN, DM), \( p = 0.001 \).

Conclusion: Our study showed that testosterone therapy is not causal of MI. If carefully monitored, testosterone treatment in a younger population was safe and established risk factors such as smoking, hypertension and diabetes are associated with higher rates of MI in our testosterone treated patients.

Introduction: In nowadays herbal medicines in modern countries come to help for diseases treatment, herbal are known a sources of antioxidant and minerals. Objectives: To study the effect of Cinnamon zeylanicum on spermatogenesis in rats.

Methods: in this experimental study, Wistar male rats \( (n=20) \) were divided into two groups, a control group \( (n=10); g−1 \) and a Cinnamon zeylanicum group\( (n=10); g−2 \) that received 75mg/kg/day cinnamon by gavage for 28 days; however, the control group just received an equal volume of distilled water daily. Animals were kept in a standard condition. On day 28, 5 ml blood sample of each rat taken from tail area to measure testosterone, SOD (superoxide dismutase), CAT (catalase), GPX (glutathione peroxidase), and MDA (malondialdehyde) levels. Testes were collected and were then prepared for sperm analysis by the WHO method.

Results: Sperm parameters, total serum testosterone, SOD, CAT, and GPX levels were significantly increased in the group−2 group in comparison to the group−1 \( (P<0.05) \). Besides, group−2 showed a significant decreased in the level of plasma MDA \( (P<0.05) \) in comparison to the group−1. There were no significant differences between the groups in testis weight \( (P>0.05) \).

Conclusion: Since in our study, 75mg/kg/day Cinnamon has significantly increased the sperm population, motility and viability, it seems using it in mammalian has beneficial potential on spermatogenesis.

Introduction: Brominated flame retardants are compounds used to prevent the initiation and propagation of fires. However, it was demonstrated that their efficacy has been overestimated and, more importantly, that they interfere with the hormonal balance of the body. Some of these flame retardants, such as BDE−47, disrupt the regulation of testosterone production by Leydig cells. As an alternative, new chemicals, the organophosphate flame retardants (OPFRs), are being used as “safer” replacements. The objective of this study was to characterize the effects of some common OPFRs, e.g. isopropylated phenyl phosphates, 2−ethylhexyl diphenyl phosphate and triphenyl phosphate, on male reproductive health. We hypothesized that the newer compounds will not affect Leydig cell function.

Methods: To test this hypothesis, we used a commonly used Leydig cell model, the MA−10 cell line, to investigate potential toxic effects on steroidogenesis. MA−10 cells were treated with concentrations of OPFRs ranging from 0.1 to 100 \( \mu \)M for 48h; we then assessed the effects on mitochondrial activity, cell count, superoxide production and steroidogenesis. As a reference, cells were also treated with BDE−47.
ABSTRACTS

Results: Our results showed that all OPFRs inhibited mitochondrial activity to a greater extent (~95% at 100 µM) than BDE-47 (~75% inhibition at 100 µM). All of the OPFRs except triphenyl phosphate reduced total cell numbers by 20% or more at 10 µM, while BDE-47 and triphenyl phosphate had close to no effect at this concentration. Cytotoxicity at 10 µM was correlated with an increase in superoxide production, suggesting that oxidative stress is a factor leading to the observed cell death. OPFRs increased basal steroid production by at least 1.5-fold (reaching up to 3-fold), while inhibiting the ability of cells to respond to external stimuli such as LH by up to 4.4-fold.

Conclusion: These results suggest than none of the OPFRs tested is safer than BDE-47 in this cellular model: all affected mitochondrial activity, cell survivability and redox status. A significant increase in basal steroid production could have drastic effects on organs such as the prostate, while the decrease in responsiveness to stimuli could lead to long-term consequences on reproductive health, as well as on other tissues such as the brain or muscles. These studies were supported by CIHR and REDIH.

17 FINASTERIDE 1MG/DAILY CONSUMPTION IMPAIRS SPERMATOGENESIS BY HISTOLOGICAL EVALUATION AND SEMEN QUALITY IN MEN IN REPRODUCTIVE AGE

Juliana Pariz, MSc, PhD student¹,² and Jorge Hallak, MD, PhD¹,²
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(Presented By: Juliana Pariz, MSc, PhD student)

Introduction: Finasteride, an oral type 2,5 -reductase inhibitor, is used in 1 mg/daily doses for the treatment of male pattern hair loss. Its consumption affects the conversion of testosterone to dihydrotestosterone (DHT), which is a more potent androgen, impairing the male reproductive system. Objective: to study the effect of finasteride 1mg/daily consumption on the spermatogenesis and seminal quality.

Methods: We studied 23 male subjects (mean 33 y.o.) who came for an initial andrological evaluation and who self-reported use of finasteride 1mg for more than one year. These subjects were paired and compared, by Paired-Samples T Test (p adopted <0.05), with fertile (pre-vasectomy candidates with no risk factors for sperm/testicular dysfunction) and infertile (for over a year) patients with same age and varicocele grade. In addition, we included six testicular biopsies with spermatogenesis assessment. The study was approved in Ethics Committee (n°12331). We conducted ANOVA test of the means and adopted p <0.05.

Results: The mean of Finasteride consumption was 72.22 months. Finasteride users had significantly decreased in total sperm count (p=0.026 and 0.014) and total motile sperm count (p=0.053 and 0.036) when compared with infertile and fertile group, respectively. Furthermore, morphology by strict (p=0.001) and WHO (p<0.001) criteria and testicular volume (p<0.001) decreases in Finasteride users when compared with fertile men. Creatine-kinase activity which is a marker of sperm immaturity was increased in Finasteride users (p<0.001). In spermatogenesis analysis, four showed spermatogenesis altered and round spermatids; predominant Johnsen score was 7. We conducted T Test paired and adopted and p <0.05.

Conclusion: Our results demonstrate that Finasteride consumption affects negatively the spermatogenesis and semen quality and may be contraindicated to young men that are willing to father their own offspring. We do not yet know the long-term effects on male reproductive health and recovery rate after discontinuation, but this study suggest that Finasteride is of concern for the proportion and widespread prescription by dermatologists and others health professionals unaware of its potential reproductive effects.

18 EFFECT OF ALCOHOL CONSUMPTION ON MALE REPRODUCTIVE POTENTIAL

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(Presented By: Artemis da Silva, BSc)

Introduction: Alcohol is a psychoactive substance that may create dependence and affects the overall health of man by different mechanisms, which can be fatal in excessive chronic use. The possible association between alcohol consumption and reduced male fertility has been the subject of several studies and still remains unclear. Objectives: to demonstrate the effects of alcohol consumption in fertile men of reproductive age, evaluating semen parameters and hormonal profile.

Methods: For this study, were included data of semen analysis, hormone profile and testicular volume of pre-vasectomy candidates with no risk factors for sperm/testicular dysfunction from Clinical Hospital of Urology Department, Universidade de São Paulo, Brazil. We excluded patients with infertility, azoospermic, presence of varicocele or any clinical or surgical history that cause sperm changes. The study was approved by Ethics Committee (12331/14). We conducted T-Test for independent samples and adopted p <0.05.

Results: Subjects who reported not being drinkers constituted the control group (n=86), mean age of the patients 34.7 ±5.43. Who declared themselves consumers was included in alcohol group (n=18), mean age 37.35 ±6.55. Statistical differences were seen in following seminal parameters: pH (7.65 vs. 7.97; p <0.07), motility grade A (8.59 vs. 4.61%; p<0.003), strict criteria (6.46 vs 4.11%; p<0.01) and WHO (21.44 vs. 15.22%; p<0.004) normal morphology and total number of round cells (14.75 vs. 5.66 million; p <0.001). In hormonal parameters, there was an increase of 17-OH progesterone in alcohol consumption (1.05 vs. 1.54ng/mL; p<0.011).

Conclusion: In view of results, we suggest that alcohol intake affects adversely the production of 17-OH progesterone and spermatogenesis, resulting in reduced mobile and morphological quality of sperm. Thus, the intake of alcohol appears to be associated with reduced male reproductive potential.
19 YOGA AND MEDITATION - THERAPEUTIC FOR SPERM DNA HEALTH
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Introduction: Life style habits adopted by father before the conception may lead to the increase in seminal free radical level and culminate in seminal oxidative stress. Oxidative stress damages sperm nuclear and mitochondrial DNA. Complementary and Alternative Medical therapies such as yoga/meditation are being increasingly used as adjuncts to modern medicine. Therefore, we analyzed the effects of yoga and meditation on sperm DNA integrity in fathers of children with Retinoblastoma (Rb) after interventions (3 and 6 months).

Methods: A total of 26 men (father of child with non-familial Rb) were recruited in this study. Semen samples were collected at base line (day 0), 3 months and after 6 months of yoga practice. Reactive Oxygen Species (ROS), DNA Fragmentation Index (DFI) and 8-hydroxy-2’-deoxyguanosine (8−OHdG) estimation was done at each interval.

Results: Seminal mean ROS levels were reduced after 3 months [p=0.081] and after 6 months [p<0.001] compared to base level (day 0). There was reduction in mean DFI levels [p=0.059] at 3 months and after 6 months DFI significantly reduced [p<0.01] compared to base level (Fig 1). We also observed reduction in levels of 8−OHdG after 3 months [p<0.05] and after follow up of 6 months [p<0.01] with respect to the base level.

Conclusion: The results of this study highlight that the yoga/meditation may significantly lower the DFI, 8−OHdG and ROS levels and thus are therapeutic for maintaining/restoring sperm DNA integrity. To the best of our knowledge, this is the first study to report a reduction in DFI mutagenic load following adoption of yoga and meditation. Recently United Nation’s proposal to celebrate International Yoga Day (21st June) might be an encouraging step and to reduce childhood morbidity by improving DNA integrity.

20 GENITOURINARY INFECTION ALTERS SEMEN PARAMETERS AND INCREASES PRESENCE OF ANTI-SPERM ANTIBODIES
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Introduction: Pro−inflammatory cytokines produced from bacterial infections induces changes in permeability of blood−luminal barrier (testis/epididymal/prostate) and may alter the male reproductive potential. Objective: to evaluate the effect of genitourinary infection in semen parameters and presence of anti−sperm antibodies (ASA).

Methods: This retrospective study included 172 infertile men (22 – 56 years-old) of Andrology Clinic between 2006 and 2014 who underwent semen analysis, ASA test and microbiological evaluation of male genitourinary system. Patients who had bacterial infection were classified as infection group and the absence of infection composed the control group. The means of seminal parameters and ASA were compared between groups using the T test for independent samples and was adopted p <0.05 for statistical significance.

Results: Sixty-eight patients were included in infection group and 104 in control group. 59% of patients had infection caused by Staphylococcus spp, 30 % by Enterococcus spp and 11% by Escherichia coli. Reduction in total number of progressive sperm (48.18 million/ejaculate vs 23.38 million/ejaculate; p=0.003), total motility (39.62% vs 23.71%; p<0.001), progressive motility (61.50% vs 50.04%; p <0.001) and motility grade B (35.45% vs 21.06%; p <0.001) and increase in motility grade C (22.04% vs 26.34%, p = 0.011) and grade D (38.34% vs 49.96%, p <0.001) were observed. Increase in percentage of ASA was observed, with 12.31% in control group to 20.34% in infected group (p<0.001)

Conclusion: Our results suggest that the presence of infection in the genitourinary system can interfere with the blood−luminal barrier, resulting in increased presence of testicular ASA, which can affect the quality of sperm motility and male reproductive potential.

21 CHARACTERIZATION OF PRIMARY CULTURES OF ADULT HUMAN EPIDIDYMIS EPITHELIAL CELLS
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Introduction: This study aimed to establish cultures of epithelial cells from all regions of the human epididymis, to provide enough materials for molecular approaches to epididymis function.

Methods: Human epididymis tissue was obtained from nine patients undergoing orchectomy for a clinical diagnosis of testicular cancer. The three different anatomical regions: caput, corpus and cauda, were separated, and the tissues of each segment were digested with collagenase type I and seeded onto collagen I−coated cell culture plasticware. The cells were grown in CMRL 1066 medium and maintained in a humid 5% CO2 incubator at 33°C.
Results: Cultures of caput, corpus and cauda epithelial cells were passaged up to 8 times and maintained differentiation markers. They were also cryopreserved and recovered successfully. Androgen receptor, clusterin and CRISP1 were expressed in cultured cells as shown by immunofluorescence, western blot and quantitative reverse-transcription PCR (qRT–PCR). The distribution of other epididymis markers alone different regions was also investigated by qRT–PCR. Cultures developed transepithelial resistance (TER) when cells were grown on filter inserts, which was androgen responsive in the caput but androgen insensitive in the corpus and cauda where unstimulated TER values were much higher.

Conclusion: The results demonstrate a robust in vitro culture system for differentiated epithelial cell types in the caput, corpus and cauda of the human epididymis. These cells will be a valuable resource for studying biological mechanisms relevant to epididymis in health and disease, which has a pivotal role in male fertility.

22 COMPARATIVE ANALYSIS OF EPIDIDYIMIS TRANSCRIPTOME IN FERTILE AND SUBFERTILE BULL

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(Presented By: Christine Legare, MSc)

Introduction: The epididymis is a single long convoluted tubule that connects the testis to the vas deferens and is responsible for sperm maturation and storage. Gene expression is highly segmented along this organ resulting in the formation of luminal microenvironments that sequentially modify maturing spermatozoa. In this study, we compare the transcriptome signature in the epididymal segments of fertile and subfertile bulls in order to highlight putative subfertility explanations.

Methods: Epididymis from 6 Holstein bulls with documented fertility was used. According to their ‘fertility solution’ (SOL), as calculated by the Canadian dairy network, bulls were divided into 2 groups: high fertility (HF) (SOL>3.0; n=3), and medium–low fertility (−2.8>SOL>−4.9; n=3), SOL=0 being the average. Microarray analysis was performed on GeneChip Bovine Genome Array (Affymetrix®). Hierarchical clustering and Principal Component Analysis revealed an excellent separation between caput, corpus and cauda segments. Among the 23000 bovine qualifiers spotted on the chip, 14725 transcripts were differentially expressed in caput, corpus and cauda, respectively. To compare gene expression between fertile and subfertile groups, nine (including AKAP4, SMCP, SPATA3, TCP11, ODF1, CTCFL, SPATA18, ADAM28, SORD and FAM161A) were found to exert function related to reproduction activity and 5 genes (including DEAD, CYST11, DEFB119, DEFB124 and MX1) were found to be associated to the defense response.

Conclusion: Dysregulation of these two epididymal functions can jeopardise sperm ability to reach and fertilise the oocyte. As fertility can be quantified in bulls used for artificial insemination, this species is a unique model to understand male fertility/subfertility in man.

23 CONTRIBUTION OF PRINCIPAL AND CLEAR CELLS IN THE REGULATION OF LUMINAL PH IN THE MOUSE EPIDIDYMIS

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(Presented By: Yoo-Jin Park, PhD)

Introduction: While spermatozoa undergo epididymal maturation, they remain quiescent thanks to the establishment of a low luminal pH. In the cauda epididymis (Cd), clear cells (CCs) secrete protons via the proton–pumping ATPase (V−ATPase) to acidify the lumen. However, upon activation principal cells (PCs) can secrete HCO3−, a process that is mediated by CFTR and would contribute to increasing luminal pH. We examined here the relative contribution of CCs and PCs in the regulation of the overall acidic luminal pH.

Methods: The lumen of the Cd was perfused with an acidic (pH 5.5), control (pH 6.6) and alkaline (pH 7.8) phosphate buffer solution. The perfusate was then collected via an incision made at the epididymal/vas deferens junction, and its pH was measured immediately using high sensitivity pH strips (0.2 unit increment).

Results: While the pH of the control solution remained constant at 6.6 after its passage through the Cd lumen, the pH of the acidic perfusate progressively increased from 5.5 to 5.70 ± 0.20 after 10 min and 6.23 ± 0.03 after 20 min. Addition of cpt−cAMP induced a faster recovery, the pH reaching 6.1 ± 0.06 (versus 5.70 ± 0.20 without cAMP) after 10 min, and the CFTR inhibitor (CFTRinh172) abolished recovery (pH 5.5 ± 0.00 after 20 min). Alternatively, the pH of the alkaline perfusate rapidly decreased from 7.8 to 6.4 at 10 min and then remained constant after 20 min. cAMP or CFTRinh172 had no significant effect on this response. V−ATPase labeling of fixed cryostat sections showed “activated” CCs with numerous and long V−ATPase−labeled microplicae when perfused at pH 7.8, and “resting” CCs with very few V−ATPase−labeled microplicae at pH 5.5.

Conclusion: Our results suggest: 1) CFTR−dependent bicarbonate secretion by PCs when the Cd is perfused at acidic pH (pH 5.5); 2) V−ATPase−dependent proton secretion by CCs when the Cd is perfused at alkaline pH (pH 7.8). Both processes would contribute to the re-establishment of luminal pH towards its control value. Our study, therefore, indicates the participation of both CCs and PCs in acid/base transport in the Cd epididymis, and shows that in addition to CCs, PCs can also respond to variations in luminal pH.

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24
TESTICULAR FLUID REGULATES APICAL BLEBBING IN THE PORCINE EPIDIDYMIS
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(Presented By: Jennifer Hughes)

Introduction: The epididymis functions to mature spermatozoa. Apical blebbing of the epithelium produces vesicles, termed epididymosomes, which transfer proteins important for fertilizing capacity to maturing spermatozoa. Our understanding of the mechanism by which apical blebs are produced is limited. Prior work from our laboratory indicates that apical blebbing is a mature phenotype not regulated by androgens or estrogens within the epididymis. Reduction of the testicular luminal flow down regulates the appearance of apical blebbing after effertent duct ligation.

Methods: To confirm that luminal content drives apical blebbing we ligated the left caput epididymis at two sites and removed the intervening epididymal tubule during early peri–pubertal development (12 weeks.) Tissues were collected from both epididymides and the corpus morphological appearance of apical blebbing was assessed four weeks later, at 16 weeks of age, a developmental stage when blebbing is easily detectable. The non–manipulated epididymis served as an internal animal control.

Conclusion: Initial findings further support regulation of apical blebbing by a testicular luminal factor.

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25
DIFFERENTIAL PLATING RATIO OF NON-ADHERENT TO ADHERENT CELLS ISOLATED FROM AN AZOOSPERMIC MICROTESTICULAR TISSUE EXTRACTION (MICROTESE) SAMPLE HAS A PROFOUND EFFECT ON IN VITRO GERM CELL COLONY FORMATION
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(Presented By: Itai Gat)

Introduction: Somatic cell overgrowth limits spermatogonial stem cell (SSC) propagation ex-vivo. We evaluated the effect of differential plating (DP) ratios of non-adherent (NAd) to adherent (Ad) cells isolated from azoospermic microTESE tissue to determine the effect of this on in vitro germ cell colony formation.

Methods: Excess tissue from a hypospermic human testicular sample after microTESE was obtained. After enzymatic digestion and overnight incubation, GFR1+ve and GFR1−ve cell subpopulations were enriched by magnetic activated cell sorting and cultured on 12 well laminin-coated dishes in 3 groups: GFR1+ve with density of 7.5K cells per cm2; GFR1−ve with density of 7.5K; GFR1−ve with density of 15K (P0). After 10 days, repetitive DP was conducted and passaged (P1) using pre–determined NAd-to-Ad cell ratios. Number and characteristics of germ cell–like colonies were assessed.

Results: 3 patterns were seen at P0: GFR1+ve cultures had small irregular NAd germ-like cells, together with a low Ad cell concentration followed by cell death withoutpassaging to P1. GFR1−ve at 7.5K density included 5 colonies associated with somatic cells. GFR1−ve at 15K density resulted in mostly somatic cells without germ cell–like colony formation. During repetitive DP dramatically different NAd-to-Ad ratios were observed between GFR1−ve at 7.5K and 15K plating densities. After passage to P1, Ad cell density was reduced to 2.5K per cm2 while passing all NAd cells from both GFR1−ve culture conditions using NAd-to-Ad ratios of 15:1 and 4:1 (figure 1). After 10 days of P1, 200 colonies were observed in the 15:1 culture while none were observed in the 4:1.

Conclusion: Low (GFR1a+ve) or high (GFR1a−ve, 15K) somatic–like Ad cell concentration ratios affected colony formation. We hypothesize that this is due to a lack or over–dominance of somatic cells in culture, respectively. GFR1−ve 7.5K condition demonstrated intermediate somatic cell numbers and enabled colony formation at P0. We show for the first time that repetitive DP and NAd-to-Ad cell ratio can have a profound effect on germ cell colony formation. These results are being repeated with additional samples, with concomitant assessment of miRNA and protein expression.

26
REQUIREMENT FOR MOV10L1 RNA HELICASE ACTIVITY IN THE PROCESSING OF PIRNA PRECURSORS
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Introduction: PIWI–interacting RNAs (piRNAs) are a class of small non–coding RNAs highly expressed in the germlines of many species from C. elegans to mammalian species. They most notably protect the integrity of the germline genome through transcriptional and post–transcriptional silencing of active transposable elements (TEs) during germ cell development. piRNA biogenesis is tightly coupled to TE silencing. In mammals, the RNA transcripts of active TEs are targeted into the piRNA processing pathway as piRNA precursors for degradation and biogenesis of primary piRNAs. Primary piRNAs then enter the ping–pong amplification loop to generate secondary piRNAs with complementary sequences. Finally, secondary piRNAs are believed to guide their PIWI proteins to transcriptionally silence active TEs in the nucleus. However, the mechanisms underlying piRNA biogenesis remain unclear.

Methods: Moloney Leukemia Virus 10–like 1 (MOV10L1) is a testes–specific RNA helicase required for piRNA biogenesis. Mov10l1−deficient males are viable but infertile, due to meiotic arrest. Disruption of Mov10l1 leads to the accumulation of piRNA precursors.
ABSTRACTS

Results: Our CLIP–seq results reveal that MOV10L1 directly associates with piRNA precursors. The majority of MOV10L1–bound RNAs map to piRNA hotspots, but do not represent mature piRNA sequences. MOV10L1 binds to RNA near regions with high secondary structure potential. Because RNA helicases recognize and resolve secondary structures, we hypothesize that MOV10L1 processes piRNA precursors through its RNA helicase activity. I designed two Mov10l1 knock−in mouse models containing conserved point mutations in the ATP binding and ATP hydrolysis sites of the MOV10L1 RNA helicase domain. Male Mov10l1 homozygous knock−in mice exhibit meiotic arrest, mislocalization of piRNA pathway proteins, a derepression of the LINE1 TE, and a lack of piRNAs associated with MILI.

Conclusion: In conclusion, MOV10L1 RNA helicase activity is required for piRNA biogenesis. In the future, I plan to determine if MOV10L1 RNA helicase activity is required for the processing of piRNA precursors by measuring the levels of piRNA precursors in Mov10l1 knock−in mice. Our previous and current studies demonstrate that MOV10L1 is a master regulator of piRNA biogenesis in mammals. As an essential germ cell−specific gene, mutations in human Mov10l1 are expected to cause male infertility. Thus, MOV10L1 may be a molecular target for male contraception.

27 SEMEN PARAMETERS, PATIENT CHARACTERISTICS, AND ASSOCIATED SPERM EPIGENETIC PROFILES
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(Presented By: Timothy Jenkins, PhD)

Introduction: Objective: To evaluate sperm epigenetic changes associated with patient age and BMI at the time of collection as well as general semen parameters including, sperm motility and total count. Methods: Illumina 450k human methylation array was used to assess sperm DNA methylation patterns across the entire genome in patients attending the University of Utah Andrology and IVF Laboratories for a routine semen analysis. We analyzed regional and single CpG DNA methylation patterns to identify any associations with BMI (normal, n=23; vs. obese, n=17) and age (<25 years of age, n=20; vs. >40 years of age, n=17) as well as total sperm count (normal, n=58; vs. low, n=6) and progressive motility (normal, n=44; vs. low, n=20).

Results: Our findings suggest very a subtle association between BMI (obese vs. normal) and sperm methylation patterns, with a total of 4 genomic regions that are significantly differentially methylated between the two categories. Similarly, only 1 genomic region showed an association between total sperm count and DNA methylation. In contrast, we have identified relatively strong sperm epigenetic associations with progressive motility where 85 genomic regions are significantly differentially methylated between samples with normal and low motility. Interestingly, these alterations were frequently found at imprinted loci and regions important for spermatogenesis. Similarly, paternal age is strongly associated with sperm epigenetic alterations, with 131 genomic regions that are significantly altered with age.

Conclusion: Our data demonstrate that there are some associations between sperm epigenetic profiles and general semen parameters and patient characteristics. The associations identified with aging are in agreement with previous work from our lab and others. Of particular interest in this study are the unanticipated associations identified between sperm motility and DNA methylation patterns. While the etiology of these alterations is unclear, they may be an indication of more general problems during spermatogenesis. Interestingly, the loci where these alterations are found in low motility samples comport with this hypothesis. Further, targeted studies are required to fully address these findings and the potential impact these alterations may have on general fertility, fertilization capacity, embryogenesis, and offspring health.

28 AN ASSOCIATION BETWEEN THE GLUCOSE TRANSPORTER GLUT3 AND MALE INFERTILITY
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(Presented By: Alexander W. Pastuszak, MD, PhD)

Introduction: Approximately 15% of couples have fertility problems, with a 50% male factor contribution. In many of these men, a genetic cause is suspected. While numerous known genetic alterations cause male infertility, many other genetic etiologies likely exist. Glucose transporters (GLUTs) may be involved in sperm motility, though their role in this process is unclear and no genetic studies exist supporting a role for GLUTs in fertility. Here, we present data associating GLUT3 with male infertility.

Methods: Genomic DNA from 22 men with nonobstructive azoospermia (NOA) and normal Y−chromosome microdeletion and karyotype assays, as well as 4 fertile controls, was used for array comparative genomic hybridization (aCGH) to assess copy number variations (CNVs). Candidate fertility genes were selected based on 1) the frequency of CNVs in the gene, 2) the magnitude of the gain / loss, and 3) evidence supporting a role in male fertility. CNVs were validated using qPCR and candidate genes were sequenced. Immunohistochemical staining of testis sections used a rabbit polyclonal antibody against GLUT3 and standard protocols.
Results: Microduplications of 115kb and 130kb at position 12p13.31 encompassing GLUT3 were identified using aCGH in 2 of 22 (9%) NOA men and in no controls. qPCR of DNA from 43 infertile men revealed 5 more GLUT3 gains. In contrast, in a cohort of 52 fertile men, a gain in GLUT3 was identified in one (2%) patient (p=0.04). The frequency of GLUT3 CNVs in the general population is approximately 0.06%, whereas nearly 14% of the NOA cohort (p<0.0001) had CNVs in the GLUT3 region of chromosome 12. Sequencing of GLUT3 identified single nucleotide polymorphisms (SNPs) predicted to be benign in exons 2, 6, and 10, suggesting that the effects of GLUT3 in male fertility are mediated by gene dosage rather than protein function. In support of this, staining of testis from a male with 3 GLUT3 copies and hypospermatogenesis showed cytoplasmic interstitial cell and spermatocyte staining, with less intense interstitial staining, but more intense spermatocyte staining observed in testis from a normal male with 2 GLUT3 copies.

Conclusion: CNVs in GLUT3 were identified in infertile males more frequently than in fertile males or the general population. Future work assessing the impact of GLUT3 on fertility-related cellular signaling pathways, as well as animal model studies, will further elucidate the role of GLUT3 in male fertility.

29 PATERNAL FACTORS IN EARLY EMBRYONIC DEVELOPMENT
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Introduction: Recurrent miscarriage (RM) or consecutive pregnancy loss before 20th week of gestation occurs in 1−5% women. RM is usually seen as a mother’s problem but the developing knowledge shows the role of paternal contribution for the fetal loss. However it is still difficult to diagnose male partners who contribute to pregnancy loss with classical semen analysis. And there is need to establish other molecular diagnostic markers apart from DNA fragmentation index (DFI) which help in the best diagnosis of RM. Our objective is to study the role of sperm gene expression profile in idiopathic RM patients.

Methods: Total RNA was isolated from 24 male partners of RM couples and 24 male partners of healthy couples who have fathered healthy child and was reverse transcribed to cDNA. cDNA was amplified and quantified by qPCR and gene expression analysis was performed for TOMM7, RBM9 (RBFox2), RPS6, RPL10A, EIF5A, AKAP4, STAT4, Sox3, and FOXG1 genes.

Results: Sperm gene expression of TOMM7, RBM9, RPL10A and AKAP4 were up regulated around one fold, the genes FOXG1, SOX3 and STAT4 were up regulated more than one fold as compared to EIF5A and RPS6 in the male partners of iRM couples compared to the controls.

Conclusion: In this study we observed increase in the gene expression of genes which are important for normal fetal development. Future studies can help elucidating their functional importance and how they regulate the fetal development and their over expression leads to embryonic lethality and recurrent miscarriage.
LIFETIME FOLATE DEFICIENCY AND SUPPLEMENTATION INDUCES ABERRANT SPERM DNA METHYLATION AND REPRODUCTIVE HEALTH

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(Presented By: Lundi Ly, BSc)

Introduction: Epigenetic modifications such as DNA methylation have an essential role in developmental programs. Recent evidence shows that embryos are highly sensitive to signals from the gametes and the environment. Furthermore, disruptions in gamete epigenetic reprogramming which occur primarily in prenatal development, are further associated with adult disease and transgenerational effects. The fetal period is the key to DNA methylation pattern acquisition in developing male germ cells and adequate supply of methyl donors is required. The folate cycle is involved in the production of methyl groups necessary for methylation reactions. Previous studies showed that either postnatal folate deficiency (FD) or supplementation (FS) could alter the sperm epigenome. The main objective of this study was to determine if lifetime FS or FD induce an aberrant epigenetic landscape in germ cells detrimental to offspring health.

Methods: Female mice (F0; n=15) were placed on one of four amino acid controlled diets: a basal diet (FCD; 2mg folate/kg diet), a 20-fold folate supplemented diet (20FS), a 10-fold folate supplemented diet (10FS) or a 7-fold deficient diet (7FD). F0 females were mated to produce F1 litters whose germ cells were exposed to the folate diets at all stages of development. F1 males were weaned onto their respective prenatal diets. F2 and F3 litters, unexposed to the folate treatments, were subsequently generated. Tissues and organs of interest were collected, and genome-wide DNA methylation analysis by reduced representation bisulfite sequencing (RRBS) was performed.

Results: Despite no apparent health effects in the F1 males, F2 litters derived from 7FD and 20FS exposed sperm were significantly smaller than FCD F2 litters at weaning. Preliminary analysis of RRBS results from F1 sperm (n=5) demonstrated that perinatal exposure to 7FD, 10FS, and 20FS diets resulted in 153, 132 and 114 differentially methylated (DM) loci, respectively. Affected regions included intergenic, intron, exon, promoter, 5’ and 3’ UTR sequences. Ingenuity Pathway Analysis of associated genes from DM loci implicated various affected pathways such as those involved in embryo development and cell cycle regulation.

Conclusion: These results suggest that lifetime FD and FS can impact sperm development and offspring health. DNA methylation changes in the sperm following these lifetime exposures offer a potential mechanism of action. (Supported by CIHR and CEEHRC).

IMPACT OF HIGH DOSE FOLATE SUPPLEMENTATION ON THE HUMAN AND MOUSE SPERM EPIGENOME

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(Presented By: Mahmoud Aarabi, MD, PhD)

Introduction: Supplementation with high doses of folic acid is widely used in clinics to improve the sperm parameters of infertile men. While dietary folate is a major source of methyl groups for epigenetic processes such as DNA methylation, little is known about the impact of high dose folate on the sperm epigenome and whether alterations can be transmitted to the offspring.

Methods: To address the epigenetic consequences of excess folate supplementation, semen and blood samples were collected from 30 men with idiopathic infertility who received 5mg/day of folic acid for 6 months at the McGill University Reproductive Centre and the Clinique OVO, Montreal, Quebec. Folate and hormone levels, semen parameters and the sperm epigenome were investigated before and after treatment. Germ line-specific differentially methylated regions of the imprinted genes H19, DLK1/GTL2, SNRPN, KCNQ1OT1, PLAGL1 and MEST were screened in sperm by pyrosequencing. A next generation sequencing—based method, reduced representation bisulfite sequencing (RRBS), was utilized to study over 3 million DNA methylation sites across the sperm epigenome. RRBS was also used to study the sperm epigenome in Balb/C mice (n=7) treated with high dose (20-fold control) or control folate diets for 12 months.

Results: Blood folate levels increased significantly following the supplementation period (P<0.0001). Sperm parameters and blood homocysteine, vitamin B12 and hormones remained unchanged. Neither infertility nor excess folate affected the methylation levels of imprinted loci. Interestingly, preliminary analysis of RRBS revealed slight but significant loss of methylation across genic and intergenic regions of sperm DNA in both human and mouse although some specific sites demonstrated gain of methylation. Ingenuity Pathway Analysis of differentially methylated sites suggested changes in methylation of genes involved in pathways related to cancer and developmental disorders.

Conclusion: Six month of folate supplementation in infertile men increases blood folate levels while sperm parameters and DNA methylation at imprinted loci remain unchanged. Unexpected loss of methylation across the sperm epigenome suggests the involvement of other factors such as folate metabolic pathways. We are now performing in-depth analysis as well as validation of the RRBS findings. Supported by Canadian Institutes of Health Research (CIHR).
THE IMPACT OF LEUKOCYTOSPERMIA ON CHROMATIN CONDENSATION, STRUCTURE INTEGRITY, DNA FRAGMENTATION AND CORRELATION WITH OTHER SPERM PARAMETERS

Ruben Burnazyan, MD, PhD and Mohamed Hammadeh, Prof
(Presented By: Ruben Burnazyan, MD, PhD)

Introduction: Infertility is a worldwide problem, affecting up to 8% of married couples and in some countries much more (WHO 2010). Male factors account for 20% – 50% of cases of infertility and in 25% of cases, the etiology of male infertility is unknown. (Jung, Seo 2014). In some patients infertility is presented on the background of normal conventional semen parameters: concentration, motility, morphology. In some cases the only disturbance, which been documented in semen was leukocytospermia. Infections/inflammations of the man genitourinary tract account rather high value −for approximately 9% of cases of male infertility and are associated with elevated seminal white blood cells (WBC)–leukocyte counts (Weidner et al.2013). DNA in sperm cells is packed heterogeneously. Peripheral portion of it (15%) is packed with histones and its inner core has a highly compact crystalline structure bound to protamines (Montellier et al.2013). So disturbances in nuclear condensation or changes in histone (H2B) to protamine ratio could lead to male infertility. And it is very advisable to evaluate sperm quality by investigating such parameters as DNA damage, abnormal nuclear packaging and nuclear decondensation in patients with leukocytospermia.

Methods: Semen was analyzed according WHO guidelines, WBC (leukocytes) in semen were estimated by peroxidase method. Chromatin condensation assessment was performed, using chromomycin (CMA3) staining method (Bianchi et al.1993). Assesment of chromatin structure integrity was performed by modified Sperm Chromatin Structure Assay (mSCSA) after staining of smears with acridine orange. DNA fragmentation of spermatozoa was assessed using the TUNEL assay. Results: The obtained results showed statistically significant correlation between MDA and WBC (p<0.001) and “round cells” (p=0.0084). Besides, a significantly positive correlation was found also between chromatin condensation (CMA3) of spermatozoa and sperm concentration (p=0.002), total and progressive motility (p=0.039), morphology (p=0.027) and vitality (p=0.019). DNA strand breaks (TUNEL) correlates not significantly, negatively with motility (p=0.600), vitality (p=0.467), and membrane integrity (p=0.105), but not with morphology and density.

Conclusion: Correlation between MDA and leukocytes as well as “round cells” points to an association of these cells and the induction of lipid peroxidation in sperm cells and may explain the decrease of the fertilizing potential of spermatozoa in infertile patients.

CAFFEINE AND MELATONIN SUPPLEMENTATION IMPROVES MOTILITY PARAMETERS AND MITOCHONDRIAL ACTIVITY IN POST-THAW SEMINAL SAMPLES: INITIAL REPORT

Juliana Pariz, MSc, PhD student¹,² and Jorge Hallak, MD, PhD¹,²
¹Androscience; ²Universidade de São Paulo
(Presented By: Juliana Pariz, MSc, PhD student)

Introduction: Although semen cryopreservation in an effective method, able to fertilize an oocyte and generate a healthy child, the damage it can impair the structural and functional integrity of spermatozoa. Our objective is to evaluate the effects of caffeine (CAF), a stimulant, and melatonin (MEL), an antioxidant, in motility and mitochondrial activity post−thaw semen samples.

Methods: Were selected seven semen samples of patients with infertility. As inclusion criteria, we consider only samples with total motility ≥50%. After collection by masturbation, the samples were analyzed according to WHO criteria, processed and cryopreserved in liquid nitrogen with Test Yolk Buffer (1:1). Each sample was divided into four aliquots: Post-thaw (without supplementation), CAF (incubation for 15 minutes with CAF 2 mM after thawing), MEL (MEL 2 mM before cryopreservation) and CAF+MEL (MEL 2mM before cryopreservation and incubated for 15 min with CAF 2mM after thawing) groups. Were performed sperm motility analysis and mitochondrial activity by 3,3´−diaminobenzidine (DAB) method in all samples. We conducted ANOVA test of the means adopted and p <0.05.

Results: The cryopreservation method decreased seminal volume, total progressive motile sperm number, total motile sperm number, total sperm number and sperm concentration parameters of CAF, MEL and CAF+MEL groups when compared with fresh semen (p<0.001). There was a significant improvement of non−progressive motility, DAB I and DAB III parameters of CAF+MEL group when compared to other study groups, resembling the values of fresh semen.

Conclusion: In this initial study, we suggest that supplementation with caffeine and melatonin in cryopreserved semen samples improves motility parameters and mitochondrial activity and may contribute to future implementation in routine semen cryopreservation and assisted human reproduction procedures.
Introduction: Sperm DNA integrity is important for accurate transmission of genetic information to the offspring. There are different molecular factors that explain the origin and impact of sperm DNA damage. Telomeres are highly conserved hexameric repeats part of sperm DNA, which confer chromosome stability & maintain genomic integrity. Telomerase a reverse transcriptase maintain telomere length. As telomeres are Guanine rich repeats, they are highly prone to oxidative damage. Sperm transcript & DNA repair enzymes have a role in DNA integrity. So, this study was planned to evaluate seminal oxidative stress, sperm DNA damage, sperm telomere length & expression of DNA repair genes in infertile men.

Methods: The study included 112 infertile men and 53 controls. Reactive oxygen species estimation was done by chemiluminescence method. The average telomere length from the sperm DNA was measured using a quantitative Real Time PCR. 8-Hydroxy-2-deoxy-Guanosine level was assessed by Cayman’s ELISA kits. DFI was assessed by Sperm Chromatin Structure Assay (SCSA). Microarray of sperm transcript was done & genes selected were validated by real time PCR.

Results: The mean ROS was significantly elevated in cases (66.61±28.32 RLU/sec/million sperm) compared to controls (14.04±10.67 RLU/sec/million sperm). The 8-OHdG level in patients were 30.92±3.27 pg/ml and in controls 14.29±2.24 pg/ml. The mean DNA Fragmentation Index (DFI %) in patient was 36.11 ± 13.69 and in controls 24.17 ± 8.7. The mean telomere length was significantly lower in patient group (ROS>35) as compared to patient group (ROS<22) but it significantly increased in the patient group (ROS=22−35) as compared to patient group (ROS<22). 1077 genes were dysregulated from which 282 were up regulated &790 were down regulated.

Conclusion: In this study we found that in infertile patients oxidative stress leads to sperm DNA damage and telomere shortening. Elevated ROS levels lead to telomere shortening but seminal ROS to a particular level (ROS=22−35 RLU/sec/million sperm) are protective in maintaining telomere length. As an important pathway in the DNA damage repair network, genes of repair mechanisms also play a critical role in the maintenance of genome integrity.

Method: The study included 112 infertile men and 53 controls. Reactive oxygen species estimation was done by chemiluminescence method. The average telomere length from the sperm DNA was measured using a quantitative Real Time PCR. 8-Hydroxy-2-deoxy-Guanosine level was assessed by Cayman’s ELISA kits. DFI was assessed by Sperm Chromatin Structure Assay (SCSA). Microarray of sperm transcript was done & genes selected were validated by real time PCR.

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**Methods:** We conducted a cross-sectional study at two tertiary care hospitals & fertility clinics in Mumbai, India and interviewed 100 male patients of both primary and secondary infertility. The study was approved by ethics committee of the hospitals. In-depth interviews were conducted and verbatim was transcribed. Thematic analysis was done to identify the most expressive and elaborate themes of the data and identified three major areas under which themes were clustered: 1) Individual level, 2) Couple relationship, 3) Financial impact.

**Results:** Knowing and accepting the infertility status was the toughest thing for men. Many of the respondents suggested creating awareness regarding infertility and its treatment among people. Some of them also suggested for managing separate male infertility section with a male doctor. The study also showed decreased sexual satisfaction and stress during sexual activity in few men while trying for the conception. In addition to these findings, men undergoing IUI/IVF with donor sperms expressed the fear of not having their biological child. One of the major concerns was exorbitant cost of treatments.

**Conclusion:** The study suggests that clinics/hospitals should provide proper information to men regarding treatment protocol & budget before starting the infertility treatment. It is necessary to provide counselling services at every stage of treatment to satisfy the queries and to control the anxiety of men who are undergoing infertility treatment.

**ABSTRACTS**

**38**

**PROFILE DIAGNOSIS OF PATIENTS IN A PRIVATE SEMEN BANK**

Artemis da Silva, BSc¹,², Juliana Pariz, MSc, PhD student¹,² and Jorge Hallak, MD, PhD¹,²

¹Androscience; ²Universidade de São Paulo

(Presented By: Artemis da Silva, BSc)

**Introduction:** Semen cryopreservation as fertility preservation method has been used in various situations, especially in cases where the individual has or will have progressive loss of fertility. Within a sperm bank is important to determine the profile of patients, so that the conduct of the healthcare team is more specialized. Objective: To determine the patients profile of a private semen bank and to compare seminal parameters of patients with different diagnoses.

**Methods:** We included 132 samples of men aged 16 to 69, between 2000 and 2014, submitted to cryopreservation process. Collected samples were from ejaculate, parenchyma and epididymis fragments, and patients who did not sign the Informed Consent were excluded. Samples were classified into two groups: patients with cancer diagnoses (I) and patients who sought the clinic due to some andrological/urological situation (II). The means were compared between groups using T test for independent samples and adopted p<0.05.

**Results:** Group I consisted of 60 subjects with a mean age of 31.19 y.o. (±9.84). The main diagnostics in group I was testicular tumors (33%). Seventy-two patients, with 40.65 y.o. (±10.45) were included in group II. The main diagnostics were oligozoospermia (29.2%), vasectomy (22.2%) and azoospermia (18.1%). We observed that groups I and II have different profiles in pre-cryopreservation parameters, respectively: pH (7.80 vs. 9.8; p<0.056), total progressive motile sperm count (53.71 vs. 17.68; p<0019), total motile sperm count (104.91 vs. 32.53; p<0.009), total sperm count (160.90 vs. 63.15; p<0.033), sperm concentration (30.42 vs. 70.79; p <0.017), total motility (43.34 vs. 53.58; p<0.049), thus group II showed lower sperm quality when compared with group I. In post-thaw analysis, we observed statistical difference in motility grade B (10.40 vs. 5.97; p <0.042) between the groups.

**Conclusion:** Neoplasms, combined with immunosuppressive therapies and andrological/urological conditions associated with habits and lifestyle can reduce the male fertile potential. Sperm quality after cryopreservation was similar, except motility grade B, suggesting that, regardless diagnosis of patient, cryopreservation seems to be effective. Thus, in an overview, we can conclude that fertility preservation option can be applied in various diagnostic and needs to be widely disseminated to the lay population and especially for the medical population specialized in oncology and other medical professionals.

**39**

**WORLD HEALTH ORGANIZATION GUIDELINES AND ITS INFLUENCE IN CONDUCT OF VARICOCELE TREATMENT**

Tábata Martins, BSc student¹,²,³, Juliana Pariz, MSc, PhD student¹,² and Jorge Hallak, MD, PhD¹,²

¹Androscience; ²Universidade de São Paulo; ³Faculdades Metropolitanas Unidas

(Presented By: Tábata Martins, BSc student)

**Introduction:** Varicocele affects about 20% of the general population, and up to 40% of infertile men or sub-fertile. Surgical correction of varicocele, or varicocelectomy, is widely performed procedure in the treatment of male infertility, with significant improvement in motility and sperm morphology. The results of semen analysis are adopted as the main indicator for varicocelectomy. Objective: To verify the influence of reference values changes for semen analysis established by the World Health Organization (WHO) in conduct of varicocele treatment.

**Methods:** This retrospective study included infertile men (23–65 y.o.) of Andrology Clinic between the years 2000 and 2014, diagnosed with some degree of varicocele who underwent semen analysis according to the criteria of the World Health Organization (WHO 1999/2010). Patients with varicocele were included in the study group (n=77) and without varicocele composed the control group (n=30). The means of seminal parameters were compared between groups using the T test for independent samples analysis and adopted p<0.05.

**Results:** When compared with control group, presence of varicocele had detrimental effects on sperm concentration (61.63 vs. 189.74 millions/ml; p<0.001), total sperm count (196.39 vs. 457.91 million/ejaculate; p<0.001), total motile sperm count (118.41 vs. 284.77 millions/ejaculate; p<0.001), total progressive motile sperm count <0.042) between the groups.

**Conclusion:** Variocoele affects about 20% of the general population, and up to 40% of infertile men or sub-fertile. Surgical correction of varicocele, or varicocelectomy, is widely performed procedure in the treatment of male infertility, with significant improvement in motility and sperm morphology. The results of semen analysis are adopted as the main indicator for varicocelectomy. Objective: To verify the influence of reference values changes for semen analysis established by the World Health Organization (WHO) in conduct of varicocele treatment.

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(47.49 vs. 124.85 millions/ejaculate; p<0.001), increase in non-progressive sperm (25.93 vs. 20.96%; p=0.044) and activity of enzyme Creatine Kinase (0.50 vs. 0.14 U/108; p=0.041). In addition, the differences of seminal analysis diagnosis in according WHO guidelines were illustrated in Table 1.

**Conclusion:** New reference values published by WHO in 2010 are being questioned by the medical community. It is well established in the literature that varicocele is a factor of male infertility and the varicocelectomy should be indicated in cases of seminal changes. This procedure has a direct injury front changes the values of reference of the WHO, whereas patients who were referred for surgery in 1999 values, does not currently perform varicocelectomy.

| Table 1: Seminal analysis diagnosis frequency in according to 1999 and 2010 World Health Organization guidelines |
|-------------------|-------------------|
| WHO               | 1999              | 2010              |
| Normozoospermia   | –                 | 14.9%             |
| Oligozoospermia   | 1.7%              | 5.8%              |
| Asthenozoospermia | 0.8%              | 21.5%             |
| Teratozoospermia  | 17.4%             | 7.4%              |
| Oligoasthenozoospermia | 0.8% | 8.2% |
| Oligoteratozoospermia | 9.1% | 9.1% |
| Asthenoteratozoospermia | 42.1% | 20.7% |
| Oligoasthenoteratozoospermia | 28.1% | 12.4% |

**Results:** Flow cytometric analysis of normozoospermic and mTESE samples showed high frequencies of cells displaying a DNA content profile corresponding to diploid cells, which include somatic cells, spermatogonia as well as secondary spermatocytes. Two additional cell populations could be identified: a low percentage of cells showing DNA content corresponding to tetraploid cells (4N) which include primary spermatocytes and up to 15% haploid cells (1N) in mTESE tissue indicative of cells that have completed second meiosis (spermatids). High frequencies of haploid cells were detected in a significant proportion of samples where the embryologists failed to retrieve spermatozoa.

**Conclusion:** Flow cytometric analysis of NOA diagnosis in mTESE cases. The detection of haploid cells in mTESE samples where the embryologists failed to retrieve spermatozoa suggests that a flow cytometry approach could improve the efficiency and success of finding gametes for ICSI. We plan to sort the haploid cells using PI or novel viable cell sorting markers and evaluate morphology and differentiation status.

41 **THERAPEUTIC POTENTIAL OF MUCONA PRURIENS (LINN.) ON ERECTILE DYSFUNCTION DUE TO SCHWANN CELL DAMAGE IN DORSAL NERVE OF PENIS INDUCED BY AGEING**

Prakash Seppan, PhD, Ibrahim Muhammed, PhD, Karthik Ganesh Moharanj, MSc, Ganesh Lakshmanan, MSc, Dinesh Premavathy, MSc, Sakti Jothi Muthu, MSc and Khayinmi Wungpam Shimray, MSc University of Madras

(Presented By: Prakash Seppan, PhD)

**Introduction:** Erectile dysfunction (ED) can lead to poor quality of life in elderly population. One of the important factors behind ED is the loss of integrity in dorsal nerve of penis (DNP), affecting towards achieving and maintaining erection. Mucuna pruriens (M. pruriens), a leguminous plant identified for its properties like aphrodisiac and improving fertility, in Indian traditional medicine. Bioavailability and natural source of essential bioactive compounds in the seed of MP has been the motivating force for the design of this study. Objective: To analyze the therapeutic efficacy of M. pruriens on the structural and functional alterations of DNP in ageing in relation to penile erection, using Wistar albino rat

**Methods:** Grouping: Young (3 months), Aged (24–28 months), Aged+ M. pruriens and Young+ M. pruriens (ethanolic extract of the seed at dose of 200 mg/kg b.w for 60 days). Rats were subjected to hypo–thalamo–hypophysial–gonadal axis, nerve conduction velocity (NCV) and penile reflex. DNP processed for electron (EM) & light microscopic studies, nNOS and NADPH diaphorase. Osmium tetroxide stained slides were used for histometric analysis.

**40 FLOW CYTOMETRIC EVALUATION OF DNA CONTENT FOR CLASSIFICATION OF NON–OBSTRUCTIVE AZOOSPERMIA MTESE SAMPLES**

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(Presented by: Ekaterina Shlush, MD)

**Introduction:** Non-obstructive azoospermia (NOA) caused by failed or arrested spermatogenesis contributes to 60% of azoospermia. Successful retrieval of rare spermatozoon by microdissection testicular sperm extraction (mTESE) is a time-consuming, variable and labour intensive process but has allowed thousands of men to father biological children. There is a need to develop efficient automated methods to confirm hypospermia and to retrieve sperm in a clinical setting.

**Methods:** Testicular tissues were obtained by mTESE in patients with NOA. The extensive search for mature spermatozoon or elongated spermatids was performed by two qualified embryologists. Waste testicular tissue material was used to perform a flow cytometric analysis of DNA content (ploidy) in dissociated testicular cells using propidium iodide. Normozoospermic orchietomy tissue samples were used as positive control. Data was analysed using FlowJo.
**Results:** Aged rats showed significant disturbance in hypo-thalamo-hypophysial-gonodal axis, degenerative changes in DNP includes demyelination, reduced diameter and number of myelinated fibres and in EM study, vacuolization and indentation of myelin sheath and Schwann cells degeneration were seen. nNOS and the cofactor (NADPH diaphorase) were reduced along with low levels of antioxidants. NCV was slow in aged rats and concomitant poor penile reflex. These pathological changes were remarkably reduced or recovered in M.pruriens treated aged rats. No pathology was seen in Young+M.pruriens.

**Conclusion:** Schwann cells degeneration plays crucial role in ED in ageing (reflexogenic erections) and its involvement in nitricergic/neuroparaxial degenerations. Significant reduction or recovery in DNP after M.pruriens treatment indicates the therapeutic potential of extract related with increased antioxidants level or reduced oxidative stress and possible remyelination by androgenic potential, indicative of neural androgen receptor as a promising therapeutic target for myelin repair.

**ABSTRACTS**

**Results:** Twenty-five patients met study criteria. Twelve patients (50%) presented for infertility, and the remaining 13 had symptomatic varicocele associated with hypogonadism. Average post–surgical follow up was 18.8 ±10.6 months. Baseline characteristics are presented in Table 1. Significant improvements in the total MSHQ score (4.0 ±8.4, p=0.041), the MSHQ erectile function (1.5 ±2.8, p=0.012), and the MSHQ ejaculatory function (1.9 ±3.9, p=0.033) domains were seen. Eleven (44%) men saw improvement in their erectile function and 14 (56%) saw improvement in ejaculatory function. The improvement in serum testosterone was also significant (177.5 ±226.9, 0.009). Eleven (44%) men saw improvement in their erectile function and 14 (56%) saw improvement in ejaculatory function. The improvement in serum testosterone was also significant.

**Conclusion:** Microsurgical repair of varicocele not only improves testosterone, but also improves patient reported erectile and ejaculatory function. Patients can confidently be counseled that varicocelectomy has the potential to improve sexual function along with serum testosterone.
Results: The mean ±SD age of the men was 53.6 ±8.2 years. Sixteen (100%) of the men had a positive ADAM questionnaire, with an average of 8.3 ±1.7 positive responses out the ten questions. Thirteen men (81%) had two serum AM testosterone <300 ng/dL, with an average of 191.0 ±76.9 ng/dL. The luteinizing hormone for the hypogonadal patients was 2.55 ±1.64 IU/mL, all consistent with hypogonadotropic hypogonadism. The average SHIM score was 14.3 ±6.8, with only 4/13 (31%) having no or mild erectile function. The mean MSHQ-EjD score was 6.4 ±3.5 (possible range 1–15, higher is better), with an average MSHQ bother score of 2.7 ±1.8 (on Likert scale 0–5, higher is more bother). Eight (50%) of men experienced ejaculatory dysfunction at least half time, and nine (56%) men were at least moderately bothered by their symptoms. While the MSHQ bother score did correlate inversely with the SHIM (r=−0.61, p=0.026), some two (13%) men experienced ejaculatory dysfunction despite preserved erectile function.

Conclusion: Opioid induced androgen deficiency is common in men with symptoms of hypogonadism using chronic opioids. Erectile dysfunction and ejaculatory dysfunction are commonly noted in these men. However, querying men for erectile dysfunction alone may miss significant ejaculatory dysfunction in this population.

44 EFFECT OF AQUEOUS CISSAMPELOS CAPENSIS EXTRACT ON PROSTATE CANCER,LEYDIG AND SERTOLI CELL FUNCTION
Keenan Pearce, MSc¹, Donavon Hiss, PhD¹, Frans Weitz, MSc¹, Uta-Christina, Hipler PhD², Cornelia Wiegand, PhD² and Ralf Henkel, PhD¹
¹University of the Western Cape; ²University of Jena (Presented By: Ralf Henkel, PhD)

Introduction: In Africa, 80% of the population depends on traditional remedies for their primary health care. Cissampelos capensis is a widely used medicinal plant in South Africa that is a rich source of different alkaloids. Traditionally, this plant is used to treat diabetes, menstrual cramps, pain and different types of cancer. Since no scientifically documented information concerning the efficacy of C. capensis on normal testicular function or its use as an anti-cancer agent are available, this study aimed to investigate these aspects using the TM3 Leydig and TM4 Sertoli cell lines, and the prostate cancer cell line LNCaP.

Methods: TM3 Leydig cells, TM4 Sertoli cells and LNCaP prostate cancer cells were cultured under standard conditions in a mixture of 50% DMEM and 50% Ham’s F–12 medium supplemented with 2.5% FBS and 5% Horse serum, and RPMI–1640 medium, respectively, and were exposed to concentrations ranging from 0.001–1000µg/ml of an aqueous extract of C. capensis over 24 and 96 hours, after which the XTT assay was performed to. LNCaP cells were cultured without and with 1000 nM testosterone. The effect on testosterone production in Leydig cells was determined with standard ELISA technique.

Results: Cell viability showed a significant change in TM3 cells at 1000µg/ml over 24–hours, while changes were found at concentrations higher than 100µg/ml after 96–hours. TM4 cells showed a significant change in cell viability at 1000µg/ml over 24–hours, along with changes at concentrations greater than 10µg/ml over 96–hours. Over both 24 and 96–hour incubations, C. capensis produced no biologically significant change in testosterone production in Leydig cells and when used in the presence of testosterone, increased the effectiveness of the extract.

Conclusion: C. capensis produces no observable negative effects towards Leydig and Sertoli cell function and shows no potential for an androgen replacement therapy. However, it might have the potential to prevent the formation of prostate cancer or slowing the progression of prostate cancer. The latter feature might be possible in conjunction with other therapy options as this plant might cause a decrease in the therapeutic concentrations to be used.

45 RAPID METHOD FOR THE ISOLATION OF SPERM DNA
Haotian Wu, Matthew de Gannes, Gianna Luchetti and J. Richard Pilsner
Department of Environmental Health Sciences, UMass Amherst (Presented By: Haotian Wu)

Introduction: There is a growing interest in elucidating the role of sperm epigenetics and genetics on reproductive success and the trajectory of health outcomes over the lifecourse. However, the condensed nucleus of sperm is resistant to lysis by buffers from commercially available column-based DNA purification kits due to the formation of disulfide bridges between protamines. Our objective was to develop a rapid method for extracting high quality DNA from human sperm for downstream DNA methylation and genetic analyses.

Methods: Sperm from semen samples provided by three human volunteers were isolated and homogenized in the presence of a commercially-available guanidine thiocyanate lysis buffer supplemented with different reducing agents. After homogenization, sperm DNA were extracted using silica–based column kits. DNA methylation analyses of imprinted loci were assessed using the MassARRAY platform (Sequenom).

Results: Our method resulted in yields > 90% of high quality DNA using three different commercially available spin kits. DNA yields did not differ between immediate extraction (2.84 ± 0.04 pg/cell) and after four weeks of homogenate storage at 4°C (2.91 ± 0.13 pg/cell). DNA methylation analyses revealed similar methylation levels at baseline and 4 weeks of storage for the imprinted loci: SNURF (3.5% ± 0.7% and 2.6% ± 1%), PEG10 (3.7% ± 1.8% and 6.9% ± 3.2%), and H19 (94.1% ± 0.1% and 92.2% ± 1.9%).

Conclusion: Our room temperature homogenization protocol resulted in > 90% yield of high quality sperm DNA extracted by user–preferred silica–based spin columns. Our homogenization method produces stable nucleic acids to allow for optional storage of homogenate for future DNA extraction. This method is also amendable for sperm DNA extraction of other mammalian species and RNA extraction using a previously published protocol. Together, our improved method has important implications for research in clinical settings where sample processing constraints likely exist.

46 – WITHDRAWN

ABSTRACTS

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ASSOCIATION BETWEEN PROSTATE–SPECIFIC ANTIGEN AND BIOMARKERS OF SUBCLINICAL SYSTEMIC INFLAMMATION IN MIDDLE−AGE HEALTHY MEN FROM THE GENERAL POPULATION

Saad Elzanaty, MD, PhD, Babak Rezanezhad, MD, Ronnie Willenheimer, MD, PhD and Rasmus Borgquist, MD, PhD

(Presented By: Saad Elzanaty, MD, PhD)

**Introduction:** To determine the association between PSA and biomarkers of subclinical systemic inflammation based on data from 119 middle-age healthy men from the general population.

**Methods:** Serum levels of PSA and biomarkers of systemic inflammation (CRP and fibrinogen) were measured. Demographic data were also collected. Subjects were divided into two groups according to PSA levels; < 2 µg/L and ≥ 2 µg/L.

**Results:** The mean (SD) age of men was 55±4.0 years. We found a positive significant correlation between PSA and fibrinogen (r = 0.20, p = 0.04), and between CRP and fibrinogen (r = 0.60, p = <0.001). On the other hand, no significant correlation between PSA and CRP was found. Men with PSA values ≥ 2 µg/L had a significant higher levels of fibrinogen as compared to those with PSA < 2 µg/L (2.9 g/L vs. 2.0 g/L, p = 0.01). In a multivariate regression analysis model adjusted for age, BMI, marital status, smoking, snuff, and alcohol intake with serum levels of PSA as dependent variable, serum level of fibrinogen predicted higher PSA−values (odds ratio = 3.30, 95 % CI = 1.05−10.20, p = 0.042).

**Conclusion:** The present results indicate that biomarkers of subclinical systemic inflammation are associated with elevated levels of PSA among healthy men from the general population. Further investigations are warranted in order to elucidate how this information could be applied in the daily clinical practice.

DO FAMILY MEMBERS OF INFERTILE MEN HAVE AN INCREASED RISK OF CANCER?

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(Presented By: Ross Anderson, MD, MCR)

**Introduction:** Male factor infertility is associated with an increased risk of genitourinary cancers. We sought to investigate if this risk of specific cancers extends beyond the men to their first-degree relatives. We hypothesized that family members of men with reduced sperm quality would have an increased risk of site-specific cancers.

**Methods:** We linked the University of Utah and Intermountain Healthcare semen analysis database with the Utah Population Database (UPDB), a multi-generational epidemiological database, which provides demographic, pedigree and medical information on over 7 million individuals. State death certificate data and the Utah and Idaho Cancer Registries identified cancer diagnoses. We stratified our semen analysis cohort by sperm count: azoospermia, cryptozoospermia (<0.5m/mL), oligozoospermia (<20m/mL), normozoospermia (>20m/mL), and hyperzoospermia (>100m/mL). First−degree relatives comprised siblings or parents, and only those family members with complete UPDB information were included. Cox models were run separately by gender, stratified by birth year, and adjusted for familial clustering.

**Results:** 24,732 men with 63,433 first−degree relatives underwent semen analysis between 1996 and 2014. The top 5 cancers diagnosed in the relatives of men undergoing semen analyses were: prostate (761), breast (683), melanoma (452), thyroid (184), and cervical (150). For female relatives of azoospermic men, there is a two-fold increase in thyroid cancer (HR=2.07,p=0.023), and test for trend showed a significant inverse relationship between thyroid cancer risk and sperm quality (HR=0.80,p=0.005). There is suggestive evidence that relatives of azoospermic men have a decreased risk of melanoma (HR=0.35,p=0.07). There was no significant difference in the risk of site−specific cancers we investigated in the family members of men with low sperm counts. We also considered the risk of cancer for siblings and parents separately, and the results do not substantively change.

**Conclusion:** This cohort of men with semen analyses linked with generations of population health data is the largest US study of its kind. We found that lower sperm quality is associated with higher rates of thyroid cancer in first−degree female relatives and possibly lower rates of melanoma. Sperm quality is not associated with increased risk of prostate or the other site−specific cancers we investigated, at least in first−degree relatives.

EFFECT OF SPERM DNA DAMAGE ON ART OUTCOMES: A SYSTEMATIC REVIEW AND META−ANALYSIS

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(Presented By: Luke Simon, PhD)

**Introduction:** Sperm DNA damage is prevalent amongst infertile men and is known to influence natural reproduction. However, the impact of sperm DNA damage on assisted reproduction (AR) outcomes remains controversial. The purpose of this study is to further evaluate the relationship between sperm DNA damage and ART outcomes by systematic review and meta−analysis.

**Methods:** We conducted a systematic review and meta−analysis of studies on sperm DNA damage (assessed by SCSA, TUNEL, SCD or Comet assay) and fertilization rate, embryo quality and clinical pregnancy after IVF and/or ICSI treatment.
Results: We identified all relevant papers published until April 2014 from MEDLINE, EMBASE, and PUBMED database searches for the systematic review. Two–by–two tables were constructed and odds ratios (ORs) were derived from 56 estimates of clinical pregnancy. These studies measured DNA damage (by one of 4 assays: 23 SCSA, 18 TUNEL, 8 SCD and 7 Comet) and included a total of 8,068 treatment cycles (3,734 IVF; 2,282 ICSI and 2,052 mixed IVF+ICSI). The combined OR of 1.68 (95% CI, 1.49–1.89; p<0.0001) indicates that sperm DNA damage is predictive of clinical pregnancy following IVF and/or ICSI. Moreover, the combined OR estimates of IVF (16 estimates, OR = 1.65; 95% CI, 1.34–2.04; p<0.0001), ICSI (24 estimates, OR = 1.31; 95% CI, 1.08–1.59; P=0.0068) and mixed IVF+ICSI studies (16 estimates, OR = 2.37; 95% CI, 1.89–2.97; p<0.0001) were also highly significant. Our systematic review demonstrated that sperm DNA damage has an adverse impact on fertilization rate and embryo quality in 38% and 36% of the evaluable studies, respectively.

Conclusion: This systematic review and meta–analysis is the first to demonstrate that sperm DNA damage is predictive of clinical pregnancy following IVF and/or ICSI. The data are strong enough to justify the clinical application of sperm DNA testing in the context of IVF and ICSI. These data also provide a rationale for conducting further research aimed at evaluating the underlying mechanism(s) responsible for the effect of sperm DNA damage on IVF and ICSI pregnancy outcomes.

50 SPERM CHROMATIN QUALITY ASSESSMENT: OPTIMIZATION OF THE HIGH THROUGHPUT COMET ASSAY

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(Presented By: Océane Albert, PhD)

Introduction: Various agents, including physical or chemotherapeutic agents, irradiation, or xenobiotics, can put human sperm DNA integrity at risk, echoing the growing concern about male fertility. To date, the standard semen parameters used to assess fertility, as described by the World Health Organization, do not include any information about the quality of sperm nuclear material. However, a number of studies show that there is sperm DNA damage in men with apparently normal standard semen parameters, and that this damage can imperil the outcome of pregnancy. The COMET assay (single cell gel electrophoresis) involves the collection of data on sperm DNA damage at the level of the single cell, allowing the use of samples from severe oligospermic patients. However, this particularity makes comet scoring a low throughput procedure that renders large cohort analyses tedious. Our objective is to develop a standardized high throughput COMET assay for human sperm that will increase both its accuracy and efficiency.

METHODS: The assay we have developed includes (i) automated mixing and distribution of sperm and low melting point agarose on a 96–well plate by the Janus® workstation, to ensure evenness across the plate and avoid artificial DNA damage trends; (ii) optimized cell lysis and DNA decondensation treatment parameters for human sperm; (iii) optimal horizontal electrophoresis conditions; (iv) automated detection of SYBR gold stained comets by the Operetta® high content imaging system; and (v) automated scoring of comets by the Columbus™ image data analysis system that comprises assessment of typical comet criteria (% DNA in the tail, tail length and tail extent moment) and that compares to the broadly used Comet™ software.

Results: This standardized high throughput COMET assay offers many advantages compared to the classical 2–well COMET, including higher accuracy and evenness due to automation of sensitive steps, a decrease of up to 90% in overall experimental time, and a 144 fold increase in sample analysis power.

Conclusion: Hence, this assay constitutes a more efficient option to assess sperm chromatin quality, and paves the way to using this assay to screen large cohorts.

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51 ROLE OF SPERM THIOLS’ REDOX STATUS IN KEEPING RAT SPERM QUIESCENT IN CAUDA EPIDIDYMIS

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(Presented By: Santosh Yadav, PhD)

Introduction: The sperm require energy-intensive motility to reach the female gamete for delivering the male genome. This energy is conserved by keeping the sperm quiescent in cauda epididymis before ejaculation. A variety of mechanisms have been proposed to explain the activation of mammalian sperm motility at ejaculation, however the molecular mechanisms controlling this process remain an enigma. Cauda sperm produce H2O2 and we have made an attempt to study the redox regulation of sperm motility in conjunction with other factors.

Methods: Quiescent and motile sperm were collected from Sprague Dawley rat cauda epididymis and experimented in vitro. Care was taken to prevent motility initiation of quiescent sperm on isolation from epididymis.

Results: The quantitative estimation of free thiols showed that motile rat sperm possess about 2-fold free thiols than quiescent sperm. Caudal sperm failed to initiate motility in presence of 0.1% sulfhydryl–alkylation N-ethylmaleimide (NEM), and when applied vaginally before mating 50 mg NEM prevented pregnancy in rabbits. Motile sperm quickly lost motility when placed in 0.1% H2O2, while quiescent sperm did not initiate any motility in 3% H2O2. Caudal sperm remained immotile at pH 4.0, and between pH 4 and 6.5 the motility ranged from zero to control level. Caudal sperm could initiate appreciable motility in a gel of viscosity equal to caudal semen (82 cP).

Conclusion: Besides low pH and caudal fluid viscosity, redox status of thiols plays a crucial role in rat sperm quiescence and motility initiation.
**ABSTRACTS**

52

ENERGY METABOLISM OF QUIESCENT SPERM IN CAUDA EPIDIDYMIS OF RAT
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(Presented By: Lokesh Kumar, MSc)

**Introduction:** Mature mammalian spermatozoa are held quiescent in the cauda epididymis to conserve energy. While the energy metabolism of motile sperm has been studied well, little is known about the energy metabolism of quiescent sperm, especially the changes associated with motility initiation. We hypothesize that during the quiescent state, sperm may have a different mode of energy generation as the energy requirement is very low, in comparison to motile sperm.

**Methods:** Motile and quiescent rat sperm were isolated from rat cauda epididymis. Care was taken to prevent any motility activation in quiescent sperm sample. All studies were conducted in vitro.

**Results:** Motility initiation of caudal sperm in rat was associated with a 1.5 to 2 fold increase in activities of the rate limiting enzymes of glycolysis along with an increased expression of HIF–1. Motility activation also increased expression of HSP70 and phosphorylation of MAPK/ERK in sperm. Active pAMPK was associated chiefly with quiescent sperm and was mostly dephosphorylated in the motile rat sperm.

**Conclusion:** Quiescent sperm are at a low energy level with high AMPK activity indicating high AMP:ATP ratio, which may drive sperm to use oxidative metabolism for ATP generation. A rapid drop in redox potential of sperm thiols on motility initiation (unpublished observations) coincides with the activation of MAPK–ERK pathway, activating (by phosphorylation) a number of enzymes including those of energy metabolism. The high energy demand of motile sperm creates hypoxic condition which increases HIF–1 and HSP–70 expression and apparently shifts energy metabolism towards glycolysis.

53

CRYOPRESERVATION OF SPERMATOZOA: DO PERMEABLE CRYOPROTECTANTS IMPROVE MOTILE SPERM YIELDS?
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(Presented By: Cigdem Tanrikut, MD)

**Introduction:** Preserving sperm while maintaining adequate post–thaw motility remains a challenge in patients with severe male factor infertility. This study sought to assess whether: 1) a permeable cryoprotectant yields better results than the non–permeable cryoprotectant, trehalose, and 2) the combination of trehalose and a permeable cryoprotectant improves post thaw sperm motility.

**Methods:** Motile sperm were isolated from fresh ejaculates of 10 healthy men using a density gradient. Two to three repeats per sample per treatment were applied. In part 1, 7 different freezing media were compared, made up of human tubal fluid (HTF), 5% human serum albumin (HSA) and one of the following: 5% 1,2–propanediol (PrOH), 5% glycerol, 5% dimethyl sulfoxide (DMSO), 10% PrOH, 10% glycerol, 10% DMSO, or 0.25M trehalose. Aliquots of sperm were mixed 1:1 with freezing medium then loaded into a 200 μm silica capillary. The capillary was incubated in liquid nitrogen (LN2) vapor for 5 minutes then lowered into LN2 and stored. To thaw, each capillary was quickly immersed in a room temperature water bath. Capillary contents were expelled into a drop of 12 µl HTF with 5% HSA on a glass slide, then covered by a coverslip and sperm motility was examined.

In part 2, 10 different media were compared. Baseline medium was HTF supplemented with 5% HSA and 0.25M trehalose, and the others were baseline with: 2.5% PrOH, 2.5% glycerol, 2.5% DMSO, 5% PrOH, 5% glycerol, 5% DMSO, 10% PrOH, 10% glycerol, or 10% DMSO. The same vitrification was performed. One–way ANOVA was used to compare group means. Differences were considered significant at P < 0.05.

**Results:** For the 7 freezing media in part 1, recovered sperm motility (post thaw motility/pre–freeze motility × 100%) was 36.7%, 42.8%, 43.3%, 22.2%, 43.8%, 46.2%, and 63.6%, respectively. Samples containing 0.25M trehalose achieved the greatest recovered motility. For the 10 media in part 2, recovered sperm motility was 70.3%, 58.7%, 73.5%, 74.7%, 49.2%, 64.2%, 66.0%, 33.3%, 64.8%, and 65.3%, respectively. The media composed of 0.25M trehalose, 0.25M trehalose with 2.5% glycerol, and 0.25M trehalose with 2.5% DMSO had the highest rates of recovered motility without significant differences among them.

**Conclusions:** PrOH, glycerol, or DMSO alone was not as effective as 0.25M trehalose in preserving sperm motility. Adding 2.5% glycerol or DMSO to a medium containing 0.25M trehalose did not significantly improve sperm motility.

54

THE IMPACT OF OXIDATIVE STRESS ON CHAPERONE–MEDIATED HUMAN SPERM–EGG INTERACTION
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The University of Newcastle
(Presented By: Brett Nixon)

**Introduction:** An inability to bind to the zona pellucida (ZP) is commonly encountered in the defective spermatozoa generated by male infertility patients; however, the underlying mechanisms remain unresolved. Recent studies have revealed that ZP-binding is mediated by molecular chaperones, particularly HSPA2, that facilitate the formation of multimeric zona pellucida (ZP)-receptor complexes on the surface of mammalian spermatozoa during capacitation. Given the well–established link between oxidative stress and male–factor infertility, we sought to determine whether such stress might impair sperm function by dysregulating the expression of ZP receptor complexes on the sperm surface.

**Methods:** For the purpose of this study, low levels of oxidative stress were induced in populations of human spermatozoa by treatment with 4-hydroxynonenal (4HNE) or hydrogen peroxide (H2O2).

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55 LOWER SEMEN QUALITY AS A MARKER FOR INCREASED FAMILIAL MORTALITY
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(Presented By: Mitchell Bassett, MD)

Introduction: Poor semen quality has been linked to increased mortality and disease burden in the infertile male. In order to determine if this association extends to relatives, we investigated the relationship between semen quality and mortality in first-degree relatives of men with poor sperm quality.

Methods: We identified 24,732 men with semen analyses between 1996–2014 at the University of Utah Health Sciences Center or Intermountain Healthcare linked to the Utah Population Database (UPDB). The UPDB is a comprehensive genealogical and epidemiological database, containing over 7 million individuals dating back to the 19th century. Men were grouped as azoospermic, cryptozoospermic (<0.5m/mL), oligozoospermic (<20m/mL), normozoospermic (>20m/mL) or hyperozoospermic (>100m/mL). All first-degree relatives of these men with complete information (N=63,433) were selected from UPDB for this analysis. Relatives were assigned an exposure group based on the semen quality of the male relative. Cox models were used to test for mortality of relatives by semen quality of tested men. All models were gender–specific and stratified by birth year and adjusted for familial clustering of observations.

Results: Male first degree relatives of azoospermic men have a 25% increase (HR=1.25, p=0.01) in mortality compared to relatives of normozoospermic men (control group). In addition, relatives of hyperozoospermic men have lower mortality (HR=0.91, p=0.02). A test for trend showed that there is an inverse relationship between mortality and sperm concentration levels (HR=0.93, p<0.0001). First–degree male relatives of azoospermic and oligozoospermic men have elevated rates of cardiovascular related mortality (HR=1.52, p=0.02; HR=1.38, p=0.005, respectively). First degree female relatives of azoospermic and oligozoospermic men have elevated rates of mortality caused by congenital malformation (HR=2.4, p=0.07; HR=2.0, p=0.02, respectively).

Conclusion: We find evidence of increased mortality for first degree relatives of men with poor sperm quality, however more research is necessary to understand the familial nature of this association and if it extends to 2nd and 3rd degree relatives.
57 AUTOMATIC SPERM TRACKING AND ANALYSIS OF SWIMMING PATTERN TRANSITIONS
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(Presented By: Leonardo Urbano, PhD)

Introduction: Our objective is to develop a fully automated sperm tracking algorithm for analyzing the transitions between sperm swimming patterns over time and with minimal operator intervention. Understanding how and why individual sperm swimming patterns change over time is of significant interest to researchers studying sperm motility and to medical practitioners evaluating male infertility.

A single sperm may transition between a number of different progressive and non-progressive swimming patterns, including linear, meandering, circular, and hyperactivated, and may stop swimming and restart again. Analysis of swimming transitions is difficult using today’s computer-assisted semen analysis (CASA) instruments, which require significant user intervention to track sperm swimming in close proximity, or whose paths apparently collide. Similar problems have been addressed by modern target tracking algorithms originally developed for radar and video processing, and their methods can be used for sperm tracking and analysis.

Methods: Videos of 10 washed sperm samples were recorded and digitized at 200x magnification at 15 frames per second. A custom-made MATLAB program was developed to automatically detect sperm in recorded video frames and perform multi-sperm tracking. A joint probabilistic data association (JPDA) filter was used to de-conflict sperm tracks during apparent cell-to-cell collisions.

Results: Sperm motility parameters, including curvilinear velocity (VCL), straight-line velocity (VSL), linearity (LIN), average velocity (VAP), straightness (STR), wobble (WOB), mean angular displacement (MAD), and amplitude of lateral head displacement (ALH) were calculated for each sperm tracked. These parameters changed abruptly at the onset of swimming transitions. For example, VCL and ALH typically decreased in sperm transitioning from linear to non-progressive swimming.

Conclusion: The JPDA algorithm was effective at tracking simultaneously hundreds of sperm through apparent collisions while calculating a host of sperm motility parameters, enabling analysis of transitions between sperm swimming patterns. The biological and clinical significance of these transitions merit further study.

58 EFFECT OF RESVERATROL ON SPERMATIC PARAMETERS OF ADULT RATS SUBMITTED TO EXPERIMENTAL VARICOCELE INDUCED IN THE PERIPUBERTY
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(Presented By: Talita B. Mendes, Master Student)

Introduction: Varicocele is the most common cause of male infertility. It happens primarily in the adolescence and has a progressive harmful effect on fertility. In addition, oxidative stress is the main factor responsible for these damages. Resveratrol, a natural fitoalexin, has several beneficial effects on the human body, including antioxidant and antiapoptotic properties. Objectives: To investigate the protective action of resveratrol against the reproductive damage caused by surgically induced varicocele.

Methods: Seventy-two peripubertal Wistar male rats (41dpp) were distributed into 4 groups: Sham−Control (S); Varicocele (V); Resveratrol (R) and Varicocele treated with Resveratrol (VR). Varicocele was induced in V and VR animals at 41 days postpartum (dpp), through the partial ligation of the left renal vein; for this goal, a 2−0 cotton thread and a catheter with diameter similar to an epidural catheter were utilized. The rats from S group were submitted to the similar surgical procedure, excepting the partial renal vein ligation. The groups R and VR received 300mg/kg/day of resveratrol by gavage, in the morning until the age of 100dpp (euthanasia). Spermatic parameters (morphology, mitochondrial activity, acrosome integrity and motility), testicular biometry and oxidative stress were investigated in these rats at 100dpp.

Results: There was a reduction of testicular major axis in the group V when compared with the S, R and VR groups; the testicular volume was reduced in the V group in comparison to the S and R groups but not in comparison to the VR group. The frequency of morphologically abnormal sperm was higher in the V and VR groups than in S and R groups. The frequency of sperm showing 100% of mitochondrial activity and showing normal acrosome integrity was lower in the V group than in VR, S and R groups. Sperm motility was also reduced in the V group when compared to the other groups. Defects of acrosomal integrity and of mitochondrial activity and morphological abnormalities occurred in sperm collected from the right (contralateral side) and left epididymides (varicocele side). The testicular levels of malondialdehyde were higher in V and VR groups but no alteration in oxidative stress levels was observed between V and VR groups.

Conclusion: The preliminary results suggest that daily resveratrol administration to rats with induced−varicocele from peripuberty improves the sperm quality in adulthood. Complementary analyzes are being performed.

59 EFFECTS OF BETAINES SUPPLEMENTATION ON SPERM FUNCTIONAL PARAMETERS IN HUMANS AND MICE WITH DEFECTS IN CHOLINE METABOLISM
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(Presented By: Summer Goodson, PhD)

Introduction: An estimated 15% of couples experience reproductive issues and in approximately half of these the problem is attributed to male infertility. Genetic factors, including single nucleotide polymorphisms (SNPs) and alterations in genes that disrupt metabolism, may be responsible for some cases of idiopathic male infertility. Males carrying two minor alleles of the common rs12676 SNP, located in the gene encoding for the mitochondrial enzyme choline dehydrogenase (CHDH), have low sperm ATP concentrations, abnormal mitochondrial function and altered sperm motility. Male Chdh−null mice display a similar phenotype: infertility due to defects in sperm motility, low sperm ATP concentrations and dysmorphic sperm mitochondria. Administration of dietary betaine (N,N,N−trimethylglycine) during spermatogenesis partially restores sperm
functional parameters in Chdh−null mice. Betaine is a naturally occurring nutrient in food and is the product of CHDH, and therefore presents a potential dietary intervention for the treatment of infertility.

Methods: We are now investigating whether betaine supplementation restores normal motility profiles (using CASAnova, a recently developed software program for assessing multiple patterns of sperm motility) and fertility in Chdh−null mice with demonstrated fertility defects. We also assess the impact of betaine on sperm metabolic flux in both normal and Chdh−/− sperm using the Seahorse XF24 Metabolic Flux Analyzer. We are also performing preliminary studies of the safety and efficacy of dietary betaine supplementation on sperm function in men harboring two minor alleles of rs12676. Men are consuming 6g of betaine per day for 75 days (approximately one round of spermatogenesis and epididymal transport in humans).

Conclusion: We will present data on the effect of betaine supplementation on ATP, motility, choline metabolites, and metabolic flux in sperm at Day 0, 10, 30, 50, and 75 of supplementation. These data will form the foundation for future studies of the therapeutic benefits of betaine supplementation on fertility in humans.

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60 ORIGIN OF THE STEROIDOGENIC POOL OF CHOLESTEROL USED IN CAMP−INDUCED ACUTE STEROID FORMATION

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Introduction: The hormone-sensitive and rate-limiting step in steroid biosynthesis is the movement of cholesterol from intracellular sources to the inner mitochondrial membrane (IMM) where it is converted to pregnenolone by the cytochrome P450 side chain cleavage enzyme (CYP11A1). Despite the numerous studies carried out to analyze cholesterol trafficking in steroidogenesis, the exact source of cholesterol as well as the mechanism by which it is transported to IMM remains to be elucidated.

Methods: D4 is the fourth domain of perfringolysin O protein, which has the ability to bind with high affinity cholesterol−enriched membranes, i.e. containing cholesterol greater than 30 mol% of total lipid. A fluorescent mCherry−tagged D4 was used to visualize cholesterol trafficking in hormone−responsive MA−10 mouse tumor Leydig cells.

Results: Confocal imaging microscopy showed that in D4−mCherry−transfected MA−10 cells, fluorescence localizes at the plasma membrane, but upon 30−45 minutes treatment with the cAMP analog dibutyryl−cAMP (dbcAMP) a significant reduction in plasma membrane labeling was observed. Functional inhibitors of the steroidalogenic acute regulatory protein (STAR), translocator protein (TSPO), voltage dependant anion channel (VDAC) and CYP11A1, proteins involved in cholesterol import into mitochondria and cholesterol metabolism to steroids, blocked steroid formation, and slowed down the movement of D4−mCherry from the plasma membrane. Treatment with the substrate 22R−hydroxycholesterol, which results in maximal steroid formation, also slowed down the D4−mcherry movement, suggesting that elevated formation acts as a feedback mechanism to control plasma membrane cholesterol release. D4−mCherry also localized at the late endosomes upon dbcAMP stimulation suggesting a route for the cholesterol from plasma membrane to mitochondria.

Conclusion: These data suggest that the bulk of the steroidalogenic pool of cholesterol, mobilized by cAMP for acute steroidogenesis, likely originates from the plasma membrane.

61 HENMT1 IS INVOLVED IN THE STABILIZATION OF PACHYТЕNE PIRNAs, RETROTRANSPOSON SILENCING AND REGULATING THE SPERMIOGENIC PROGRAM

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(Presented By: Shu Ly Lim, PhD)

Introduction: The Piwi-interacting RNA (piRNA) pathway is an RNA silencing pathway that represses the expression of transposable elements (TE) in the gonads via binding of piRNAs to their complimentary RNA targets. Mammalian piRNAs are 26−31 nt in length and are 2′−O−methylated at their 3′ termini. Although the biogenesis of piRNAs remains unclear, Hen1, a plant microRNA (miRNA) 2′−O−methyltransferase, is known to play an important role in piRNA stabilization. in Zebrafish and Drosophila. Herein, we aim to understand the role of murine HENMT1 and adult pachytene piRNAs in mammalian spermatogenesis.

Methods: We have generated a mouse model containing a point mutation in the Henmt1 gene that produces truncated and unstable Henmt1 transcripts.

Results: Henmt1 homozygous mutant males are sterile. They produce greatly reduced numbers of sperm with round heads, and the epididymis are virtually devoid of sperm. Deep sequencing and biochemical analyses revealed the loss of HENMT1 function results in piRNA instability i.e. the presence of 3′−end truncated piRNAs and significantly increased piRNA uridylation which may lead to RNA decay. Furthermore, increased expression of several transposable element (TEs) subtypes was found in Henmt1 mutants using real−time PCR, transcriptome analysis and in situ hybridisation. Transcriptome analyses also suggested the premature expression of many spermiogenic genes during meiosis in Henmt1 mutants. Our preliminary findings with histone chromatin immunoprecipitation (ChIP) and quantitative PCR suggested a role for piRNA in promoting a more heterochromatic state in meiotic cells similar to that seen in Drosophila.
Conclusion: In summary, our data suggests an important role for HENMT1 in regulating pachytene piRNA stability, post–natal TEs repression and the translational regulation of haploid germ cell mRNAs.

62 ACETAMINOPHEN VERSUS IBUPROFEN: EFFECTS ON NEONATAL TESTICULAR GONOCYTE DEVELOPMENT
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(Presented By: Gurpreet Manku, PhD)

Introduction: Newborn baby fever is often treated with acetaminophen (AC) (Tylenol®) or ibuprofen (IB) (Motrin®). Both drugs inhibit cyclooxygenases (COXs), enzymes responsible for producing prostaglandins, thromboxanes, and leukotrienes. COXs are involved in platelet aggregation, fever, and inflammation, processes that can be decreased by COX inhibitors.

There are two types of COX enzymes, COX1 and COX2. Although COX2 is not commonly known for a role in male reproductive biology, it has been reported to play a role in the steroidogenic function of Leydig cells. However, the possible role of COX2 in germ cells is not yet known.

Here, we report that COX2 is abundantly expressed in postnatal day (PND) 3 rat gonocytes, the precursor cells to spermatogonial stem cells which provide a life–long source for sperm production. PND3 gonocytes undergo proliferation, migration followed by differentiation, while abnormal cells are removed by apoptosis. Interestingly, COX2 expression was downregulated in PND8 spermatogonia, indicating a possible role in gonocyte development.

Considering the presence of COX2 in neonatal germ cells, our objective was to determine whether gonocyte proliferation or differentiation could be altered upon exposure to either AC or IB. This is an important question to address as improper gonocyte development has been suggested to lead to testicular tumor formation.

Methods: Isolated PND3 gonocytes were treated with either AC or IB alongside PDGF–BB and 17 estradiol (PE; proliferation) or Retinoic Acid (RA; differentiation).

Results: Although neither drug had any effect on cell survival (trypan blue exclusion analysis), we found that IB stimulated gonocyte proliferation to levels similar to those seen with PE treatment (determined by Proliferating Cell Nuclear Antigen (PCNA) immunohistochemistry analysis). Furthermore, IB reduced the effect of RA on mRNA expression of the differentiation marker Stra8 (Stimulated by RA 8) (quantitative PCR), indicating a negative effect of this COX inhibitor on differentiation, that was not seen using AC.

Conclusion: These data suggest that COX2 activity plays a dual role in gonocytes, being positively involved in gonocyte differentiation, while preventing proliferation. It will be interesting to identify which COX2 products are mediating these effects. Taken together, our data suggests that anti–pyretic medications such as IB could disrupt neonatal gonocyte development, which could potentially lead to the formation of testicular germ cell tumors.
64 IMPORTANCE OF SOMATIC NICHE IN REGULATING TESTICULAR STEM CELLS DIFFERENTIATION INTO SPERM
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(Presented By: Sandhya Anand, MSc)

Introduction: We have undertaken studies on busulphan treated mice testes to address fertility issues of cancer survivors. Very small embryonic-like stem cells (VSELs, <8 μm, Lin−/CD45−/SCA−1+) exist as a sub-population amongst spermatogonial stem cells (SSCs) in adult mice testes and survive busulphan treatment (BT) because of their quiescent nature (0.03±0.002% in normal versus 0.05±0.005% BT by flow cytometry). Spermatogenesis is suppressed in chemoablated testes, possibly due to compromised somatic niche which is crucial for normal proliferation and differentiation of stem cells. Transplantation of healthy niche cells including Sertoli cells (SC) and mesenchymal cells through inter-tubular route restored spermatogenesis from surviving stem cells. Present study aimed to understand the role played by transplanted SC and mesenchymal cells to restore spermatogenesis, in-depth analysis of niche in BT testis and in vitro stem cells−niche interaction to result in spermatogenesis.

Methods: SC and mesenchymal cells from GFP mouse were injected in testis of wild type BT mouse and studied on 1, 7, 14, 30 and 60 days post transplantation. To evaluate the effect of BT on niche, SC from normal and BT testis were subjected to microarray studies. In addition, cells from BT testis were cultured for three weeks in SC conditioned medium with 10% FBS and 0.5 IU FSH.

Results: Transplanted GFP cells showed formation of neo-tubule like structures in the interstitium. Adjacent germ cells depleted native tubules showed gradual resumption of spermatogenesis from VSELs which survived chemotherapy. Microarray analysis showed up-regulation of 1835 genes and down-regulation of 1768 genes after BT. Several signaling pathways including Wnt pathway (implicated in SC function) were affected. Up-regulation of Wnt4 and beta catenin was observed. During in vitro culture, SC from BT testis attached and provided support to the surviving stem cells which underwent proliferation, clonal expansion and differentiation into sperm within 3 weeks. Various stages of spermiogenesis were observed correlating with an increase in transcript levels of Pcna, Sca−1, Gfra, Prohibitin, Scp3 and Protamine.

Conclusions: Crucial role played by the somatic niche by secreting various factors in a paracrine manner to affect stem cells proliferation and differentiation is highlighted. Potential of VSELs to differentiate into sperm (unlike ES cells) in presence of a supportive niche is also demonstrated.

65 GHRELIN−INDUCED ATTENUATION OF TESTICULAR DAMAGE IN MOUSE CRYPTORCHID TESTES
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Introduction: Cryptorchidism or undescended testis (UDT) is a common congenital abnormality that is associated with increased risks for developing male infertility and even testicular cancer. It is also associated with germ cell loss and impaired spermatogenesis. Previous studies provided evidence of Ghrelin and its receptor expression in rat testis, demonstrating an involvement of this molecule in the direct control of gonadal function. We hypothesized that ghrelin, a 28 amino acid peptide predominantly found in the stomach, may play an important role in the attenuation of testicular damage induced by surgical cryptorchidism in mice.

Methods: C57BL/6 mice were subjected to creation of surgical unilateral cryptorchidism and were randomly separated into two groups: treatment group (ghrelin) and control group (saline). Mice received intraperitoneal injections of Ghrelin (0.16 mg/Kg) or saline twice a day for 20 days post-surgery. The animals were then sacrificed at 21 days after surgery and their testes were collected. Cryptorchid testes were embedded in glycol methacrylate for histological and immunohistochemical analysis. The following histological endpoints were used to assess testicular damage: testis weight, seminiferous tubule diameter, percentage of seminiferous tubules with spermatids and with giant cells.

Results: Our results suggest that ghrelin treatment exhibits a protective role in testicular damage. Testicular weights and seminiferous tubules diameters were significantly decreased in control mice compared to the ghrelin-treated animals. In addition, histological evaluation demonstrated that ghrelin significantly reduced formation of giant cells and preserved the spermatogenesis in the cryptorchid testes, and subsequent conservation of testicular architecture.

Conclusion: These findings indicate that ghrelin treatment exhibits a protective role in testicular damage. Testicular weights and seminiferous tubules diameters were significantly decreased in control mice compared to the ghrelin-treated animals. In addition, histological evaluation demonstrated that ghrelin significantly reduced formation of giant cells and preserved the spermatogenesis in the cryptorchid testes, and subsequent conservation of testicular architecture.

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LATE EFFECT OF NICOTINE ON THE SEMINIFEROUS EPITHELIUM OF THE OFFSPRING FROM RAT DAMS TREATED THROUGHOUT PREGNANCY AND LACTATION

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(Presented By: Camila Paccola, doctoral student)

Introduction: Nicotine is largely consumed worldwide through cigarette. It reaches maternal milk, is able to cross the placental membrane, induces apoptosis in different cell types and alters spermatogenesis. Objective: To investigate whether nicotine administration to pregnant and lactating rats, in a similar dose to human consumption (one packet of cigarettes/day), provokes in the offspring a late testicular damage involving apoptosis of both Sertoli and germ cells as well as functional changes in the Sertoli cell and/or in its cytoskeleton.

Methods: Fifteen rats received nicotine (2mg/Kg/day) throughout pregnancy and lactation via subcutaneous osmotic minipumps (N group). Other 15 pregnant rats had minipumps implanted but without nicotine (Sham group) and 15 pregnant rats did not receive minipumps (Control group). The male offspring was distributed in subgroups according to the euthanasia age (30, 60 and 90dpp). Plasmatic levels of FSH and LH were measured by Luminex™ xMAP methodology. The testes were processed for histopathological study and for evaluation of the frequency of the stages of the seminiferous epithelium cycle. The immunolabeling of apoptotic cells (TUNEL, Fas and FasL) and of transferrin, vimentin and β−catenin was performed in the seminiferous epithelium. The numerical density of TUNEL+ cells and the volume densities of Fas, FasL, transferrin, vimentin and β−catenin immunolabeling were obtained using an image analysis system. Expressions of vimentin and β−catenin proteins were investigated considering the stages of the seminiferous epithelium cycle.

Results: FSH and LH plasmatic levels were significantly increased in the N group, in adulthood. Nicotine did not induce changes in the apoptotic cell number nor Fas and FasL expression, but provoked pronounced seminiferous epithelium disorganization and large sloughing of germ cells. Alterations of the frequency of some stages of the seminiferous epithelium cycle of the N group were observed in the puberty and adulthood. Although the transferrin expression in the seminiferous epithelium did not change, vimentin expression was reduced in adult rats of the N group, especially in the early stages of the cycle.

Conclusion: Nicotine exposure during intrauterine and lactation phases provokes early and accentuated germ cell loss and alters the organization of the seminiferous epithelium cycle, the Sertoli cell vimentin expression and the plasmatic levels of pituitary gonadotropins in adulthood.

REQUIREMENT FOR ADENOSINE DEAMINASE CONTAINING PROTEINS IN MALE GERM CELL DEVELOPMENT

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Introduction: Adenosine deaminase, RNA−specific (ADAR) proteins are the only known drivers of adenosine to inosine (A-to-I) RNA editing. Murine ADARs (encoded for by Adar, Adarb1, and Adarb2) contain two conserved domains: an adenosine deaminase (AD) domain, which catalyzes A to I conversion, and one or more double−stranded RNA binding motifs (dsRBM). While expression of Adarb1 and Adarb2 is confined to neural tissue, Adar is observed in a wider range of tissues, including the testis. In addition, the testis expresses two closely related AD domain−containing proteins, Adad1 and Adad2. Both carry amino acid substitutions in critical regions of the AD domain, suggesting they do not have catalytic activity, although this has not been formally proven. Both ADADs contain dsRBMs similar to those found in ADARs, implying they may bind a similar set of targets. Expression profiling in isolated testicular cell types, throughout testis development, and in germ cell ablated mutant models demonstrated both Adad1 and 2 are expressed exclusively in the meiotic and post−meiotic germ cell populations while Adar is expressed in germ and somatic cells.

Methods: The extent of RNA editing in the testis was determined by applying a computational pipeline to high throughput RNA sequence data of isolated testicular cell types. This analysis demonstrated A to I editing in both the germ line and soma, with a much higher number discovered in Sertoli cells as compared to germ cells. To address the functional role of RNA editing in the testis and the specific requirement of AD−domain containing proteins in male germ cell development, we generated germ cell and Sertoli cell−specific knockout models of Adar, as well as CRISPR−induced mutant models of Adad1 and Adad2, respectively.

Results: Despite the occurrence of editing in both cell types, germ cell or Sertoli cell ablation of Adar had no appreciable impact on germ cell development. In contrast, mutation of either Adad1 or 2 resulted in male−specific sterility.

Conclusion: Tolerance for germ cell ADAR loss demonstrates ADAR−mediated editing is not essential for male fertility. However, the absolute requirement of both Adad1 and Adad2 for male fertility confirms a fundamental role of AD−domain containing proteins in germ cell development. Whether ADADs catalyze or regulate RNA−editing events in the germ line or have evolved essential functions outside of RNA editing is unknown. Current studies are aimed at distinguishing between these disparate hypotheses.
VITAMIN B12–INDUCED SPERMATOGONIAL MITOTIC ACTIVITY IN THE TESTES OF CIMETIDINE–TREATED RATS
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Introduction: Cimetidine, an antiulcer drug, exerts an antagonist effect on histamin H2–receptors. In rodents, this drug has caused significant disorders in male reproductive tract, including structural changes in the seminiferous tubules. Vitamin B12 plays an important role in DNA synthesis and cell division; supplementation of cimetidine-treated rats with vitamin B12 has demonstrated to recover the seminiferous epithelium. In this study, we investigated the effect of vitamin B12 on the mitotic and meiotic activities of spermatogenesis in cimetidine-treated rats.

Methods: Adult rats were distributed into four groups (n=5): Cimetidine (CMTG), cimetidine/vitamin B12 (CMT/B12G), vitamin B12 (B12G) and control (CG). CMTG received cimetidine (100mg/kg bw) for 50 days. CMT/B12G received cimetidine+3µg vitamin B12G and control (CG). CMTG received cimetidine (100mg/kg bw) for 50 days. CMT/B12G received cimetidine+3µg vitamin B12. B12G and CG received vitamin and saline, respectively. Sperm concentration was obtained and the testes were fixed and embedded in paraffin or historesin for detection of: a) cell death by TUNEL, b) cellular proliferation by PCNA immunohistochemistry; c) quantitative analyses of spermatogonia (A; In/B) and spermatocytes in tubules at stages I–VI, VII–VIII and IX–XIV. Data were statistically analyzed by one way ANOVA followed by Tukey test (p≤0.05).

Results: Cimetidine caused a significant reduction in sperm concentration, which increased in the vitamin supplemented animals of CMTG/B12. In CMTG, spermatogonia and spermatocytes showed apoptotic nuclear features and were TUNEL-positive. Moreover, a significant reduction in the number of spermatogonia (A and/or In/B) and spermatocytes was observed at all stages analyzed. In contrast, a significant increase in the number of In/B spermatogonia and a high incidence of PCNA-positive spermatogonia and spermatocytes was found in the tubules at stages I–VI of CMTG/B12, in comparison to CMTG. Although the number of spermatocytes and sperm concentration increased in CMTG/B12, it was not recovered at normal levels. Differences between CG and B12G were not found.

Conclusion: The results show that cimetidine treatment reduces the number of spermatogonia and spermatocytes. However, the vitamin B12–induced epithelial recovery is due to the potential effect of this vitamin on A4 and In/B spermatogonia of the cimetidine–damaged testes. Although vitaminB12 was able to recover spermatogonia number, the following spermatogenic processes (meiosis and spermiogenesis) could not be completely restored by this vitamin.

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