**VITAMIN B12–INDUCED SPERMATOGONIAL MITOTIC ACTIVITY IN THE TESTES OF CIMETIDINE–TREATED RATS**

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(Presented By: Flavia Luciana Beltrame, PhD)

**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 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 cellular proliferation by PCNA immunohistochemistry; c) quantitative 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 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.

**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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CURCUMIN TARGETS RAT TESTICULAR 11−HYDROXYSTEROID DEHYDROGENASE 1 TO ANTAGONIZE AGAINST STRESS−INDUCED INHIBITION OF TESTOSTERONE

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(Presented By: Xiaohe Li, MS)

Introduction: It has been demonstrated that stress induces male sexual dysfunction and infertility via excessive active glucocorticoid (corticosterone, CORT, in the rat). In adult Leydig cells, 11−hydroxysteroid dehydrogenase 1 (11−HSD1) alternates between oxidative inactivation of CORT and reductive regeneration of its metabolite 11−dehydrocorticosterone (11DHC). Curcumin is a natural product.

Methods: The effects of curcumin on rat 11−HSD1 in the intact adult Leydig cells and human 11−HSD1 in the intact transfected human HSD11B1 gene were examined in vitro by adding radiolabeled substrate and separating substrate and product in the thin−layer chromatograph and detecting the signal in the radiometer. The adult male rats were randomly divided into eight groups with 10 rats of each group: 1) no stress (vehicle); 2) no stress (curcumin 50 mg/kg); 3) no stress (curcumin 100 mg/kg); 4) no stress (curcumin 200 mg/kg) and 5–8) stress with the treatment of different doses of curcumin (one gavage before immobilization). Then rats were subjected to immobilization stress (stress group) or the no−stress setting.

Results: It was found that curcumin stimulated 11−HSD1 oxidase with the EC50 values of 2.82 µM for rat adult Leydig cells and 2.11 µM for CHOP cells transfected with human HSD11B1 gene. Curcumin also inhibited 11−HSD1 reductase with IC50 value of 5.71 µM for rat Leydig cells and 4.18 µM for human one. Acute immobilization stress (3h) caused significantly suppression of serum testosterone level (0.62 ± 0.13 ng/ml, Mean ± SEM, n = 10) when compared to control (1.72 ± 0.35 ng/ml). Gavage of curcumin (50 mg/kg) did not recover the loss of testosterone. However, gavage of 100 or 200 mg/kg curcumin significantly antagonized the reduction of serum testosterone. Gavage of curcumin did not change the circulating level of CORT levels. However, curcumin significantly reduced the testicular CORT levels.

Conclusion: In conclusion, curcumin dually modulates the testicular 11−HSD1, reducing testicular CORT levels thus against stress−induced suppression of testosterone biosynthesis.
HOW LONG SHOULD HYPOGONADAL SUBJECTS BE TREATED? INTERMISSION AND RESUMPTION OF LONG-TERM TESTOSTERONE REPLACEMENT THERAPY (TRT) AND EFFECTS ON HORMONAL AND ANTHROPOMETRIC PARAMETERS IN HYPOGONADAL ELDERLY MEN

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(Presented By: Farid Saad, DVM, PhD)

Introduction:
Little is known about optimal duration of TRT, and whether its withdrawal would lead to loss of effects and recurrence of symptoms.

Methods: In an ongoing registry study, 262 hypogonadal men (mean age 59 years) received testosterone undecanoate (TU) 1000 mg injections in 12–week intervals for a maximum of 11 years representing 2088.5 patient–years. After having been on TRT for a mean duration of 66 months, TRT was temporarily intermitted for a mean of 17 months in 147 patients (Group I; I): in 140 men due to cost reimbursement issues, and in 7 men diagnosed with prostate cancer. All men resumed TRT thereafter for a mean period of 14 months. 115 men were treated continuously (Group C; C). To compare on−treatment to off−treatment periods, three periods of equal duration were defined: pre−intermission (on TRT), during intermission (off TRT) and post intermission (on TRT after resumption of TRT). For comparison, the same periods were analysed for those patients who continued TRT throughout.

4 patients dropped out, and their data up to their last visits were analysed in group C.

Hormonal and anthropometric parameters were measured at every other visit.

Results: I: Total testosterone (T) was 16.54 pre, dropped to 7.51 during and increased to 18.50 nmol/L post intermission. C: Total T was stable at 19.61, 19.76 and 19.65 nmol/L.

I: SHBG was 29.85 pre, increased to 33.75 during and decreased to 24.81 nmol/L post intermission. C: SHBG was stable at 36.84, 35.57 and 32.27 nmol/L.

I: Free T was 375.16 pre, dropped to 149.88 during and increased to 466.38 pmol/L post intermission. C: Free T was stable at 418.98, 429.04 and 447.41 pmol/L.

I: Waist circumference was 100.16 pre, increased to 105.42 during and decreased to 102.29 cm post intermission. C: Waist circumference declined progressively from 98.42 to 97.20 and 95.75 cm.

I: Weight was 92.12 pre, increased to 97.12 during and decreased to 94.41 kg post intermission. C: Weight declined progressively from 94.41 to 97.12 and 93.57 kg.

In Group I, 6 patients had major adverse cardiovascular events (MACE) and 5 had major urological events while off TRT. There were no major adverse events of either kind in Group C or in Group I while patients were on TRT.

Conclusions: Interruption of TRT resulted in worsening of symptoms. Hypogonadism may require lifelong TRT.

INTERMISSION AND RESUMPTION OF LONG-TERM TESTOSTERONE REPLACEMENT THERAPY (TRT) AND EFFECTS ON METABOLIC PARAMETERS IN HYPOGONADAL ELDERLY MEN

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Introduction: Optimal duration of TRT is unknown.

Methods: 262 hypogonadal men received testosterone undecanoate (TU) in 12–week intervals. After a mean 66 months of TRT, TRT was intermitted for a mean 17 months in 147 patients (Group I; I). All men then resumed TRT for a mean period of 14 months. 115 men were treated continuously (Group C; C). For comparison, three periods were defined: pre−intermission (on TRT), during intermission (off TRT) and post intermission (on TRT after resumption of TRT). The same periods were analysed for those patients who continued TRT.

Metabolic parameters were measured at every other visit.

Results:
Glycemic control:
I: Fasting glucose was 104.14 pre, increased to 116.74 during and dropped to 89.20 mg/dl post TRT intermission. C: fasting glucose decreased progressively from 92.65 to 88.34 and 80.20 mg/dl.

I: HbA1c was 5.94 pre, rose to 6.71 during and decreased to 5.97% post intermission. C: HbA1c decreased continuously from 5.77 to 5.70 and 5.58%.

Lipid pattern (mg/dl):
I: Total cholesterol (TC) was 223.71 pre, increased to 284.20 during and dropped to 200.86 post. C: TC decreased progressively from 197.33 to 185.97 and 173.74.

I: LDL was 131.06 pre, increased to 162.99 during and dropped to 116.15 post intermission. C: LDL decreased progressively from 119.56 to 110.81 and 101.66.

I: HDL was 50.71 pre, decreased to 38.17 during and rose to 57.51 post intermission. B: HDL increased continuously from 54.44 to 55.45 and 58.42.

I: TC:HDL ratio was 4.9 pre, increased to 7.7 during and dropped to 3.6 post intermission. C: TC:HDL decreased from 3.8 to 3.6 and 3.0.

Blood pressure:
I: Systolic blood pressure (SBP; mmHg) was 125 pre, increased to 137 during and dropped to 121 post TRT intervention. C: SBP decreased from 119 to 117 and 116.

I: Diastolic blood pressure (DBP; mmHg) was 77 pre and remained stable at 77 during and 74 post TRT interruption. C: DBP decreased from 75 to 73 and 72.

I: Pulse pressure (PP) was 48 pre, increased to 60 during and decreased to 47 post intermission. C: PP remained stable at 44, 45 and 44.

In Group I, 6 patients had major adverse cardiovascular events (MACE) while off TRT. There were no MACE in Group C or in Group I while patients were on TRT.

Conclusions: Interruption of TRT resulted in worsening of symptoms. Hypogonadism may require lifelong TRT.
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**KNOWUT OF THE TRANSCRIPTION FACTOR NRF2: EFFECTS ON TESTOSTERONE PRODUCTION BY AGING MOUSE LEYDIG CELLS**

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**Introduction:** In previous studies, increases in reactive oxygen species (ROS) and decreases in antioxidant defense molecules were shown to be associated with age-related reductions in Leydig cell testosterone formation. It remains unclear whether redox imbalance is the cause of the reduced steroidogenesis that characterizes aging. A number of previous studies have examined the effects on cell function of the acute suppression of individual antioxidant molecules. However, aging is associated with the altered expression of numerous antioxidant molecules, and therefore with the long-term exposure of cells to an altered redox environment. The transcription factor nuclear factor-erythroid2-related factor 1 (Nrf2) is a master regulator of phase 2 antioxidant genes. Therefore its knockout affects the expression of a number of antioxidant molecules, reminiscent of aging. In the current study, we investigated the long-term effect of knocking out Nrf2 on mouse Leydig cell testosterone production.

**Methods:** Nrf2−/− mice were generated. Young (3 month−old), middle aged (8 month−old) and aged (24 month−old) wild−type and knockout mice were used in these studies.

**Results:** In wild−type C57BL/6 mice, serum testosterone levels and Leydig cell testosterone formation remained unchanged through middle age (8 months), but then were reduced significantly by old age (21−24 months). In contrast, serum testosterone levels were reduced significantly in the Nrf2 knockout (Nrf2−/−) mice as early as middle age, as was Leydig cell testosterone production. Both serum testosterone level and Leydig cell testosterone production were reduced in aged wild−type mice, but significantly more so in the aged Nrf2−/− mice. Reduced Leydig cell steroidogenesis in the knockout mice was associated with increased expression of protein nitrotyrosine residues, a marker of reactive oxygen species (ROS), and by reduced antioxidant levels.

**Conclusion:** These results strongly suggest that, over time, increases in oxidative stress resulting from Nrf2 knockout contribute to, or cause, the reduced testosterone production that characterizes aging Leydig cells.

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**THE MITOCHONDRIAL PEPTIDE ANALOGUE HNG PROTECTS AGAINST CYCLOPHOSPHAMIDE−INDUCED DECREASE IN SPERM OUTPUT AND NEUTROPENIA**

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(Presented By: Yan−He Lue, MD)

**Introduction:** Onco−infertility and neutropenia are the most common adverse events in cancer patients after chemotherapy. We have previously demonstrated that HNG, a potent Humanin analogue, protected male germ cells from apoptosis after a single dose of cyclophosphamide (CP) treatment. The objective of this study was to investigate whether HNG has protective effect on sperm output and peripheral blood cells after multiple doses of CP in mice.

**Methods:** Thirty adult male mice (C57BL/6J) were randomized into 4 groups: 1) 5 as control; 2) 5 received daily subcutaneously injection of HNG (10mg/kg); 3) 10 were given 6 doses of CP (150mg/kg) intraperitoneally at 5−day intervals; 4) 10 received both HNG and CP. All mice were killed at 28 days. Blood was collected for complete blood cell count using an automated cell counter. Plasma HNG and IGF−1 levels were measured by ELISAs. The cauda epididymal sperm count was performed using hemocytometer.

**Results:** Plasma HNG levels increased significantly (p<0.001) in HNG treated (80.8±7.8ng/ml), and HNG+CP treated (64.7±1.8ng/ml) mice compared to control (1.3±0.1ng/ml), and CP treated mice (1.7±0.1ng/ml). Compared to control (41.7±4.9ng/ml), plasma IGF−1 levels were significantly (p<0.001) suppressed by HNG (347.2±20.1ng/ml), CP (182.4±10.5ng/ml), and further suppressed by CP+HNG treatment (148.8±8.1ng/ml). Epididymal sperm counts were significantly elevated by HNG (1.7±0.2x10^6/mg), p=0.04), and significantly suppressed by CP (0.5±0.1x10^6/mg, p<0.001) as compared to control (1.2±0.2x10^6/mg). HNG+CP significantly increased sperm count (0.8±0.1x10^6/mg, p=0.02) as compared to CP. HNG alone had no effect on total white cell count (WBC:2.3±0.6x10^6/mg), granulocytes (GRA:0.5±0.2x10^6/mg), monocytes (MON:0.1±0.2x10^6/mg), and lymphocytes (LYM:1.7±0.4x10^6/mg) compared to control (WBC:2.4±0.3; GRA:0.2±0.1; MON:0.1±0.2; LYM:2.1±0.4x10^6/mg). CP treatment significantly (p<0.001) decreased the number of leucocytes (WBC:0.3±0.2; GRA:0.2±0.2; MON:0.1±0.1; LYM:0.07±0.01x10^6/mg) compared to control. Addition of HNG to CP significantly (p<0.05) rescued the CP induced neutropenia (WBC:0.6±0.4; GRA:0.4±0.3; MON:0.05±0.1; LYM:0.1±0.01x10^6/mg) as compared to CP.

**Conclusion:** We conclude that HNG prevents not only CP−induced suppression of sperm output but also CP−induced suppression of granulocytes, monocytes and lymphocytes. Our findings suggest that HNG is a promising adjuvant to chemotherapy by reducing chemotherapy−induced neutropenia and preventing onco−infertility.
ABSTRACTS

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PRO-ANDROGENIC EFFECTS OF LOW DOSE DEHP ARE ANTAGONIZED BY GENISTEIN IN YOUNG ANIMALS EXPOSED IN-UTERO

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(Presented By: Steven Jones, MSc)

Introduction: Early life exposure to environmental endocrine disruptors (EDs) is believed to predispose males to reproductive abnormalities. Although males are exposed to countless combinations of environmental chemicals from the time of conception to adulthood, relatively few studies have attempted to evaluate the effects of ED mixtures at relevant doses. Previous work in our laboratory demonstrated the ability of in utero exposure to a mixture of the phytoestrogen, Genistein (GEN), and plasticizer, DEHP, to induce long term alterations that are substantially different from individual exposures.

Methods: In this follow-up study, we examined F1 postnatal-day (PND) 3 and 6 male offspring exposed in-utero from gestational day 14 to parturition with either control corn oil, 10 mg/kg GEN, DEHP or combined GEN and DEHP to gain insight into the early molecular events driving long term alterations.

Results: Interestingly, DEHP had a stimulatory effect on the mRNA and protein expression of the steroidogenic enzyme, HSD3B1, uniquely in PND3 animals. The pro-androgenic effect of MEHP, the principal bioactive metabolite, was further investigated in PND3 testis organ cultures: 10 µM MEHP stimulated basal testosterone production, an effect that was attenuated by co-treatment with GEN (10 µM). In PND3 DEHP treated animals, concomitant mRNA increases of proliferation (Pcna), Sertoli cell (Wt-1, Nestin) and early germ cell (Hsp90a, Plzf, Foxo1) markers were observed. Lastly, a correlated increase in redox (Nqo1, Sod2, Sod3, Trx, Gst and Cat) and xenobiotic transporter (Abcb1b, Abcg2) gene expression was observed in PND3 DEHP treated animals, while attenuated when combined with GEN, suggesting the involvement of cellular stress in short-term DEHP mediated bi-phasic effects and a possible protective effect of GEN.

Conclusion: In contrast to previous reports of androgen suppression by DEHP used at elevated doses, lower dose gestational DEHP and in-vitro MEHP treatment had a stimulatory effect on androgen related processes, somatic and germ cell markers. We propose a potential mechanism by which GEN, through antioxidant action, normalizes the effects of ROS induced up-regulation of androgen related processes in DEHP treated animals. This notion that EDs do not follow classical dose-response effects and involve different mechanisms of toxicity from perinatal ages to adulthood highlights the importance of assessing impacts across a range of doses during appropriate windows of exposure.

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EFFECTS OF IN UTERO EXPOSURE TO DISISONONYL PHTHALATE ON RAT FETAL LEYDIG CELL FUNCTION AND AGGREGATION

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(Presented By: Tiao Bu, MD)

Introduction: Diisononyl phthalate (DINP) is a synthetic material that has been widely used as a substitute for other plasticizers prohibited due to reproductive toxicity in consumer products. Some phthalates have been associated with testicular dysgenesis syndrome in male fetus when female pregnant dams were exposed to them. The present study investigated effects of DINP on fetal Leydig cell function and testis development.

Methods: Female pregnant Sprague Dawley rats received control vehicle (corn oil) or DINP (10, 100, 500, and 1000 mg/kg) by oral gavage from gestational day (GD) 12 to GD 21. At GD 21.5, testicular testosterone production, fetal Leydig cell numbers and distribution, testicular gene and protein expression levels were examined.

Results: DINP showed dose-dependent increase of fetal Leydig cell aggregation with the low observed adverse-effect level (LOAEL) of 10 mg/kg and multinucleated gonocyte with LOAEL of 100 mg/kg. At 10 mg/kg, DINP also significantly increased fetal Leydig cell size, but inhibited insulin-like 3 and 3 β-hydroxysteroid dehydrogenase gene expression and protein levels. DINP inhibited testicular testosterone levels at 1000 mg/kg.

Conclusion: The results indicate that in utero exposure to DINP affects the expression levels of some fetal Leydig cell steroidogenic genes, gonocyte multinucleation and Leydig cell aggregation.

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EFFECTS OF IN UTERO MIXED EXPOSURE TO DIETHYL AND DIETHYLHEXYL PHTHALATES ON RAT FETAL LEYDIG CELL GENE EXPRESSIONS AND FUNCTIONS

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Introduction: Phthalate diesters are chemicals to which humans are ubiquitously exposed. Humans expose simultaneously to the mixtures of multiple phthalates, especially diethyl phthalate (DEP) and di-(2-ethylhexyl) phthalate (DEHP). However, the cumulative effects on fetal Leydig cell aggregation and gene expression levels have not been well understood. The objective of the present study was to investigate cumulative effects of the mixed exposure to DEP and DEHP on fetal Leydig cell aggregation and gene expressions.

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Methods: Pregnant female Sprague Dawley rats received control vehicle (corn oil) or DEP (10, 100, 500, and 1000 mg/kg) or DEHP (10, 100, 500, and 1000 mg/kg) or DEP+DEHP (10, 100, 500, and 1000 mg/kg) by oral gavage from gestational day (GD) 12 to GD21. At GD 21.5, testicular testosterone levels, fetal Leydig cell numbers and aggregation, Leydig cell related gene expression levels, and their protein expression levels were examined.

Results: DEP and DEHP showed synergistic effect in the induction of fetal Leydig cell aggregation with the low observed adverse−effect level (LOAEL) of 10 mg/kg. DEP and DEHP significantly decreased fetal Leydig cell size starting at 10 mg/kg, and inhibited Cyp11a1, Cyp17a1, Hsd17b3, and InsI3 expression levels dose−dependently. At the highest dose, DEP and DEHP inhibited testicular testosterone levels at 1000 mg/kg.

Conclusion: These data demonstrate that individual phthalates with a similar mechanism of action can elicit cumulative, dose additive effects on the expression levels of some fetal Leydig cell steroidogenic genes, and Leydig cell aggregation when administered as a mixture.

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81 EFFECTS OF DIBUTYL AND MONO-BUTYL PHTHALATES ON RAT IMMATURE LEYDIG CELL STEROIDOGENESIS IN VITRO
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Introduction: Phthalate esters such as di–n–butyl phthalate (DBP) are commonly found in cosmetics and in flexible plastics distributed by the food, construction, and medical products industries. It has been suggested that DBP is metabolized into mono–butyl phthalate (MBP) to activate its toxicity. Immature Leydig cells have steroidogenic capacity during the pubertal development and produce mainly the weak androgen 5β−androstane−3β,17β−dihydroxy−17β−ol (DIOL) because they have all testosterone biosynthetic enzymes (CYP11A1, HSD3B1, CYP17A1 and HSD17B3) and metabolizing enzymes (SRD5A1 and ARK1C14).

Methods: We compared the potencies of DBP and MBP in inhibition of steroidogenesis by rat immature Leydig cells. Rat immature Leydig cells (ILCs) were isolated from pre−pubertal male rats (35−day−old). ILCs were cultured with 50 nM−50 µM DBP or MBP for 24 hrs.

Results: We found that only the highest concentration (50 µM) of DBP inhibited androgen production. However, MBP at 50 nM had the inhibitory effects on androgen production. Quantitative PCR revealed that at this lowest concentrations, MBP inhibited Scarb1, Cyp11a1 and Hsd3b1 expression levels. However, DBP only at the highest concentration (50 µM) inhibited Scarb1, Cyp11a1 and Hsd3b1 expression levels. DBP at the highest concentration (50 µM) inhibited Scarb1, Hsd3b1 as well as Arkc1c14 expression levels. 50 µM DBP inhibited the enzymatic activities of HSD3B1, HSD17B3 and ARK1C14, and 50 µM MBP inhibited HSD3B1 activity.

Conclusion: In conclusion, DBP is metabolized into the more potent metabolite MBP, which mainly inhibits androgen biosynthesis by suppressing the expression of Scarb1, Cyp11a1 and Hsd3b1.

82 WALNUTS ADDED TO A WESTERN DIET ARE ASSOCIATED WITH DECREASED DNA STRAND BREAKAGE IN SPERM
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Introduction: The importance of diet to human male reproductive success has become increasingly apparent. Because sperm DNA damage is a strong predictor of reproductive outcomes, we investigated influences of dietary fatty acids (FAs) on sperm DNA integrity. The purpose of the research was to describe relationships between blood and seminal plasma fatty acids (FAs) and sperm DNA strand breakage in men consuming a Western diet and to determine if walnuts as a source of dietary α-linolenic acid (ALA) and other nutrients might have a beneficial effect on sperm DNA integrity.

Methods: This work was nested within a three month dietary RCT investigating efficacy of 75 gm walnuts per day to improve conventional sperm parameters (count, motility, morphology) in 117 healthy young men eating a Western diet. For the current work, measures of body weight, BMI, FAs (gas chromatography, FAs reported as a percent of total FA) and sperm DNA strand breakage (comet assay, reported as moment and %tail DNA) were explored in 105 participants from the parent study (walnut intervention group=n=54, controls=n=51).

Results: At baseline, intervention and control groups were similar on age, race, education, weight, BMI, sperm DNA strand breakage, FAs in serum and seminal fluid. Cross−sectional baseline data showed trends toward positive correlation between sperm DNA damage and saturated FAs and negative correlation with polyunsaturated FAs reaching statistical significance only for seminal plasma palmitic and arachidonic acids after correction for multiple comparisons. The intervention group showed a statistically significant increase in blood serum ALA (p<0.0001) and seminal fluid ALA (p<0.04) and decrease in sperm DNA strand breaks (moment p<0.02, %DNA tail p<0.04) after three months eating walnuts compared to the control group who had no significant change.

Conclusions: In a group of men eating a Western diet, addition of walnuts was associated with a decrease in sperm DNA strand breakage compared to a control group. This research suggests that dietary interventions may be beneficial for sperm DNA integrity. It is not clear that the magnitude of change found in this work would improve reproductive success. However, findings point to the importance of continued research into diet and male reproductive health.

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83 OPEN CHROMATIN MAPPING IDENTIFIES TRANSCRIPTIONAL NETWORKS REGULATING HUMAN EPIDIDYMIS EPITHELIAL FUNCTION
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Introduction: The epithelium lining the epididymis in the male reproductive tract maintains a luminal environment that promotes sperm cell maturation. This process is dependent on the coordinated expression of many genes that encode proteins with a role in epithelial transport. We previously generated genome−wide maps of open chromatin in primary human epididymis epithelial (HEE) cells to identify potential regulatory elements controlling coordinated gene expression in the epididymis epithelium. Subsequent in silico analyses identified transcription factor−binding sites (TFBS) that were over−represented in the HEE open chromatin, including the motif for paired box 2 (PAX2). PAX2 is a critical transcriptional regulator of urogenital tract development, which has been well studied in the kidney but is unexplored in the epididymis.
Methods: Due to the limited lifespan of primary HEE cells in culture, we investigated the role of PAX2 in an immortalized HEE cell line (REP). First, REP cells were evaluated by DNase I digestion followed by high-throughput sequencing and the PAX2-binding motif was again identified as an over-represented TFBS within intergenic open chromatin, though on fewer chromosomes than in the primary HEE cells. To identify PAX2-target genes in REP cells, RNA-seq analysis was performed after siRNA-mediated depletion of PAX2 and compared with that with a non-targeting siRNA. 

Results: In response to PAX2-repression, 3135 transcripts were differentially expressed (1333 up-regulated and 1802 down-regulated). Novel PAX2 targets included multiple genes encoding proteins with predicted functions in the epididymis epithelium.

Conclusion: PAX2 plays a critical role in the proliferation and differentiation of SLCs and PLCs, but inhibits the differentiation of SLCs into PLCs.

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A SUSCEPTIBILITY LOCUS, RS7099208, IS ASSOCIATED WITH NON-OBSTRUCTIVE AZOOSPERMIA VIA REDUCTION IN THE EXPRESSION OF FAM160B1

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(Presented By: Yan Zhang)

Introduction: Non-obstructive azoospermia (NOA) is a severe defect in male reproductive health that occurs in 1% of adult men. In a previous study, we identified three risk loci associated with NOA. One, rs7099208, is located within the last intron of FAM160B1 at 10q25.3.

Objectives: This study was undertaken to investigate the biological roles of rs7099208 in spermatogenesis and the potential as targets for NOA.

Methods: We analysed expression Quantitative Trait Loci (eQTL) of FAM160B1, ABLIM1 and TRUB1, the three genes surrounding rs7099208. With immunohistochemistry FAM160B1 expression showed significant weakening trend in NOA testes. And morpholinos were constructed significantly to inhibited FAM160B1 expression at two levels of the mRNA and protein in GC2 cell line.

Results: The expression level of FAM160B1 was reduced for the homozygous alternate genotype (GG) of rs7099208, but not for the homozygous reference or heterozygous genotypes. The expression levels of ABLIM1 or TRUB1 were unaffected by the rs7099208 genotype. And we show that FAM160B1 is predominantly expressed in human testes, where it is found in spermatocytes and round spermatids. We examined testes from 17 patients with NOA and five with obstructive azoospermia (OA) and found that expression of FAM160B1 is significantly reduced, or undetectable, in NOA patients, but not in OA cases or normal men. Then we determined that FAM160B1 is expressed in germ cells in mouse testes. Using a mouse germ cell line (GC2) as a model, knockdown of FAM160B1 resulted in a dramatic reduction in cell number, reduced cell viability, and ultimately cell death.

Conclusion: We conclude that rs7099208 is associated with NOA via a reduction in the expression of FAM160B1.

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REGULATION OF THE PROLIFERATION AND DIFFERENTIATION OF RAT STEM AND PROGENITOR LEYDIG CELLS BY ACTIVIN

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(Presented By: Linxi Li, PhD)

Introduction: Stem Leydig cells (SLCs) have been demonstrated to differentiate into adult Leydig cells (ALCs) via intermediate stage of progenitor (PLCs) and immature (ILCs) Leydig cells. However, the exact regulatory mechanisms are unclear. We hypothesized that the proliferation and differentiation of SLCs or PLCs depended upon locally produced factors.

Methods: Microarray analysis revealed that the expression levels of activin type I receptor (Acvrl1) were SLC > PLC > ILC = ALC and those of activin A receptor type II–like 1 (Acvrl1) were SLC > PLC = ILC > ALC. This indicates that that their ligand activin might play important role in stem and progenitor proliferation and differentiation. PLCs were incubated with 10 or 100 ng/ml activin in the absence (basal) or presence (LH–stimulated) of 1 ng/ml LH for 24 hrs.

Results: Activin concentrated–dependently increased the 3H–thymidine incorporation into PLCs by 136% and 203% when cells were treated with activin alone. LH also significantly increased the 3H–thymidine incorporation into PLCs by 268%. However, activin significantly reduced LH–stimulated effects when activin was combined with LH. Activin significantly inhibited the basal testosterone production to 61.77% and 63.08% of control value. At 10 and 100 mg/ml, it also significantly inhibited LH–stimulated testosterone production to 52.36% and 45.34% of control value, respectively.

Methods: We also applied a unique in vitro culture system in which SLCs proliferated on the surface of a cultured seminiferous tubule during one week of culture, and their progeny subsequently differentiated to androgen–forming PLCs during the following 2–4 weeks. ALC–free seminiferous tubules from adult rat testes were cultured with 10 ng/ml activin for three days, and EDU incorporation was used to visualize the proliferative SLCs.

Results: Activin significantly stimulated EDU incorporation by 500% when compared to control. When 10 ng/ml activin were incubated with the seminiferous tubules in the presence of Leydig cell–differentiating medium which contain 10 ng/ml LH plus insulin–transferrin–selenium. Activin inhibited the differentiation of SLCs into PLCs, and this effect was antagonized by the treatment of activin receptor antagonist 100 nM SB431542.

Conclusion: In conclusion, activin primarily stimulates the proliferation of SLCs and PLCs, but inhibits the differentiation of them into Leydig cell lineage in rat testes.
Introduction: Abnormalities of autosomal chromosomes can affect spermatogenesis even in the presence of intact Y chromosome. There are scarce reports in the literature describing the effect of chromosomal translocations on male infertility. The aim of the present study was to record the effect of reciprocal translocations; whether Robertsonian or non-Robertsonian on male infertility.

Methods: The medical records of infertile male patients with chromosomal translocations were reviewed. The patients were classified into 2 groups; group “A” included patients with Robertsonian translocations and group “B” included patients with non-Robertsonian translocations. Semen parameters, hormonal assays and testicular histopathology were reviewed and compared between both groups.

Results: The study included 13 patients with chromosomal translocation, 6 patients in group “A” (Robertsonian translocation) and 7 patients in group “B” (Reciprocal translocation). In general all 13 cases of translocations showed abnormal semen parameters. In Group “A”, 3 patients had Azoospermia and 3 patients had oligoasthenoteratozoospermia. ICSI was scheduled for 4 patients. Sperm were retrieved from 3 patients only without successful pregnancy. In group “B”, 4 patients had Azoospermia and 3 patients had oligoasthenoteratozoospermia. ICSI was scheduled for 5 patients. In all, sperm could be retrieved however without successful pregnancy.

Conclusion: Reciprocal translocations of chromosomes negatively affects spermatogenesis. Chromosomal translocations as a whole has poor prognosis for pregnancy by ICSI.

Introduction: Recent advances in DNA methylation studies suggest that mitochondrial DNA (mtDNA) may be subjected to epigenetic modifications, and related to or causative of disease development, though the process is poorly understood. It is clear that epigenetic modifications of mtDNA that affect expression of respiratory chain complex subunits may impact energy production and thus cell function. In the sperm however, these potentially important epigenetic marks have not yet been described, which prompted the current investigation.

Methods: Targeted sequencing of bisulphite-converted sperm mtDNA using MiSeq was performed on sperm from normozoospermic (n=5) and asthenozoospermic (n=5) men. Differential methylation analysis was performed to identify regions with methylation differences between the two groups.

Results: A very low magnitude of sperm mtDNA methylation at CpG and non-CpG cytosines was observed in both the study and control groups. Though, globally the difference in methylation was statistically non significant between both groups, we did observe some regions that displayed modest but statistically significant alterations. In protein coding genes, significant differences in methylation at CpGs of CytB gene and non-CpG cytosines of ATPase8, ND4L, ND4, ND6 genes were identified. Non-CpG cytosines of 12S rRNA were significantly hypo-methylated in asthenozoospermic men. Of the 22 tRNAs coded by mtDNA, significant differences between both groups were observed at CpGs of 1 tRNA gene and at non-CpG cytosines of 7 tRNA genes. In the D-loop, non-CpG cytosines were significantly hypermethylated in asthenozoospermic men, and in regulatory regions of the D-loop, significant methylation differences were observed in CpGs at the H-strand origin and non-CpG cytosines of the L-strand promoter region, transcription factor binding sites and at termination associated sequence.

Conclusion: These findings confirm that the level of sperm mtDNA methylation is well below the level reported for nuclear DNA of sperm or other human cell types. The physiological, functional and gene regulatory consequences of the methylation alterations in mtDNA regions, between asthenozoospermic and normozoospermic men are yet to be established. Further study is needed to understand changes in mtDNA methylation patterns, both global and gene specific, and the associated affects on sperm function.

Introduction: Identifying the cues governing progression of germ cells through meiosis is critical to understand the mechanisms involved in formation of healthy gametes. We previously characterized the mouse ENU-induced repro42 mutation which results in a nonsense codon in spermatogenesis associated 22 (Spata22). Mutant repro42 males and females are infertile due to meiotic arrest at the late zygote stage, suggesting that SPATA22 plays an essential function during prophase I of meiosis. We set forth to confirm the requirement for Spata22 during gametogenesis by describing a novel allele of this gene.

Methods: Histological and cytological analyses were used to characterize mice carrying the targeted gene trap allele Spata22Gt.

86 CHROMOSOMAL TRANSLOCATIONS AND MALE INFERTILITY
Mohamed Arafa, MD, Haitham ElBardisi, MD, Sami ElSaid, MD, Ahmad Majzoub, MD and Ahmad AlMalki, MD
Urology Department, HMC, Qatar
(Presented By: Mohamed Arafa, MD)

87 MITOCHONDRIAL DNA EPIGENETICS: CpG AND NON−CpG CYTOSINE METHYLATION IN THE SPERM MITOCHONDRIAL GENOME
Monis B. Shamsi, PhD, Timothy G. Jenkins, PhD, K.I. Aston, PhD and Douglas T. Carrell, PhD
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(Presented By: Monis B. Shamsi, PhD)

88 SPATA22 LOCALIZES TO MEIOTIC RECOMBINATION NODULES AND IS REQUIRED FOR FERTILITY IN THE MOUSE
Vinita Daniel, Zachary Ferguson, Patrick Davis, Chelsea Schonert, Emily Hays and Sophie La Salle, PhD
Midwestern University
(Presented By: Vinita Daniel)
**ABSTRACTS**

**89 SEMEN CRYOPRESERVATION IN MEN WITH CANCER; THE USAGE RATE AND OUTCOME OF ASSISTED REPRODUCTIVE TECHNOLOGY IN 898 PATIENTS**

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(Presented By: Tycho M.T.W. Lock, MD, FEBU)

**Introduction:** Nowadays, an increasing number of patients survive cancer due to improved treatment techniques. An undesired side effect of these treatments is potential sub- or infertility. Timely cryopreservation of semen is the only way to ensure fertility. Earlier studies showed high success rates of assisted reproductive technology (ART) with cryopreserved semen. The objective of this study was to determine how often cryopreserved semen from cancer patients is used and the success rate of ART in this group in achieving parenthood.

**Method:** All oncological patients who banked their semen between 1983 and 2013, before undergoing treatment, were included in the study. The semen was obtained by masturbation and analysed according to World Health Organization (WHO) guidelines. The semen was frozen in a Planer Kryo560−16 freezer (Planer, United Kingdom) at a rate of 0.5 oC / minute to +5oC, followed by 10 oC / minute to −8oC, and finally stored in liquid nitrogen. Patients characteristics and information about the ART were collected from the patient’s medical records in the hospital’s central electronic registration system and the fertility clinic’s specific data management system.

**Results:** 898 patients cryobanked their semen. 96 patients used their cryopreserved semen for ART (10.7%). The clinical pregnancy rate for intra−uterine insemination (IUI), in vitro fertilisation (IVF), intracytoplasmic sperm injection (ICSI) and cryo embryo transfer (ET) were 14%, 37%, 38% and 18%, respectively. A total of 77% of the couples achieved parenthood.

**Conclusion:** Although the success rates of ART are impressive, the usage rate of cryopreserved semen in cancer patients is still low. Health professionals should continue to encourage cancer patients to cryopreserve semen before cancer treatment. Furthermore cancer patients should be advised to return to the fertility clinic if they desire children.

**90 OUTCOME OF MICROSURGICAL TESTICULAR SPERM EXTRACTION IN FAMILIAL IDIOPATHIC NON-OBSTRACTIVE AZOOSPERMIA**

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(Presented By: Haitham El Bardisi, MD)

**Introduction:** Objective: To study the sperm retrieval rate by microsurgical testicular sperm extraction (Micro−TESE) in familial idiopathic non−obstructive azoospermia (NOA).

**Methods:** Study design: This is a cohort retrospective study. The study included 115 patients with idiopathic NOA who underwent micro−TESE over the past 5 years. Medical records of these patients were reviewed. Patients were then divided into two groups; Group “A” with familial idiopathic NOA and Group “B” with non−familial idiopathic NOA. Clinical data as well as data of micro−TESE were recorded. Main outcome measure(s): Testicular sperm retrieval rate in both groups.

**Results:** Group “A” included the members of seven families, each family contains two brothers (total=14 patients). Group “B” consisted of 101 patients. There was no statistically significant difference in the patients’ demographics between the two groups. Also there was no difference between both groups as regards testicular size, FSH, LH, testosterone and prolactin. In group “A” sperm retrieved rate was 14.29% (2/14 patients) compared to 43.56% in group “B” (44/101 patients) (p= <0.05). The two patients in group “A” with successful sperm retrieval belonged to one family. The histopathological diagnosis was the same in the brothers in each family.

**Conclusion:** The testicular sperm retrieval rate in familial idiopathic NOA is significantly lower than in non−familial idiopathic NOA.
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ICSI OUTCOME IN KLINEFELTER’S SYNDROME: QATAR EXPERIENCE
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(Presented By: Ahmad Majzoub, MD)

Introduction: Klinefelter syndrome (KF) is the most common chromosomal disorder associated with male hypogonadism and infertility. Parenthood can be achieved in men with KF by intracytoplasmic sperm injection (ICSI) using testicular sperm. Aim: To evaluate surgical sperm retrieval rate (SSR) in KF patients in Qatar and to investigate methods to improve SSR in this group of patients.
Methods: This is a retrospective study where all the medical records of KF patients who underwent SSR for ICSI, in our center in the past 14 years, were reviewed.
Results: 41 patients were included. 23 underwent conventional testicular sperm extraction (TESE) and 18 underwent microsurgical TESE (Micro−TESE). SSR was significantly higher in the Micro−TESE group than TESE group (33.3% versus 0% respectively). In the Micro−TESE group 14 patients received hormonal stimulation prior to Micro−TESE and 4 patients did not receive. SSR was 42.9% versus 0% in both groups respectively. Within the 14 patients who received hormonal stimulation 8 patients received aromatase inhibitors while the other 6 received other hormonal stimulation. SSR was 62.5% versus 16.7% in both groups respectively.
Conclusion: SSR in KF patients is significantly more when using hormonal stimulation by aromatase inhibitors followed by microsurgical testicular sperm extraction.

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SPERM RETRIEVAL SHOULD BE PERFORMED AT THE TIME OF TUMOR RESECTION IN MEN WITH CONGENITAL ADRENAL HYPERPLASIA AND BILATERAL TESTICULAR ADRENAL REST TUMORS
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(Presented By: Parviz Kavoussi, MD)

Introduction: In males with congenital adrenal hyperplasia (CAH) there is an impaired production of cortisol and mostly of aldosterone resulting in increased pituitary adrenocorticotrophic hormone (ACTH) production leading to hyperplasia of the adrenal glands and overproduction of adrenal androgens. Some men with CAH will develop benign testicular adrenal rest tumors (TART’s), which are typically bilateral and originate in the rete testis. TARTs commonly result in obstructive azoospermia and destruction of normal testicular tissue with growth and may cause orchialgia. There have been reports of testicular aspiration for use with in−vitro fertilization/intracytoplasmic sperm injection (IVF/ICSI) with the tumors in situ and reports of men remaining azoospermic after tumor resections. Our objective was to evaluate the effectiveness of concomitant sperm retrieval and tumor resections with the goal of minimizing the numbers of procedures in these men.
Methods: Case report with chart review.

Results Obtained: 35 year old male with the diagnosis of CAH and bilateral TARTs with bilateral orchialgia was found to be azoospermic. He had been on treatment with dexamethasone and human chorionic gonadotropin (HCG) was found to be azoospermic on two separate semen analyses. He underwent concomitant bilateral testicular tumor resection and open testicular sperm extraction. His sperm retrieval was successful and was cryopreserved for future use with IVF/ICSI and his orchialgia resolved after resection. Pathology revealed architecture consistent with adrenal rests.
Conclusions: To our knowledge, this is the first case reported of men with CAH and bilateral TARTs undergoing a successful sperm retrieval concomitantly with bilateral tumor resection. As it has previously been shown that men remain azoospermic following tumor resections, sperm retrieval and tumor resection in one surgical setting would seem to be the optimal approach rather than subjecting such patients to two separate procedures.

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WHY WE ESTABLISHED THE MALE CONTRACEPTION INITIATIVE
David Sokal, MD and Aaron Hamlin, MEd, MPH, JD
Male Contraception Initiative
(Presented By: David Sokal, MD)

Introduction: Resources for research and development of new, reversible male contraceptives are limited. Currently women have 15 modern contraceptive methods to choose from, while men have only two. Fifty years of research on male hormonal methods have not led to a marketed product. The Gates Foundation supports the development and marketing of new female contraceptives, but has stated it would not fund research on new male methods. The Michaelson Foundation is supporting a $100 million effort to develop new contraceptives for dogs and cats. No similar effort is focused on men, yet at least five promising methods await funding to move forward. Although women bear most of the consequences of unintended pregnancy, unintended fatherhood also has economic and mental health consequences. Given recent advances in biotechnology, it seems timely to explore novel approaches for new reversible, non−hormonal male contraceptives.
Objective: To establish a nonprofit organization focused on development of new male contraceptives, which will: (1) facilitate collaboration between researchers and encourage cross-disciplinary collaboration; (2) encourage new researchers to enter this field; (3) raise public awareness; and (4) help raise funds for priority research projects.

Methods: We are (1) recruiting experts for an Advisory Committee; (2) providing our website for public outreach and fundraising; (3) requesting help from researchers to ensure we include all promising research; (4) building a database of supporters and potential donors; and (5) encouraging researchers to collaborate on this initiative.

Results: With an active Board of Directors and Executive Director, we have initiated 501(c)3 nonprofit registration in the US; recruited internationally known experts for our Advisory Committee; launched our website, www.malecontraceptive.org; and facilitated the initiation of collaborative work between RTI International in North Carolina, and researchers at Airlangka University, Indonesia, on a novel oral contraceptive that has completed Phase 1 and 2 human trials.

Conclusion: The Male Contraception Initiative is beginning to help catalyze research and development of new male contraceptive methods. We ask for your help in this effort.
96 PROTEOMIC PATHWAYS OF OXIDATIVE STRESS IN THE HUMAN SEMINAL PLASMA.
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(Presented By: Paula Intasqui, MSc)

Introduction: Oxidative stress is widely considered one of the main cellular mechanisms of male infertility. It promotes negative effects on sperm function mainly through lipid peroxidation and oxidation of seminal plasma and sperm proteins. Thus, our aim was to investigate if the seminal plasma proteome may reflect semen lipid peroxidation (LPO) levels.

Methods: We performed a cross-sectional study with 156 normozoospermic patients. Semen was collected by masturbation, analyzed according to WHO 2010 guidelines and centrifuged for seminal plasma separation. LPO levels were assessed using a colorimetric assay for malondialdehyde, a by-product of LPO, and patients were grouped as low LPO levels (control group, bottom 15%, n=23) and high LPO levels (study group, top 15%, n=23). Seminal plasma proteomics was performed by a label-free quantitative approach, in which 50ug of total proteins were pooled, digested into tryptic peptides and analyzed by liquid chromatography followed by tandem mass spectrometry (LC−MS/MS). Four pools were prepared for each group, including biological variation between the pools, and these were run in technical triplicates. Significant proteins (Student’s T test) were used for functional enrichment analysis and confirmed by logistic regression and discriminant analysis.

Results: LPO levels (mean ± standard deviation) were 14.9 ± 1.60 ng/mL in controls and 32.0 ± 2.68 ng/mL in the study group (p<0.001). Progressive motility and total motility were statistically (p<0.05) lower, while immotility was higher in the study group. 629 proteins were quantified, of which 4 were absent, 19 were downexpressed, 8 were exclusive and 63 were overexpressed in the study group, with several enriched functions (Figure 1). The logistic regression model presented a total predictive value of 91.7%, and an area under the ROC curve of 99.3%. We suggest Mucin−5B (MUC5B_HUMAN) as a biomarker of semen oxidative stress.

Conclusion: The seminal plasma proteome and post−genomic pathways reflect semen LPO levels, mainly with the enrichment of reactive oxygen species detoxification. MUC5B may be a semen biomarker of LPO damage.

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97 TIME FOR PUBLIC HEALTH ACTION ON INFERTILITY: UPDATES FROM THE CENTERS FOR DISEASE CONTROL AND PREVENTION
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(Presented By: Steven Schrader, PhD)

Introduction: Infertility has become a public health issue in the US. Recently, the nation’s premier public health agency, the Centers for Disease Control and Prevention (CDC) released the long anticipated “National Public Health Action Plan for the Detection, Prevention, and Management of Infertility” This presentation will describe highlights of the action plan and what CDC is doing to further promote the issue of infertility as a public health problem for both men and women.

Methods: To describe the development and contents of the national public health action plan as well as ongoing surveillance, research and programmatic efforts surrounding infertility.

Results: The plan, released in July 2014, was coordinated by CDC in consultation with many stakeholders including governmental and non−governmental organizations, professional societies, health care professionals, academic programs, and individuals affected by infertility. Its goal is to highlight the need to better understand and address issues at a population level that contribute to and are caused by infertility and that may affect the health of the resulting pregnancy. This plan represents the first major effort by the agency to consider the broader implications of infertility from a public health perspective. Overall goals include promoting healthy behaviors that can help maintain and preserve fertility; promoting prevention, early detection, and treatment of medical conditions that can threaten fertility, and reducing exposures to environmental, occupational, infectious, and iatrogenic agents that can threaten fertility. Several publicly available, population−based surveys conducted by CDC, including the National Survey of Family Growth (NSFG), National Health and Nutrition Examination Survey (NHANES), National Health Interview Survey (NHIS), National Vital Statistics System (NVSS), and Pregnancy Risk Assessment Monitoring System (PRAMS) can be used to examine key aspects of or risk factors for infertility.

Conclusion: The national public health action plan provides a foundation and framework for discussion and collaboration between stakeholders and CDC regarding the prevention, management and treatment of male and female infertility in the United States.

98 CRYOPRESERVATION OF SPERMATOZOA: OPTIMIZATION OF MOTILITY WITH A NONPERMEABLE CRYOPROTECTANT
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(Presented By: Cigdem Tanrikut, MD)

Introduction: Cryopreservation of sperm from men with severe male factor infertility eliminates the need for repeat surgical extractions in azoospermic men and the risk of inadequate viable sperm the day of oocyte retrieval in men with cryptozoospermia. However, current sperm cryopreservation techniques do not reliably freeze/thaw very low numbers of sperm. In an effort to help solving this problem, we
used trehalose and silica micro-capillaries with a 2 µl capacity to preserve sperm from healthy men and optimized post thaw motility.

**Methods:** Motile sperm were isolated with 80%/40% density gradients from fresh ejaculates. Only those samples with 80% or greater motility were used. Aliquots from 8 samples (three repeats per sample per treatment) were diluted 1:1 with a freezing medium consisting of human tubal fluid (HTF), 5% human serum albumin (HSA), and varying concentrations of trehalose: 0.0M, 0.125M, 0.25M, 0.5M, and 1M. Two microliters of the suspension were loaded into each 200 µm silica capillary. After sealing each capillary end with Tygon tubing, the capillary was incubated in liquid nitrogen (LN2) vapor for 5 minutes before being plunged into LN2. To thaw, each capillary was quickly immersed into a room temperature (~22°C) water bath. Capillary contents were expelled into a drop of 12 µl HTF with 5% HSA on a glass slide, then covered with a coverslip and motility of the sperm was examined. Sham controls consisted of sperm/freezing medium suspension were loaded into capillaries and expelled immediately for assessment without freezing.

**Results:** When 0.0M, 0.125M, 0.25M, 0.5M, and 1M trehalose was supplemented into the freezing medium, recovered motility (post thaw motility/pre–freeze motility × 100%) of the sperm was 41.0%, 62.0%, 67.8%, 52.3%, and 29.1%, respectively. For sham controls corresponding to the above trehalose concentrations, sperm motility was 98.0%, 98.9%, 98.9%, 93.8%, and 84.2%, respectively. The freezing medium containing 0.25M trehalose achieved the highest recovered motility among all media.

**Conclusion:** Trehalose is an effective nonpermeable cryoprotectant in preserving sperm using silica capillaries. This protocol eliminates washing after thawing, avoiding unintended sperm loss. We have demonstrated a system potentially beneficial to preserve small numbers of sperm from azoospermia and oligozoospermia patients.

**99 NON–MOTILE SPERM CELL SEPARATION USING A SPIRAL CHANNEL**

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(Presented By: Jiyoung Son, MS)

**Introduction:** Microfluidic sperm sorting has historically relied on sperm motility. However, motility–based sperm separation technology will not work when viable non–motile sperm need to be separated in from testicular tissue from testicular sperm extraction (TESE) and microdissection TESE techniques. Here, we demonstrate the use of inertial microfluidic technology using spiral channels to separate sperm. The separation method is label–free does not rely on sperm motility for sorting.

**Methods:** Basic principles of spiral channel separations were used to define specific channel and flow parameters for separating non–motile sperm, including the ratio of inertial lift and Dean drag (Rf), the ratio of particle and channel dimension (I) and the aspect ratio of the channel cross–section. When Rf, I, and the aspect ratio are >–0.08, >–0.07 and 3:1 respectively, theory suggests that the sperm could be focused and separated from red blood cells (RBCs). Channels to implement these features were designed and validated. Mixed samples of RBCs and sperm were used to test the device. The inlet flow rate ranged from 0.1–0.3ml/min. The processed sample contained 2 million (M)/ml of sperm and 5.3M/ml of RBC in a 1 ml volume. The original sample was diluted for some later experiments to avoid interference between the different particles. After running the sample through the spiral channel, the samples were collected from four different splitter outlets, and were inspected using microscopy.

**Results:** For a 0.3ml/min injection, sperm concentration was focused at outlet 1 (1.7M/ml) and outlet 2 (2.4) with lower concentrations at outlet 3 (0.4) and outlet 4 (0.45). In terms of concentration ratio the overall collected sperm at outlet 1–4 were 34, 48, 8, and 9%, respectively. In contrast, the concentration of RBCs was clearly higher at outlet 3 (0.85M/ml) and outlet 4 (10.3) than at outlet 1 (0) and outlet 2 (0.4). The concentration ratio of the overall collected RBC at outlet 1–4 were 0, 3.4, 7.3 and 89.2%, respectively.

**Conclusion:** Sperm and RBCs were successfully separated from each other in a microfluidic spiral device with over 96% of RBCs removed from the sample and 82% of the non–motile sperm recovered, suggesting that this technique might be useful for separating non–motile sperm found in TESE and mTESE samples.

**100 QUANTITIVE EVALUATION OF EXPRESSION OF THE CATSPER CHANNEL IN HUMAN SPERM AND RELATION WITH FUNCTIONAL PARAMTERS**

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(Presented By: Elisabetta Baldi)

**Introduction:** CatSper is a sperm–specific calcium channel activated by progesterone (P) in human spermatozoa, and has been indicated as putative progesterone sperm receptor (Strunker et al, 2011; Lishko et al, 2011). KO mice for any of the Catsupe family genes, fail to acquire hyperactivated motility (HA) and are infertile. Less clear is the role of CatSper in human sperm hyperactivated/activated motility and in asthenospermia. Here, we re–examined the involvement of CatSper in sperm motility parameters, intracellular calcium levels and acrosome reaction (AR) by directly investigating their relationship with CatSper expression.

**Methods:** We set up a method for quantitative evaluation of CatSper expression in sperm by immunofluorescence/flow cytometry. CatSper expression was found reduced in asthenozoospermic men (53±16.0% , n=24 vs 68.3±17.1% in normozoospermic, n=85, p<0.01) and was significantly correlated with progressive (r=0.31, p<0.01), total (r=0.36, p<0.01) and hyperactivated (r=0.44, p<0.01) motility. Besides a higher percentage of sperm not expressing CatSper, asthenozoospermic men showed a larger amount of sperm with localization of the immunofluorescence signal in the midpiece (44.9±1.9%) respect to normozoospermic (27.7±4.3% , p<0.005). By using a probe to distinguish live and dead cells, expression of CatSper was found to be prevalent in live sperm (53.3±14% vs 20.4±2.0 % in dead).

**Results:** A significant correlation was found between CatSper expression and the increase of [Ca2+]i in response to progesterone (r=0.4, p<0.05) but not with basal [Ca2+]i. No correlation was found with AR, either basal or in response to progesterone. Receiver operating characteristic analysis demonstrated that at the threshold
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102 THE GENERAL METHOD OF PLACING THE RESERVOIR IN INFLATABLE PENILE PROSTHESIS OPERATION
Chen Bin, MD, Zhan Junxin, Chen Chaoyue, Huang Fengjin and Zhu Xihong
(Presented By: Chen Bin, MD)

Introduction: Objective: To summarize the basical putting method of reservoir to retropubic space (bladder before), is carried out in the IPP operation.

Methods: 20 cases of IPP surgery, adopt the “blind” way for putting the water sac to retropubic space (bladder before).

Results: the anatomical position, osseous marks, puncturing technique for the back–membrane of inguinal canal, the temperature change of entering into the retropubic space, and how to determine the correct position, were summarized.

Conclusion: The basical method of putting reservoir to retropubic space, should be standardized. We think the reservoir can be placed in the right place if using our general method in IPP.

103 FAILURE TO ATTAIN STRETCHED PENILE LENGTH AFTER INTRACAVERNOSAL INJECTION OF A VASODILATOR AGENT IS PREDICTIVE OF VENO−OCCLUSIVE DYSFUNCTION ON PENILE DOPPLER ULTRASONOGRAPHY
Faysal A. Yafi, MD, FRCSC, Ian R. McCaslin, MD, Russell P. Libby, MD, PremNat Sangkum, MD, Suresh Sikka, PhD and Wayne J.G. Hellstrom, MD, FACS
Tulane University School of Medicine
(Presented By: Faysal A. Yafi, MD, FRCSC)

Introduction: Penile duplex Doppler ultrasound (PDDU) is frequently used to assess the etiology of erectile dysfunction (ED). Peak systolic velocity (PSV), end–diastolic vascular velocity (EDV), and resistive index (RI) are commonly used PDDU parameters. We sought to assess whether reaching stretched penile length (SPL) at peak erection after intracavernosal injection (ICI) of vasodilator during PDDU had any correlation with the etiology of ED.

Methods: We performed a retrospective review of 278 consecutive patients who underwent PDDU for the work–up of ED or Peyronie’s disease (PD) between 2011 and 2013. Flaccid and stretched penile length and circumference were measured by standardized ruler, prior to ICI and at peak erection during PDDU. All measurements were performed by the expert ultrasonographer (SS) using standardized protocol (Sikka et al, JSM 2013). Collected data included patient demographics, vascular, and anatomic parameters.

Results: The mean age of our population was 54 years (median 56.0, range 18–80). SPL matched with peak length after ICI in 171 patients (62%, group 1) and did not in 103 (38%, group 2). There were no significant differences between the 2 groups in terms of age, presence of Peyronie’s disease, degree or direction of curvature, IIEF–5 score, percent rigidity or tumescence, and vasodilator dose used. Surprisingly, patients who did not match SPL at peak erection were found to have more veno−occlusive dysfunction (VOD) (62% vs. 42%, p=0.0013). On multivariate analysis, failure to reach SPL was predictive of VOD (OR 0.480, CI 0.271−0.850, p=0.0118) in these patients.

Conclusion: Failure to reach SPL during PDDU after ICI of a vasodilator agent appears to be predictive of VOD which is independent of rigidity and tumescence.

104 THE HISTORY OF PENILE ENHANCEMENT – TO CUT A SHORT STORY LONG
Paul Cleaveland, MBChB¹, Zubeir Ali, MBChB² and Ian Pearce, MBChB³
¹Royal Preston Hospital; ²South Manchester Teaching Hospitals NHS Trust; ³Central Manchester Teaching Hospitals
(Presented By: Paul Cleaveland, MBChB)

Introduction: Throughout history the penis has been a sign of masculinity characterised by its length, shape and performance. Insecurity regarding penile dimensions and methods of penis enlargement are well reported. We present the various methods of penile enhancement from ancient times to modern day era.

Methods: A literature search was conducted describing penile size and methods for penile enhancement throughout history. We reviewed the evolution of these techniques and present our findings.

Results: Procedures employed for male enhancement date back to ancient rituals, such as the African custom of hanging weights from genitals and the Topinama tribesmen (Brazil) practice of increasing penile size by allowing a snake to bite the penis. Approaches to penis enlargement have since evolved, with more sophisticated methods currently employed. A vacuum device utilising a compression ring was first patented in 1917. The first recorded penile augmentation procedure was performed in 1971 for the treatment of microphallus in bladder extrophy children. Over the years, division of the suspensory ligament in cosmetic surgery has become established. Other initiatives include penile rings, penile extenders/traction devices, and jelqing. The mainstay of girth enhancement is fat injection into the penis, described in 2006. These methods have various degrees of success as well as associated morbidity.

Conclusion: Penile enhancement is an interesting and controversial subject. It is clear that since ancient times across cultural divides, penile dimensions have been topical. The evolution of the techniques currently available to enhance penile size is ongoing fuelled by intrigue and demand.

105 THE EFFECT OF AN AQUEOUS TYPHA CAPENSIS EXTRACT ON THE AGING MALE REPRODUCTIVE SYSTEM
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University of the Western Cape
(Presented By: Ralf Henkel, PhD)

Introduction: In traditional African medicine, an aqueous Typha capensis rhizome extract (TCE) is recommended to males as it is believed to enhance male reproductive function although few studies
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exist focusing mainly on the phytochemistry of this plant. Thus, there is a definite need to investigate this plant in terms of its beneficial effects on male reproductive health.

**Methods:** Data obtained from an in vitro study led to a pilot study, which investigating the effect of TCE on 1-year old, male rats. The treatment group (32mg TCE kg−1 body weight (BW)) was force−fed daily at a maximum volume of 200µl while the control group received a similar volume of tap water over a period of 14 days. At termination, weights of back fat and the gastrocnemius muscle, as well as testosterone (T) and luteinizing hormone (LH) concentrations were determined.

This study was repeated over a 52−day period and animals were divided into a high−dose (32mg TCE kg−1 BW per day) (HD), a low−dose (2mg TCE kg−1 BW per day) (LD) and control group. At termination, in addition to the previous parameters, weights of testis, prostate, epididymis, seminal vesicles, liver, kidney, adipose tissue and gastrocnemius muscle were recorded. In addition, progesterone (P) and estradiol (E) were determined.

**Results:** Data from the pilot study showed no difference for the BW (P=0.0532) and adipose tissue weight (P=0.2239) between control and TG. However, the average BW and adipose tissue weight in the TG decreased by 14.28% and 29.26%, respectively, compared to the control. Although there was no significance difference (P=0.1429) in average muscle weight and T (P=0.3777) between the control and TG, increases in muscle weight (4.8%) and T (30%) were noted. There was no difference for LH levels.

After 52 days of treatment, BW in the LG (P=0.0350) increased and no difference (P=0.3447) was observed in the HG, although a slight increase in BW was observed. No difference was observed for weights of the reproductive organs, liver, kidney, adipose tissue and gastrocnemius muscle for both, LG and HG. No difference was observed for T, E, P and LH between all groups although the HG secreted less T and LH and slightly more P compared to the LG.

**Conclusion:** Results suggest that TCE affects the parameters tested. Higher dosages can cause negative feedback. Further studies are underway to determine if TCE may be used as herbal remedy to alleviate aging males’ symptoms.

**106 ASSOCIATION BETWEEN ERECTILE FUNCTION AND SUBCLINICAL ATHEROSCLEROSIS: A STUDY BASED ON MIDDLE−AGE HEALTHY MEN FROM THE GENERAL POPULATION**

Babak Rezanezhad, Rasmus Borgquist, MD, PhD, Ronnie Willenheimer, MD, PhD and Saad Elzanaty, MD, PhD
(Submitted By: Babak Rezanezhad)

**Introduction:** Epidemiological studies suggesting atherosclerosis as a common risk factor between cardiovascular diseases and erectile dysfunction (ED). We aimed, therefore, to determine the association between erectile function (EF) and biomarkers of subclinical atherosclerosis in 119 healthy men from the general population.

**Methods:** The EF was assessed using the International Index of Erectile Function−5 (IIEF−5). Serum levels of biomarkers of atherosclerosis; Apolipoprotein A (ApoA), Apolipoprotein B (ApoB), fibrinogen, and C−reactive protein (CRP) were measured. In addition, the body mass index (BMI) was calculated.

**Results:** The mean (SD) of age was 55 years (±4.0). The prevalence of ED was 50 %. There was a negative significant correlation between EF and ED, BMI (r = −0.20, p = 0.02), (r = −0.20, p = 0.03), respectively. No significant correlations between EF and ApoA, ApoB, and fibrinogen were found (p > 0.05). A positive significant correlation was found between BMI and CRP (r = 0.30, p = 0.001). Finally, in a multivariate logistic regression model with EF as the dependent variable, CRP was the only biomarker that predicted ED (odds ratio = 0.343; 95 % CI: 0.122−0.963).

**Conclusion:** These results indicate a direct causal association between subclinical atherosclerosis and ED. This association seems to be related to the increased values of BMI among such men.

**107 PENILE HEMODYNAMIC IN PATIENTS WITH HIGH RISK OF CARDIOVASCULAR DISEASES**

Evgeny Efremov, Professor¹, Yaroslav Melnik² and Stepan Krasnyak ¹Head of the Department of Andrology and Human Reproduction; ²Scientist, Department of Andrology and Human Reproduction
(Submitted By: Stepan Krasnyak)

**Introduction:** The most common pathogenetic factor for erectile dysfunction is a vascular lesion, and it is often systemic disease. Therefore, the identification of vascular origin of erectile dysfunction can be a reason for comprehensive examination of the cardiovascular system. The lack of published data on the connection between arteriogenic erectile dysfunction and cardiovascular disease caused of the organization of our study.

**Methods:** We examined 481 adult male with complaints of erectile dysfunction. Doppler ultrasound of penile blood vessels with pharmacologically induced erection was performed for all of them. In the analysis of the study population revealed that the maximum systolic blood flow velocity in the cavernous arteries does not correlate with the severity of erectile dysfunction by IIEF−5.

**Results:** It was revealed that only some cardiovascular diseases are predictors of arteriogenic erectile dysfunction. Abdominal obesity showed a correlation with the severity of hemodynamic disturbances to the penile arteries (r = −0.22, p < 0.001). In men with diabetes penile blood flow parameters were significantly lower than in men with normal carbohydrate metabolism (p=0.022).

**Conclusion:** Our data suggest that penile Doppler with pharmacologically induced erection is an important methods of determining the nature of the vascular erectile dysfunction. Furthermore, it appears that not all cardiovascular disease are risk factors for penile artery lesions.
Introduction: Aging associated erectile dysfunction (ED) is characterized by a progressive apoptosis of the smooth muscle cells and collagen deposition in the corpora cavernosa which leads to an impairment in erectile function. High local output of nitric oxide (NO) via inducible nitric oxide synthase (iNOS) has been shown to inhibit corporal fibrosis and this effect of iNOS may be further enhanced by treatment with PDE5 inhibitors. Nutraceuticals are substances that offer medicinal benefit, including prevention and/or treatment of diseases. The nutraceuticals ginger, muira puama and paullinia cupana have been shown in some studies to enhance NOS activity similar to that seen with the PDE5 inhibitors. We therefore evaluated whether the daily oral administration for 2 months with a combination of ginger, paullinia cupana, muira puama as well as L–citrulline, (COMP−4) can effectively delay the ongoing corporal fibrosis, smooth muscle cell loss and cavernosal veno−occlusive dysfunction (CVOD) seen in middle aged rats and compared these results to those receiving tadalafil only.

Methods: 10 Month old Fisher 344 rats were treated orally for two months with either vehicle (controls), COMP−4 (ginger 45 mg/Kg B.W, L−Citrulline 133 mg/Kg B.W. Muria Puama 45 mg/Kg B.W), or tadalafil (2.5 mg/Kg B.W). CVOD was determined by dynamic infusion cavernosometry. Penile sections of the corpora cavernosa were subjected to Masson trichrome staining to evaluate fibrosis, immunohistochemistry followed by image analysis was used for both iNOS and desmin, a marker of smooth muscle content.

Results: A decline in the control rat’s erectile function is evident by 10−12 months of age (ICPAP: 30% decrease; drop rate 1.7 fold increase) and is accompanied by a decrease in the corporal smooth muscle content (52%) and an increase in corporal fibrosis by 70%. The daily treatment for two months with COMP−4 increases iNOS expression, preserves corporal smooth muscle content, and decreases corporal fibrosis resulting in the preservation of normal erectile function. These results with COMP−4 parallels those obtained with daily tadalafil.

Conclusion: An oral combination of ginger, Muira Puama, Paullinia cupana and L–citrulline seems to be as effective as a daily PDE5 inhibitor in delaying and/or reversing the onset of the histological and functional characteristics of aging related ED.
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3-ADRENERGIC RECEPTORS ARE INVOLVED IN REGULATING NITRIC OXIDE SIGNALING AND NEUROGENIC FORCE OF CONTRACTION IN CORPUS Cavernosum OBTAINED FROM PATIENTS WITH ERECTILE DYSFUNCTION

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(Presented By: Faysal Yafi, MD)

Introduction: 3-adrenergic receptors (3-ARs) play a physiological role in mediating penile erection. Earlier data in human corpus cavernosum (HCC) from male-to-female transsexual surgical procedures showed that activation by a selective 3-receptor agonist elicits marked relaxation by a cyclic guanosine monophosphate (cGMP)-dependent but nitric oxide (NO)-independent mechanism. The aim of the study was to analyze whether 3-ARs were also functional in the HCC of men with erectile dysfunction (ED).

Methods: HCC specimens were obtained from patients (aged 53–69 years, n=7) with ED undergoing penile prosthesis surgery. HCC strips were mounted in organ baths and tested for changes in isometric tension in response to a 3-AR agonist, BRL-37344 (0.01–100 µM) on phenylephrine (Phe)-induced contraction. The influence of 3 agonists on nitricergic relaxations induced by either electrical field stimulation (EFS, 1–40 Hz) or acetylcholine (ACh, 10µM) was studied in HCC. The effects of BRL-37344 on neurogenic contractions evoked by EFS were investigated. The specificity of action of BRL-37344 on 3-adrenergic receptors was verified using SR 59230A (10 µM), a selective 3-AR antagonist.

Results: Phenylephrine-induced contraction in HCC was inhibited by BRL-37344 (77.2 ± 7.1%, at 100 µM). The biphasic relaxation in response to EFS changed in a monophasic response in the presence of BRL-37344. In HCC strips, ACh (100 µM)-induced relaxation responses were increased by 36%. The EFS-induced contractile response at 40 Hz was remarkably decreased by 83% with BRL-37344. BRL-37344’s inhibition of the amplitude of neurogenic contractions was antagonized with SR 59230A.

Conclusion: These results further support the hypothesis that 3-AR mechanisms may play an important role in the nitricergic and adrenergic neurotransmission processes in HCC. The 3-AR system may maintain the biological activity of exogenous NO in the presence of adrenergic stimulation. Thus, while 3-ARs likely play a dynamic role in modulating HCC smooth muscle tone, 3-AR agonists may be promising candidates for the clinical treatment of men with ED.

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EARLY EXPERIENCE WITH MICROSURGICAL SPERMATIC CORD DENERVATION FOR CHRONIC ORCHALGIA IN A CANADIAN CENTRE.

Darby Cassidy, MD
University Hospital of Northern British Columbia

(Presented By: Darby Cassidy, MD)

Introduction: Microsurgical spermatic cord denervation (MSCD) is an effective surgical technique for the management of chronic orchalgia but has not been readily adopted by Canadian Urosurgeons. This paper reviews the early experience of a single Urosurgeon in Canada.

Methods: 9 consecutive testicular units underwent MSCD over a 24 month period after ruling out reversible causes and after a successful diagnostic spermatic cord block.

Results: 77% (7/9) of the patients had a complete resolution and 22% (2/9) had a partial resolution of their pain symptoms following MSCD. There were no failures or complications.

Conclusions: MSCD is an effective, safe, and reproducible surgical technique that should be included in the armamentarium of the Urosurgeon for the treatment of chronic orchalgia.

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PROSTATORRHEA REVISTED

Ahmad Motawi, MSc, FECSM, Hussein Ghanem, MD, FECSM, Mohammad Abbas, MD and Ashraf Zeidan, MD, FECSM
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(Presented By: Ahmad Motawi, MSc, FECSM)

Introduction: It is surprising that prostatorrhea, a very common complaint in our society, is almost unheard of in western medical literature. A literature search for “to spermatorrhoea or prostatorrhoea” revealed only one 30 years old reference. Our objective is to assess the sexual profile among male patients with physiological urethral discharge.

Methods: This study was carried out on a group of 50 patients of the age group 18 – 30 years old who were attending the out patient clinic in Kasr Al−Aini Hospital,Cairo University complaining of urethral discharge. Another 50 patients of the same age group attending the clinic with any other complain were taken as a control age matched group. All patients were subjected to history taking, general examination, body measurements including the height, weight and waist circumference, local genital examination, 2 glass urine tests, EPS and serum Testosterone. Sexual profile questionnaire, that was developed by the authors.

Results: There was a statistically significant difference between the sexual activity of the comparison subjects with prostatorrhea (74%) and those without discharge , p value <0.01 as most of the men complaining of prostatorrhea were sexually inactive (83.2%)while those who were not complaining of physiological discharge were sexually active (98%),while there was no statistically significant difference of the frequency of sexual activity between males complaining of prosemenn (26%) and those without discharge with p value 0.069.

Conclusion: Prostatorrhea is a normal physiological discharge occurring in young males specially in our society associated to unrelieved sexual excitement which is probably related to cultural and religious factors.
114 TRANSCRIPTIONAL REGULATION OF HUMAN SPERM–ASSOCIATED ANTIGEN 16 BY S–SOX5
Ling Zhang, PhD¹, Junpin Liu, MD¹, Wei Li, MD², Jerome Strauss III, MD, PhD² and Zhibing Zhang³
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(Presented By: Zhibing Zhang)

Introduction: The mammalian sperm–associated antigen 16 gene (SPAG16) encodes an axoneme central apparatus protein that regulates cilia/flagella motility. It is highly abundant in the testes. The gene is also expressed in most somatic tissues, particularly in the tissues with motile cilia, such as tracheal and brain. Human and mouse SPAG16 gene promoters contain multiple potential binding sites for the transcription factor SOX5, which has homology to the high mobility group box region of the testis–determining factor, SRY.

Methods: Given that both the mouse and human SOX5 genes encode a 48–kDa SOX5 protein (S–SOX5) that is only present in tissues containing cells with motile cilia/flagella, we hypothesized that SPAG16 is regulated by S–SOX5. Thus, human SPAG16 gene regulation by S–SOX5 was investigated in BEAS–2B cells, a line derived from human bronchial cells.

Results: S–SOX5 stimulated human SPAG16 promoter function in BEAS–2B cells, but the effect was abrogated when the SOX5 binding sites were mutated. The SPAG16 message was up–regulated when S–SOX5 was overexpressed in BEAS–2B cells, and silencing of S–SOX5 down–regulated SPAG16 transcripts. Chromatin immunoprecipitation experiments demonstrated that S–SOX5 directly associates with the SPAG16 promoter.

Conclusion: The present study demonstrates that SPAG16 is a S–SOX5 target gene. We have previously reported that S–SOX5 also regulates sperm–associated antigen 6 (SPAG6), a gene that encodes another axoneme central apparatus protein. Thus, S–SOX5 may be a master transcription factor that controls expression of a suite of genes related to motile cilia/flagella. Its in vivo role is being studied by generating a conditional mutant mouse model that targets only S–Sox5.

115 MULTIDRUG RESISTANT BACTERIAL ISOLATES CAUSING NOSOCOMIAL URINARY TRACT INFECTION IN A TERTIARY CARE HOSPITAL, NEPAL
Manoj Sah, Master of Science in Clinical Microbiology¹ and Shyam Mishra, MScClinical Microbiology²
¹Kathmandu University, Kantipur Dental College, Kathmandu, NEPAL; ²TU, Nepal
(Presented By: Manoj Sah, Master of Science in Clinical Microbiology)

Introduction: Nosocomial infection is becoming a leading problem in medical practitioners now–a–days placing an extra burden on individual patients worldwide. Nosocomial urinary tract infection caused by multidrug resistant (MDR) pathogens is a major threat of the patients in developing country which are increasing numbers in Nepal. The aim of this study was to determine the etiology of nosocomial urinary tract infection caused by multidrug resistant bacterial pathogens.

Methods: A total of one hundred twenty two bacterial strains isolated from the patients diagnosed of nosocomial urinary tract infection were studied during 2011–2012 at Tribhuvan University Teaching Hospital (TUTH). Antibiotic sensitivity test was determined by modified Kirby Bauer Disc Diffusion method as described by Clinical and Laboratory Standards Institute (CLSI).

Results: Nosocomial urinary tract infection was caused by Escherichia coli 51(41.8%) was found to be more predominant which was followed by Acinetobacter calcoaceticus baumannii (Acb) complex 19(15.6%), Klebsiella pneumoniae 11(9%) Enterococcus spp. 18(14.8%) and Staphylococcus aureus 11(9%). Of the total isolates, 74.6% was MDR which is much higher in Klebsiella pneumoniae 100% which was followed by Escherichia coli 90.1%. Data were analyzed by using SPSS version 17.0

Conclusions: The emergence of MDR bacterial strains causing nosocomial urinary tract infection are increasing in number. The high prevalence of MDR has demanded the special attention to the management of such patients and prevention of dissemination of such strains into hospitals.

116 CRISP3 IS PRO-TUMORIGENIC IN THE PROSTATE
Moira O’Bryan, PhD¹, Marianna Volpert, BSc (hons), PhD², Duangporn Jamsai, BSc, PhD², Gail Risbridger, BSc (hons), PhD² and Luc Furic, BSc (hons), PhD²
¹Monash University; ²The Department of Anatomy and Developmental Biology, Monash University
(Presented By: Moira O’Bryan, PhD)

Cysteine-rich secretory protein 3 (CRISP3) is a vertebrate-specific member of the CRISP clade of the CRISP, Antigen 5, Pathogenesis-related (CAP) superfamily. Within healthy mammals it is expressed predominantly in the salivary glands and in components of the immune system. In instances of human prostate cancer, however, it is up-regulated several hundred fold and has been proposed as a potential marker of progression to advanced disease. Within this study we have assessed the mechanism of CRISP3 action in prostate cancer. We show that recombinant CRISP3 promotes both the migration and invasion of PC3 cells in the XCelligence system. Further, and consistent with the up-regulation of CRISP3 in human prostate cancer, CRISP3 is highly up-regulated with advanced disease in the Hi-MYC mouse models of prostatic adenocarcinoma. Importantly the deletion of Crisp3 up-regulation of CRISP3 in human prostate cancer, CRISP3 is highly up-regulated with advanced disease in the Hi-MYC mouse models of prostatic adenocarcinoma. Importantly the deletion of Crisp3 leads to the delayed transition of prostatic intraepithelial neoplasia to carcinoma in situ and a complete blocking of progression to invasive disease. Collectively these data define CRISP3 as pro-tumorigenic in the prostate with a role in invasion.

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118 HISTOLOGICAL PATTERN WITH PREDICTIVE PROGNOSTIC VALUE OF IMPROVED FERTILITY IN PATIENTS UNDERGOING MICROSURGICAL VARICOCELECTOMY

Jorge Hallak, MD, PhD1,2, Robertson Dutra, BSc, MSc student3 and Juliana Pariz, MSc, PhD student1,3

1Androscience; 2Universidade de São Paulo

Introduction: Varicocele is considered one of the most common identifiable causes in men with infertility complaint, affecting about 15% of the general population, and up to 40% of fertile men or sub-fertile. One of the biggest challenges in the surgical approach of varicocele is the identification of subjects who will show improvement of the fertile potential. Objective: To identify a histological pattern with predictive prognostic value of improved fertility in patients undergoing microsurgical varicocelectomy.

Methods: This retrospective study consisted of 16 testicular biopsies with histological analysis of men in a specialized clinic of male fertility between the years 2006 and 2012. Were included men in reproductive age with a diagnosis of infertility and presence of varicocele and excluded patients with azoospermia, sexually transmitted diseases and neoplasms of the genitourinary tract. The patients were submitted to urological physical examination with diagnosis of varicocele, performed by careful palpation of the pampiniform plexus with the patient standing, followed by Valsalva maneuver to evaluate the degree of varicocele. In addition, semen analyzes and hormonal measurements were performed. For the determination of a histological pattern can predict the improvement of fertility, cut-off points were created that combine Johnsen’s score, LH, FSH and prolactin serum levels to improved semen parameters.

Results: For improvement of sperm concentration, Johnsen’s score must be greater than 6.45 (right testis) and LH <0.48; to a satisfactory predictor of progressive motility, Johnsen’s score >9.15 in the right testis and >5.9 in the left testis. In the evaluation of sperm morphology, left Johnsen’s score should be greater than 9.3.

Conclusion: We suggest that the creation of histological patterns with predictive values of improved fertile potential, as demonstrated in this study, may benefit patients candidates for microsurgical varicocelectomy.

119 PROTEOMIC PROFILING OF DETERGENT RESISTANT MEMBRANES (LIPID RAFTS) OF PROSTASOMES AND THEIR REVESICULATION

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Uppsala University, Sweden

(Presented By: Göran Ronquist)

Introduction: Prostasomes are exosomes derived from prostate epithelial cells through exocytosis by multivesicular bodies. Prostasomes have a bilayered membrane and readily interact with sperm. The membrane lipid composition is unusual with a high contribution of sphingomyelin at the expense of phosphatidylcholine and saturated and monounsaturated fatty acids are dominant. Lipid rafts are liquid–ordered domains that are more tightly packed than the surrounding non–raft phase of the bilayer. Lipid rafts are proposed to be highly dynamic, submicroscopic assemblies that float freely within the liquid disordered membrane bilayer and some proteins preferentially partition into the ordered raft domains. We asked the question whether lipid rafts do exist in prostasomes and, if so, which proteins might be associated with them.

Methods: Prostasomes of density range 1.13–1.19g/mL were subjected to sucrose density gradient ultracentrifugation fabricated by PBS containing 1% Triton X–100 with capacity for banding at 1.10g/mL, i.e. the classical density of lipid rafts. Prepared prostasomal lipid rafts (by gradient ultracentrifugation) were analyzed by mass spectrometry and electron microscopy. The clearly visible band on top of 1.10g/ mL sucrose in the Triton X–100 containing gradient was subjected to LC–MS/MS and more than 350 lipid raft associated proteins were identified. Among them several were involved in intraluminal vesicle formation e.g. tetraspanins, ESCRTs and Ras–related proteins.

Results: This is the first comprehensive LC–MS/MS profiling of proteins in lipid rafts derived from exosomes. Ultrastructurally, prostasomal lipid rafts and control prostasomes displayed similar spherical shapes although the former were more electron lucent than the controls. Prostasomal lipid rafts also presented a bilyared membrane. Therefore, we hypothesized that prostasomal lipid rafts underwent revesiculation in hypertonic sucrose (1.10g/mL), and later the enveloped sucrose rendered them an osmotic lysis and another revesiculation in PBS.

Conclusion: We conclude that prostasomes contain lipid rafts that may be functional vesicular entities.

120 SEMEN QUALITY AND TESTICULAR CANCER: RESULTS FROM THE UTAH POPULATION DATABASE

Heidi Hanson, PhD1, Ross Anderson, MD, MCR2, Chong Zhang, MS3, Angela Presson, PhD4, Kenneth Aston, PhD5, Douglas Carrell, PhD6, Ken Smith, PhD7,8 and James Hotaling, MD, MS2

1University of Utah; 2Department of Surgery, Division of Urology, University of Utah; 3Department of Family and Preventive Medicine, University of Utah; 4Department of Internal Medicine, University of Utah; 5University of Utah, Department of Surgery, Andrology and IVF Laboratories; 6University of Utah, Department of Surgery, Director of IVF and Andrology Laboratories; 7Department of Family and Consumer Studies, University of Utah; 8Population Sciences, Huntsman Cancer Institute

(Presented By: Heidi Hanson, PhD)

Introduction: Abnormal semen concentration has been linked to increased risk of testicular cancer. Previous studies have examined risk in men with male factor infertility and azoospermic men, but were unable to investigate the association across a range of well–defined categories of semen concentration. We hypothesize that there will be an increased risk of testicular cancer for all groups with poor sperm quality and there will be a reduction in risk as semen concentration increases.

Methods: We linked 24,370 University of Utah and Intermountain Healthcare semen analyses from 2002 – 2014 to the Utah Population Database (UPDB), a multi–generational epidemiological database, which provides demographic, pedigree and medical information on over 7 million individuals. Individuals with prior diagnoses of cancer were excluded from the sample. Men were classified as: azoospermia, cryptozoospermia (<0.5m/mL), oligozoospermia (<20m/mL), normozoospermia (>20m/mL), and hyperzoospermia (>100m/ mL). Utah Cancer Registry data linked to the UPDB was used to identify cancer cases. Cox models stratified by birth year were used to investigate the relationship between sperm quality and testicular cancer diagnoses.
Results: 50 men were diagnosed with testicular cancer after their first semen analysis. Both cryptozoospermic and oligozoospermic men have an increased rate of testicular cancer, with hazard rate ratios of 12.97 (95% CI 5.2, 32.3) and 2.28 (95% CI 1.1, 4.7), respectively. Azoozoospermic men in our study are not at increased risk of testicular cancer and we do not find a linear decrease in testicular cancer risk with improving sperm quality. Removing cases diagnosed within one year of the sperm analyses did not alter the conclusion that cryptozoospermic men have an increased rate of testicular cancer, however the effect was attenuated for the oligozoospermic category.

Conclusion: The findings from this study do not support previous studies showing an increased rate of testicular cancer in azoospermic men. Contrary to our hypothesis, there is not a significant inverse association between semen concentration and testicular cancer, but there is an elevated risk for both cryptozoospermic and oligozoospermic men. We will further investigate the genetic and environmental aspects of this relationship utilizing the wealth of demographic data in the UPDB.

121 IMPACT OF GALECTIN−3 SINGLE NUCLEOTIDE POLYMORPHISM (SNP) ON INCREASED ODDS OF PROSTATE CANCER (PCA) AND ON PROTEOLYSIS BY PROSTATE SPECIFIC ANTIGEN (PSA)
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(Presented By: Matthew Kovak, MS)

Introduction: Galectin−3 is a carbohydrate−binding protein implicated in numerous human diseases, including PCa. In the male reproductive tract, galectin−3 function is regulated by proteolytic processing by PSA. Galectin−3 cleavage in the prostate tumor microenvironment is associated with cancer progression. The SNPs rs4644 and rs4652 generate proline (P)−to−histidine (H), and threonine (T)−to−P polymorphisms in galectin−3 at amino acids 64 and 98, respectively. The objectives of this study were to evaluate the impact of galectin−3 SNPs on the odds of PCa and on the susceptibility of galectin−3 to proteolysis by PSA.

Methods: 180 Caucasian men with PCa and 180 case−matched controls were genotyped for the SNPs rs4644 and rs4652. Logistic regression analysis was performed to evaluate the association of the galectin−3 genotype and covariates with increased odds of PCa. Kaplan−Meier analysis was utilized to compare age−at−diagnosis. The four galectin−3 variants generated by the SNPs rs4644 and rs4652 were incubated with PSA, and the extent of cleavage was quantitated. To identify a novel PSA cleavage site, cleaved galectin−3 H64P98 was subjected to Edman degradation.

Results: Heterozygosity for the SNPs rs4644 and rs4652 was associated with >63% higher odds of PCa than homozygosity. Moreover, heterozygosity for either SNP was associated with a 3−year−earlier age−at−diagnosis of PCa. Covariate analysis indicated that tobacco users heterozygous for either SNP had a nearly two−fold higher odds of PCa than non−tobacco users or homozygous tobacco users. Cleavage analysis indicated that galectin−3 H64 variants were up to 3.5−fold more susceptible to PSA cleavage than the P64 variants. Moreover, the H64 variants exhibited an additional cleavage product not observed for the P64 variants. Edman degradation of PSA−cleaved galectin−3 H64P98 indicated that PSA cleaves galectin−3 H64 variants between Y63−H64.

Conclusion: Results suggest that heterozygosity for galectin−3 H64P98 and P64T98 is a risk factor for PCa and that tobacco use and the galectin−3 SNP genotype are synergistic covariates for increased odds of PCa. Significantly, tobacco use has not previously been associated with PCa risk. Protease assay results indicate that the galectin−3 phenotype determines the susceptibility of galectin−3 to proteolytic cleavage by PSA. We anticipate that these studies will provide the groundwork towards personalized medicine approaches for the prevention and treatment of PCa.

122 ULTRASOUND FOR PALPABLE SCROTAL MASSES: WHAT ARE WE FINDING?
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(Presented By: Marah Hehemann, MD)

Introduction: Scrotal ultrasound (US) is the most common adjunctive test performed in the assessment of patients with palpable scrotal abnormalities.

We hypothesize that the majority of scrotal US performed for the evaluation of a palpable scrotal abnormality exhibit findings that are consistent with benign processes. On the other hand, we also hypothesize that the majority of testicular neoplasms detected on US coincide with a palpable mass on physical exam. Additionally, we predict that a small fraction of all testicular neoplasms are found incidentally.

Methods: After receiving IRB approval, we performed a retrospective review of all scrotal US performed from 2002 to 2014 at our tertiary care institution. We separately examined A) scrotal US performed for the evaluation of a palpable scrotal abnormality exhibit findings that are consistent with benign processes. On the other hand, we also hypothesize that the majority of testicular neoplasms detected on US coincide with a palpable mass on physical exam. Additionally, we predict that a small fraction of all testicular neoplasms are found incidentally.

Results: A total of 18,593 scrotal US were performed from 2002−2014 at our institution. There were 3,487/18,593 (18.7%) US performed for palpable abnormality (Group A). Of this group only, 198/3487 (5.68%) US identified discrete intratesticular masses suspicious for neoplasm. Individual US results were reviewed and analyzed.

Results: A total of 18,593 scrotal US were performed from 2002−2014 at our institution. There were 3,487/18,593 (18.7%) US performed for palpable abnormality (Group A). Of this group only, 198/3487 (5.68%) US identified discrete intratesticular masses suspicious for malignancy. A total of 259/18,593 (1.4%) discrete testicular masses (group B) were identified on US for any indication. Of this group, 198/259 (76.4%) were performed because of a palpable abnormality and the remaining 61/259 (23.5%) were found incidentally on US. The 61 incidentally found intra−testicular masses accounted for just 0.33% of all US performed.
Conclusion: To our knowledge, this is the largest study addressing findings of scrotal ultrasound. Based on our 12 year retrospective analysis, we found that only a small minority (5.68%) of US performed for scrotal masses on physical exam confirmed a discrete intratesticular mass concerning for malignancy. Based on these results, we believe that patients presenting with a palpable testicular mass should undergo initial scrotal US to rule out malignancy. Interestingly, though, the vast majority (94.3%) of palpable abnormalities correspond to non-malignant US findings. Finally, incidentally found intra-testicular masses are extremely rare (0.33%).

VARICOCELE IS ASSOCIATED WITH INDICATORS OF INFLAMMATORY ACTIVITY WHICH DECREASE AFTER VARICOCELECTOMY.

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(Presented By: Mariana Camargo, MSc)

Introduction: Varicocele is present in 15% of adult men, and is a major cause of male infertility. Varicocele has been hypothesized to lead to subclinical inflammation that can interfere with sperm function (Andrology, 2014; (2) 102: 54–360). To investigate this hypothesis, our study compared semen parameters in: (1) men with varicocele versus men without varicocele; (2) the same group of men before versus after varicocele repair.

Methods: Adult men with bilateral varicocele (n=14) provided a semen sample 1 day before bilateral subinguinal microsurgical varicocele repair (varicocelectomy), then again 90 and 180 days after varicocelectomy. Control subjects (n=14) were normospermic men without varicocele who each provided a semen sample. Semen analysis was performed according to WHO guidelines (2010). Sperm function was assessed by sperm DNA fragmentation (comet assay) and sperm mitochondrial activity (3,3’−diaminobenzidine [DAB] staining). Inflammation was assessed by ELISA assays for IL−1, IL−18 and caspase−1 in semen (=0.05).

Results: Age (mean ± SD) was 33.6 ± 8.4 and 34.2 ± 3.0 in men with and without varicocele, respectively. Results are shown in Table 1. Sperm parameters and mitochondrial activity were lower, and concentrations of IL−1 and caspase−1 were higher, in semen of men with, versus without, varicocele. Ninety days after repair, significant improvements were seen in volume, morphology and caspase−1, but these improvements did not reach values of men without varicocele until 180 days after repair, at which time mitochondrial activity, DNA fragmentation and caspase−1 also reached values of men without varicocele.

Conclusion: Our results indicate that varicocele in adults is associated with evidence of inflammatory activity that improves after varicocele repair. Varicocelectomy is associated with early improvement in sperm morphology and late improvement in ejaculate volume, sperm DNA fragmentation, and mitochondrial activity. Future studies will determine if these findings are present in a broader group of varicocele patients, and if these outcomes are associated with improved spermatogenesis and testicular function.

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ACTION OF RESVERATROL ON THE REPRODUCTIVE PARAMETERS OF LATE PUBERTAL RATS TREATED WITH ANTI–CANCER AGENTS (BEP PROTOCOL MODIFIED), FROM PERIPUBERTY: PART II

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(Presented By: Flávia Macedo de Oliveira Neves, Doctoral student)

Introduction: Testicular cancer is the most common cancer affecting men of reproductive age (15–35 years). BEP Protocol, which includes bleomycin, etoposide and cisplatin, has been promoting up to 90% chance of cure in patients with testicular cancer. However, side effects on the reproductive health of patients have been reported. Resveratrol (R), a fitoalexin, has anti–apoptotic and antioxidant properties. Objective: to investigate the protection of resveratrol against the reproductive side effects caused by BEP–treatment.

Methods: From the 36th day post partum (dpp), rats were resveratrol–treated (gavage) with a daily dose of 300mg/kg, per 5 days; from 41st dpp, the co–administration of R and BEP (R–BEP group) was applied for three weeks: etoposide (3.50mg/kg) and cisplatin (0.70mg/kg) for 5 consecutive days/week and bleomycin (0.35mg/kg; every 2nd day of each week); drugs were injected by intraperitoneal route (ip). Three other groups were treated with: 1)BEP (BEP group); 2)Resveratrol (R group) and 3–carboxi–methyl–cellulose (R vehicle, gavage) and 0.9% physiologic solution (ip route; SC– Sham–Control group). Body weight, sperm quantitative and qualitative (morphology and motility) evaluation, sperm mitochondrial activity and DNA fragmentation and acrosome integrity were investigated, as well as the dosage of sex hormones, apoptotic germ cell frequency (TUNEL) and testicular oxidative stress.

Results: BEP group presented higher frequency of TUNEL–positive germ cells when compared with the other three groups. Sperm anomalous forms, low mitochondrial activity and acrosome integrity, along with a reduction in intratesticular testosterone were noted in the BEP group when compared to the R–BEP group. Sperm motility was altered in BEP and R–BEP groups but the parameter reflecting sperm flagellar beating was only altered in BEP group. The plasma testosterone, lipid peroxidation and sperm DNA fragmentation of BEP and R–BEP groups were altered. The germ cell apoptosis frequency reduction and the improvement of the mitochondrial activity, flagellar beating, morphology and acrosome integrity in the R–BEP group point out to a reduction of the reproductive damage caused by BEP–treatment. Using such protocol, we did not observe any differences in sperm DNA fragmentation.

Conclusion: Additional studies are on the way to better clarify the chemoprotective action of resveratrol on the spermatogenesis.
**ABSTRACTS**

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**FLUORESCENCE IN–SITU HYBRIDIZATION DETECTS INCREASED SPERM ANEUPLOIDY IN MEN WITH RECURRENT PREGNANCY LOSS**

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(Presented By: Ranjith Ramasamy)

**Introduction:** Objective: To investigate, in men presenting with recurrent pregnancy loss (RPL), the prevalence of sperm autosome and sex chromosome aneuploidy.

**Methods:** Design: Retrospective Study. Setting: Male infertility clinic at a tertiary referral center. Patients: 140 men with recurrent pregnancy loss provided semen samples and five normozoospermic controls provided 140 semen samples for comparison. RPL, documented in the female partners, was defined as a prior miscarriage and/or recurrent IVF/ICSI failure. Interventions: Fluorescent In situ hybridization (FISH) was used to detect numerical abnormalities in sex chromosomes (X,Y) and autosomes (13, 18, 21) in ejaculated sperm. Main Outcome Measures: Sperm aneuploidy in men with RPL and normozoospermic controls.

**Results:** Men with RPL had a greater percentage of sperm aneuploidy within the sex chromosomes, chromosomes 18 and 13/21 (1.04% vs. 0.38%, p=0.015; 0.18% vs. 0.03%, p<0.001, 0.26% vs. 0.08%, p=0.002). In total, 40% of men with normal sperm density and motility had abnormal sperm aneuploidy in the all the chromosomes analyzed. Men with abnormal sperm density and motility had a higher proportion of sperm sex chromosome aneuploidy than men with normal density/motility (62% vs. 45%, p=0.042). Men with normal strict morphology (>4%) had lower rates of sex chromosome and sperm aneuploidy than men with abnormal strict morphology (28% vs. 57%, p=0.038). There was no association between sperm DNA fragmentation and sperm aneuploidy.

**Conclusion:** Men with RPL have increased sperm aneuploidy compared to controls. A total of 40% of men with RPL and normal sperm density/motility had abnormal sperm aneuploidy. Men with oligoasthenospermia and abnormal strict morphology had greater percentage of sperm aneuploidy compared to men with normal semen parameters.

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**STUDY OF RNA BIOMARKERS OF NORMAL SPERMIOGENESIS IN NORMAL SEMEN AND SPERM VIA TRANSCRIPTOME ANALYSIS.**

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MWRI

(Presented By: Alexander Yatsenko, MD, PhD)

**Introduction:** Previous studies have indicated that mature human sperm contains a complex population of RNAs that have been implicated in past and coming events such as spermatogenesis, fertilization, and possibly early embryonic development. Recent attempts were made to identify those RNAs associated with good fertility and good sperm quality. Here, we report the transcriptome analysis of normal semen and sperm based on total RNA–seq study.

**Methods:** Equimolar RNA from five semen and 6 mature sperm samples were studied. To increase efficiency of total RNA sequencing analysis we excluded ribosomal RNA via rRNA reduction. Preliminary, our RNA seq analysis detected a total of ~19,900 and ~17,00 genes in 2 RNA samples. After normalization, we identified 16,898 transcripts that were common to both samples. Using more stringent criteria with average sequence read coverage of >1, we identified ~10,000 transcripts and 5,000 transcripts in two samples, where ~4,500 transcripts were shared between the two samples. We classified these transcripts as protein coding, non–coding and pseudogenes. To reveal the functional annotation and pathways of these genes, all protein coding genes were subjected to Ingenuity Pathway Analysis and PANTHER analysis.

**Results:** This annotation resulted in 14 major categories, including DNA replication/repair, gene expression regulation, post–transcriptional regulation, post–translational modification, cellular maintenance, cellular structure, molecular transport, cell movement, cell signaling, cell cycle regulation, apoptosis, metabolism, spermatogenesis, and embryogenesis. Based on number of genes involved, IPA identified the top pathways including, translation regulation, cellular growth, cell cycle, DNA repair, apoptosis and transcription regulation. A number of novel transcripts were also identified in this study, however their role in spermatogenesis remain to be explored.

**Conclusion:** Our study suggests the presence of important sperm RNAs that could serve as informative biomarkers of male germ cell quality and potentially predict fertilization and early embryonic development outcome in IVF/ICSI procedures.

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**THE RELATIONSHIP BETWEEN SOME SEMEN QUALITY MEASUREMENTS, SCROTAL CIRCUMFERENCE, BODY WEIGHT AND FERTILITY OF AWASSI RAMS**

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(Presented By: Basim J. Awda)

**Introduction:** Great efforts have been taken in recent years for improving reproductive efficiency in Awassi sheep. Semen quality and testicular measurements are important parameters for fertility evaluation in rams and ewes as well. Despite there being studies on biological factors associated with these traits, little is known about the potential relationships between reproductive efficiency andram fertility traits in Awasi sheep. The objective of this study was to examine the relationship between conception rate and ram fertility traits (sperm motility, ejaculate volume (EV), pH, sperm concentration, scrotal circumference (SC) and body weight (BW)).
**Methods:** SC and BW were measured and semen collected from 4 mature Awasi rams [2.88 ± 0.24 yr old; 65.25 ± 1.93 kg (mean ± SE)] using the electroejaculation method. The ejaculates were placed in a thermos (35 °C) immediately after collection. The collected semen samples were then evaluated for EV, pH, sperm motility, and sperm concentration. Each ejaculate was extended and used immediately to inseminate 3–4 Awasi ewes/ram [2.05 ± 0.29 yr old; 48 ± 1.44 kg (mean ± SE)]. Ram fertility percentage was calculated based on non-return to estrus following 17 days post insemination. Conception rates and litter size were determent by real time ultrasonography at 34, 44 and 60 days post insemination. Data were analyzed using the CORR procedure and Tukey-Kramer multiple comparisons to test for trait effect.

**Results:** SC, BW, sperm motility, EV, pH, sperm concentration did not correlate significantly with ram fertility (P>0.05). However, ewes BW negatively correlated (r=-0.99; P=0.06) with ram fertility percentage. There were no significant differences in fertility percentage or ram fertility traits between the two groups of rams based on ewes litter size (pregnancy type; single vs. twin).

**Conclusion:** In conclusion, this study suggests that fertility of Awasi ram may be affected by ewe’s BW and further studies are needed to evaluate the relationships between the ram fertility traits and the conception rate of Awasi ewes.

129 THE RELATIONSHIP BETWEEN SOME BLOOD PARAMETERS MEASUREMENTS, SCROTAL CIRCUMFERENCE, BODY WEIGHT AND FERTILITY OF AWASSI RAMS

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(Presented By: Basim J. Awda)

**Introduction:** Great efforts have been taken in recent years for improving reproductive efficiency in Awasi sheep. Semen quality, testicular measurements and biochemical properties of blood serum are important parameters for fertility evaluation in rams and ewes as well. Despite there being studies on biological factors associated with these traits, little is known about the potential relationships between fertility and biochemical properties of blood parameters in Awasi rams. The objective of this study was to examine the relationship between ram fertility percentage and some biochemical properties of blood serum, scrotal circumference (SC) and body weight (BW) in Awasi rams.

**Methods:** SC and BW were measured; semen and blood samples were collected from 4 mature Awasi rams [2.88 ± 0.24 yr old; 65.25 ± 1.93 kg (mean ± SE)]. Semen samples were collected using the electroejaculation method. Each ejaculate was extended and used immediately to inseminate 3–4 Awasi ewes/ram [2.05 ± 0.29 yr old; 48 ± 1.44 kg (mean ± SE)]. Blood samples were analyzed for packed cell volume (PCV), erythrocyte sedimentation rate (ESR) and haemoglobin concentration (Hb). Blood serum was analyzed for glucose concentration, glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) activities and total proteins (TP).

Ram fertility percentage was calculated based on non-return to estrus following 17 days post insemination. Conception rates and litter size were determent by real time ultrasonography at 34, 44 and 60 days post insemination. Data were analyzed using the CORR procedure and Tukey-Kramer multiple comparisons to test for trait effect.

**Results:** PCV, Hb(r=-0.97; P=0.01) and ewes BW (r=-0.99; P=0.06) were negatively correlated with the fertility percentage. There were no significant differences in fertility percentage between the two groups of rams based on ewes litter size (pregnancy type; single vs. twin). However, GPT activity in single group was greater than that of twin group [38.05 ± 0.43 vs. 33.66 ± 0.62 U/L (mean ± SE); P=0.1] and WBC (cells/µl) was significantly greater in twin group than that in single group [P<0.05; 12800 ± 141.42 vs. 8500 ± 100 (mean ± SE)].

**Conclusion:** In conclusion, this study suggests that fertility of Awasi ram may be affected by PCV, Hb and ewe’s BW and GPT activity and WBC number could be markers for pregnancy type in Awasi sheep. Further studies are needed to evaluate these relationships in Awasi sheep.
131 THE EFFECT OF PULSATILE TREATMENT OF FSH AND TESTOSTERONE ON DIFFERENTIAL GENE EXPRESSION IN RAT SERTOLICELLS DURING POSTNATAL TESTICULAR MATURATION

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(Presented By: Indrashis Bhattacharya, PhD)

Introduction: The alarming rise in male infertility merits an immediate attention. Testicular Sertoli cells (Sc) regulate spermatogenesis under the control of FSH and testosterone (T). Postnatal maturation of Sc in terms of appropriate hormonal responsiveness is prerequisite for the pubertal onset of spermatogenesis. However, limited knowledge about the extent of hormone (both T and FSH) responsive gene expression during the different phases of Sc maturation, restricts our understanding about the molecular events necessary for male fertility.

Methods: Sc were isolated, cultured from neonatal (5 days old), pre-pubertal (12 days old) and adult (60 days old) rat testes and were stimulated with pulsatile FSH and T (in combination) treatment. The hormone induced differential gene expression data obtained from Sc of 12 days and 60 days of age were compared with that of Sc obtained from 5 days old rats using microarray technology. The array data was also revalidated further by Q−PCR for some of the genes selected from all three age groups.

Results: Our data revealed that hormone induced gene expression was significantly higher with pulsatile treatment of hormones as compare to that of the constant exposure at 24hr. Moreover, the magnitude of hormone mediated augmentation in gene expression was on its peak at 11hr. Microarray analysis indicated that genes like Igfr1, Igfr2, Fgf9, Acvr1, Bmpr1b, Tgfb1 and Iita4, were upregulated in immature Sc. Ntf3, Nrg1, BDNF, SCF, GDNF and CXCl12 were upregulated in maturing Sc. Mature adult Sc were found to express genes involved in glucose metabolism, phagocytosis, and cytoskeleton structuring for the maintenance of spermatogenesis. The expression profiles of some of such genes like Aass, Unc5c, Ccl5, RoBo, Fat3, Tir, Wisp, Msln, Spz, Pwpp1 and Testin etc were validated by qPCR that authenticated the reliability of the array data further.

Conclusion: Taken together, our data suggested that the pulsatile hormone treatment is a better choice over the conventional constant hormonal exposure to primary culture of Sc for studying hormone induced gene expression. The differential transcriptome data provide an important resource to reveal the molecular network of Sc maturation which is necessary to govern male germ cell differentiation, hence, will improve our current understanding of the etiology of some forms of male infertility.

132 HISTONE H4K20 DEMETHYLASE REGULATES SPERMATOGENESIS

Charlie Degui Chen
(Presented By: Charlie Degui Chen)

Introduction: It is well known that the amount of heterochromatin increases with the differentiation of spermatogonia, however, the role of heterochromatin in this process is not defined. Because heterochromatin formation is regulated by methylation in histone H4 lysine 20 (H4K20me), we set out to identify histone demethylases for this heterochromatin mark. Objectives: To identify a demethylase for H4K20 and examine its role in the differentiation of spermatogonia.

Methods: high-content cell-based screening of a cDNA library containing 4,500 nuclear proteins one by one, in vitro enzymatic assays, ChIP-seq coupled with RNA-seq, and gene knockout study.

Results: Ecotropic expression of KDM9 led to a reduction in the global level of H4K20me1. ChIP–Seq experiments revealed that KDM9 demethylated H4K20me2 and H4K20me3 at specific genomic loci in vivo. In vitro, KDM9 specifically demethylated H4K20me1/2/3 and generated formaldehyde, and the enzymatic activity required Fe(II), −ketoglutarate and ascorbic acid as cofactors. RNA-seq demonstrated that KDM9 regulated the transcription of repetitive elements, but not protein coding genes. KDM9 knockout blocked spermatogenesis in mice.

Conclusion: We identified a histone demethylase for H4K20 that regulates spermatogenesis. Since the protein sequence of the catalytic domain of KDM9 is different from LSD1 and JmjC domain-containing proteins, the two known classes of histone demethylases, this enzyme represents a new class of histone demethylase.

133 – WITHDRAWN
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NR4A1 EXPRESSION IS REGULATED BY THE CALCIUM SIGNALING PATHWAY THROUGH DISTINCT AP1/CREB AND MEF2 ELEMENTS IN LEYDIG CELLS
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(Presented By: Nicholas Robert)

Introduction: The nuclear receptor NR4A1 (NUR77) is expressed in steroidogenic Leydig cells where it plays pivotal roles by regulating the expression of several genes involved in steroidogenesis and male sex differentiation including Star, HSD3B2, and Ins13. Activation of the cAMP and Ca2+ signaling pathways in response to LH stimulation leads to a rapid and robust activation of Nr4a1 gene expression that requires CAMKI kinase. We recently showed that disruption of the Ca2+ signaling pathway impair steroidogenesis in MA−10 Leydig cells through to a decrease in STAR and NR4A1 expression and promoter activity. While the cAMP/PKA and Ca2+/CAMKI signaling pathways are important for Nr4a1 expression, the specific transcription factor(s) mediating the effects of the Ca2+ signaling pathway in Leydig cells remain poorly characterized. We previously showed that the Nr4a1 proximal promoter (−331 to +50 bp) contains three important regions (NIR) with distinct activities: NIR−A (−331 to −233 bp) and NIR−C (−121 to −65 bp) are hormone/cAMP responsive while NIR−B (−233 to −121 bp) mediates most of the basal promoter activity.

Methods: In order to identify potential Ca2+/CaM effectors that regulate Nr4a1 expression, MA−10 Leydig cells were treated with forskolin to increase endogenous cAMP levels, calmidazolium (Cd2) to increase intracellular Ca2+ influx, dantrolene to inhibit endoplasmic reticulum Ca2+ release, and/or W7 to inhibit CaM activity. Using a Luciferase−based promoter analysis, we identified Ca2+-responsive elements mainly located in the NIR−A and NIR−C regions of the Nr4a1 promoter, which contains binding sites for several transcription factors such as AP1, CREB and MEF2.

Results: We found that one of the three AP1/CRE sites located at −255 bp is the most responsive to the Ca2+ signaling pathway as well as the two MEF2 binding sites at −315 and −285 bp. Furthermore, we found that the hormone−induced recruitment of the co−activator p300 to the Nr4a1 promoter requires the Ca2+ pathway.

Conclusion: Together our data indicate that the Ca2+-signaling pathway increases Nr4a1 expression in MA−10 Leydig cells, at least in part, by enhancing the recruitment of co−activator most likely through the MEF2, AP1, and CREB transcription factors. In conclusion, these data demonstrate an important interplay between the Ca2+ and cAMP pathways in regulating Nr4a1 expression through distinct promoter elements that ultimately modulate co−activators recruitment.

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INTRAFLAGELLAR TRANSPORTER PROTEIN IFT20 IS ESSENTIAL FOR SPERMIOSGENESIS IN MICE
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(Presented By: James Foster)

Introduction: Intraflagellar transport (IFT), originally discovered in Chlamydomonas, is an evolutionarily conserved mechanism thought to be essential for the assembly and maintenance of all eukaryotic cilia and flagella. IFT polypeptide orthologues were also found in mice, and mutations in IFT proteins have been shown to cause several ciliopathies. In mouse testis, IFT20 is present in Golgi, the manchette, and the basal body of differentiating germ cells, key structures in ciliogenesis, suggesting that IFT20 might be also essential for spermatogenesis.

Methods: To investigate the role of IFT20 in male germ cells, the floxed Ift20 mice were bred to Stra8−cre mice so that the Ift20 gene is disrupted in spermatocytes/spermatids.

Results: The Ift20:Stra8−cre mutant mice did not show any gross abnormalities, all of the mutant mice survive to adulthood. There was no difference between testis weight/body weight between controls and mutant mice. Fertility of six week−old mutant was dramatically reduced, and adult mutant males were completely infertile. Sperm number, the percentage of motile sperm in the cauda epididymis, and sperm motility were dramatically reduced compared to the controls. Histological examination of the testes and epididymis revealed abnormally shaped elongating spermatid heads starting with step 10, and bulbous round spermatids in the lumen of seminiferous tubules. Spermatids appeared to be unable to form cytoplasmic lobes and residual bodies; resulting in necrosis or apoptosis and sloughing of cytoplasm. Some cells appeared to begin tail formation, but the tails were short, with only a few tails extend into the tubule lumen. Increased amount of cytoplasmic vesicles were observed in the mutant cells. Golgi body formation and chromatin condensation appeared to be normal. The epididymides contained round bodies of cytoplasm, probably derived from the sloughing of the cytoplasmic lobes and residual bodies. Some sperm with attached heads appeared normal, but tails were short and kinked. Immunofluorescence staining demonstrated that key sperm flagella components, including ODF2 and SPAG16L failed to be incorporated into sperm tails of the mutant mice.

Conclusion: Collectively, our findings suggest that IFT20 is essential for normal spermiogenesis.
ANABOLIC STEROIDS AND HYPOGONADISM IN ADVANCED CANCER: TO TREAT OR NOT TO TREAT

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Introduction: Hypogonadism (or testosterone deficiency) is prevalent in advanced cancer and is shown to contribute to decreased bone density and muscle wasting. These patients can benefit from a multimodal therapeutic approach to treatment that could include the administration of an anabolic steroid (testosterone or one of its numerous analogues). The clinical rational for this intervention not only favours the anabolic effect of testosterone, but the improvement of cognitive function and the reduction of the symptom burden that is frequently associated with advanced cancer patients. However, there is an ongoing controversy regarding the use of testosterone supplementation as a safe treatment intervention in hypogonadic patients, especially those with prostate cancer. Support for this argument is found in studies that have shown the absence of prostate cancer in those subjects that are naturally hypogonadic or after the surgical removal of the testes. Clinicians have been reluctant to prescribe any androgenic/anabolic steroid because of a perceived lack of evidence based medicine that would confirm its safe and effective use in advanced cancer patients. On the other hand, new theory and medical evidence are proving that testosterone administration in relatively high concentrations has a protective effect on the prostate.

Methods: We reviewed the historical and current literature (1940-the present) regarding testosterone and its utilization as a therapeutic intervention. We compared several algorithms and medical recommendations from different andro-endocrinological societies. Finally, we have elaborated upon a new risk/benefit ratio framework for advanced cancer patients.

Results: The landmark study by Huggins et al (1941) appears to have been the only published study that recommended the avoidance of testosterone especially as a treatment for patients with prostate cancer. This recommendation apparently has been based on the results from 3 male adult subjects. Current medical evidence suggest that the risk/benefit ratio favors the use and positive benefits provided by anabolic/androgen replacement therapy. At least over the last 15 years, junior clinicians have continued to question the use of testosterone therapy. Recently, the result of large clinical trials, epidemiological studies and more informed interpretations of drug precaution concepts are providing us with the rational to use testosterone for our patients as valid pharmacological choice. In fact, researchers are now demonstrating that testosterone replacement therapy can serve as a safe and valid pharmacological alternative that can ameliorate the quality of life and survival of advanced cancer patients.

Conclusion: There appears to be a paradigm shift that supports the use of anabolic steroid therapy in hypogonadic patients. This new pharmacological approach will positively change the clinical outcome of patients in palliative care.