ABSTRACTS

Sunday, April 6, 2014
11:00 a.m. - 12:30 p.m.

Poster Session I*
**Not CME Accredited*
Location: Venetian

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LONG−TERM TREATMENT WITH TESTOSTERONE UNDECANOATE (TU) IN HYPOGONADAL MEN WITH CARDIOVASCULAR DISEASES (CVD): OBSERVATIONAL DATA FROM A REGISTRY STUDY

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(Presented By: Farid Saad, DVM, PhD)

Introduction: Hypogonadism is associated with cardiometabolic risk. Several studies suggest that hypogonadism increases the risk of all−cause and cardiovascular mortality. While some short−term studies have been performed in men with CVD, there are no data on long−term effects of testosterone replacement therapy (TRT) in men with CVD.
Methods: In a prospective, cumulative, observational registry study from a single urologist's office, 300 men with testosterone ≤12.1 nmol/L received TU injections for up to 6 years. In this subgroup analysis, 68 men with a previous diagnoses of coronary artery disease (CAD; n=40) and/or a history of myocardial infarction (MI; n=40) were analyzed.
Results: Mean age was 60.76±4.94 years. 68 men were included for 2 years, 59 for 3 years, 54 for 4 years, 44 for 5 years and 28 for 6 years. Declining numbers reflect the nature of the registry (patients are included after receiving 1 year of TRT) but not drop−out rates. Weight (kg) decreased from 115.07±13.71 to 92.5±9.64. Waist circumference (cm) decreased from 112.07±7.97 to 99.89±6.86. BMI decreased from 37.27±4.45 to 30.14±3.21 ($p<0.0001$ for all). Mean weight loss was 17.05±0.57%. Mean fasting glucose decreased from 108.74±17.08 to 96.0±1.92 mg/dl, HbA1c from 7.81±1.17 to 6.2±0.62% ($p<0.0001$ for both). Total cholesterol decreased from 304.66±34.09 to 189.32±9.68, LDL from 184.28±37.51 to 134±27.91, triglycerides from 308.38±56.3 to 187.71±8.67 ($p<0.0001$ for all) and HDL increased slightly. The total cholesterol:HDL ratio declined from 5.16±1.55 to 3.15±0.87 ($p<0.0001$). Systolic BP decreased from 167.82±11.01 to 142.36±10.62, diastolic BP from 102.28±8.23 to 81.25±8.07 mmHg ($p<0.0001$ for both). Pulse pressure declined from 65.54±5.24 to 61.11±4.66 ($p<0.0001$). Quality of life, measured by the Aging Males' Symptoms scale (AMS) improved from 56.25±10.09 to 17.11±0.31. The minimum number of injections was 9, maximum 26. In no patient TRT was discontinued or interrupted. There were no major cardiovascular events during the observation time.

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156 HYPOGONADAL MEN WITH OBESITY AND TYPE 2 DIABETES ACHIEVE WEIGHT LOSS AND IMPROVED GLYCAEMIC CONTROL UPON TREATMENT WITH TESTOSTERONE UNDECANOATE UP TO 6 YEARS: A SUBGROUP ANALYSIS FROM TWO OBSERVATIONAL REGISTRY STUDIES

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(Presented By: Farid Saad, DVM, PhD)

Introduction: Obesity is a major risk factor for type 2 diabetes (T2D). In men, both diseases have a high prevalence of testosterone deficiency (hypogonadism). Testosterone replacement treatment (TRT) has been shown to improve weight and T2D. Numerous mechanisms have been identified as to how testosterone impacts glycaemic control. We studied the effects of TRT in obese hypogonadal men with T2D.
Methods: Cumulative, prospective, observational registry studies of 561 hypogonadal men from two urological centers. From these registries, we selected all men with obesity and T2D for subgroup analysis. All men received testosterone undecanoate injections for up to six years. All men were treated for their T2D by their respective family physician.
Results: 156 men (28% of all patients) met our criteria. Mean age was 61.2±6.2 years at start of treatment. Weight (kg) decreased from 113.56±11.53 to 97.18±9.04. This decrease was statistically significant vs baseline ($p<0.0001$) and each year compared to previous year. The model−adjusted mean change from baseline was −17.49±0.58 kg. The mean per cent weight loss (%) was 15.04±0.48 after 6 years. Waist circumference (cm) declined from 114±8.69 to 102.52±7.93. This was statistically significant vs baseline ($p<0.0001$) and each year compared to the previous. The mean change from baseline was −11.56±0.34 cm. BMI (kg/m2) decreased from 36.31±3.51 to 31.19±2.6. This change was statistically significant vs baseline ($p<0.0001$) and each year compared to previous year. The mean change from baseline was −5.59±0.18 kg/m2. Fasting glucose (mg/dl) decreased from 128.37±31.63 to 101.55±17.02 ($p<0.0001$ vs. baseline, significant for the first two years vs. previous year). The mean change from baseline was −27.14±2.48 mg/dl. HbA1c decreased from 8.08±0.9 to 6.14±0.71% ($p<0.0001$ vs. baseline, significant for the first 5 years vs. previous year and approaching significance from year 6 to year 5 at $p=0.0635$). The mean change from baseline was −1.93±0.06%. At baseline, 25 (16%) of all patients had an HbA1c ≤ 7.0% and 12 (7.7%) an HbA1c ≤ 6.5%. At the end of the observation period, 128 (82.05%) had reached an HbA1c target of ≤ 7.0% and 106 (67.95%) an HbA1c target of ≤ 6.5%.
Conclusions: Correcting hypogonadism by TRT with testosterone undecanoate injections in obese hypogonadal men with T2D resulted in significant and sustained improvements in weight, waist circumference, fasting glucose and HbA1c over the full 6 years of the study.

ABSTRACTS

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LACK OF ACTIVATION OF ENCLOMID TO ITS 4−HYDROXYLATED FORM BY CYP 2D6 DOES NOT EXPLAIN LACK OF TESTOSTERONE RESPONSE

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(Presented By: Ronald Wiehle, PhD)

Introduction and Objective: Enclomid (Androxal), an isomer of Clomid, is effective in raising serum testosterone (T) in ~80% of men with secondary hypogonadism. The drug appears to act at the level of the hypothalamus/pituitary by first raising LH and FSH. We hypothesized that those few men who do not respond with an increase in serum T could have a defective CYP 2D6 which does not allow the metabolism of the parent drug to the highly active 4−hydroxy−Enclomid form. To determine the proportion of men with secondary hypogonadism that are non−responders to Enclomid who also do not make 4−hydroxy−Enclomid.

Methods: A Phase 3 clinical trial (ZA−302) in men with secondary hypogonadism. Subjects were enrolled through the criteria of two morning serum T in the hypogonadal range and normal LH levels. All subjects were treated with 12.5 or 25 mg of Enclomid daily and orally for 12 weeks. Men were assessed for serum LH, FSH, and serum T. The trough (steady state) levels of serum Enclomid and 4−OH−Enclomid were determined by HPLC at the end of the study.

Results: Eight−one percent of men attained morning serum T in the 300−1000 ng/dL range. We looked a subset of 12 men who were non−responders in terms of serum T and 22 other men who did respond. Most men demonstrated high conversion of Enclomid to 4−hydroxy−Enclomid such that the ratio of 4−Hydroxy−Enclomid to Enclomid was 1.4 (+/− 0.78). Looking at 12 non−responders, we determined that only 2 individuals showed low levels of 4−Hydroxy−Enclomid but one man out of 22 who did respond with a higher T level also showed low 4−hydroxy−Enclomid. Essentially all men demonstrated increase in serum LH.

Conclusions: We infer that 4−hydroxy−Enclomid is probably not required for hypothalamic−pituitary release of LH and the metabolite is not necessary for increasing serum T. The inability to raise T despite increasing LH suggests an additional factor is involved. This results needs to be verified in a larger data set and attention to other metabolites. This work was supported by Repros Therapeutics.

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ESTRADIOL INCREASES THE PROLIFERATION OF RAT IMMATURE LEYDIG CELLS: A POSSIBLE ROLE FOR LEYDIG CELL TUMOR FORMATION

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(Presented By: Xiaoheng Li)

Introduction: The causes of Leydig cell tumor formation are still unclear. Estradiol (E2) has been hypothesized to cause Leydig cell tumor. However, its mechanism is unknown. Unlike adult rat Leydig cells that have no proliferative capacity, rat progenitor and immature Leydig cells have the higher proliferative ability.

Methods: In the present study, rat progenitor, immature and adult Leydig cells were isolated from the testes of 21, 35 and 90 day old Sprague Dawley rats, and treated with different concentrations of E2. After 24 hours of treatment, these cells were incorporated with a radio−labelled thymidine. E2 did not affect the proliferative capacity of rat progenitor and adult Leydig cells, while it significantly increased the thymidine incorporation into immature Leydig cells.

Results: The incorporation rates to immature Leydig cells were significantly increased by52,120, 123, and 364% after 50, 250, 500 and 1000 nM E2 treatment, respectively. Immature Leydig cells had significantly higher estrogen receptor α expression when compared to progenitor and adult Leydig cells. Whole genomic profiling analysis showed that E2 significantly upregulated neuropeptide Y receptor and insulin−like growth factor 2 receptor signalling pathway.

Conclusion: In conclusion, at higher concentration, E2 can stimulate the proliferation of rat immature Leydig cells.

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PLATELET−DERIVED GROWTH FACTOR (PDGF) STIMULATES DIFFERENTIATION OF RAT IMMATURE LEYDIG CELLS VIA INCREASING THE EXPRESSION OF STAR

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(Presented By: Xiaomin Chen, PhD)

Introduction: Platelet−derived growth factor (PDGF) is one of growth factors that regulate cell growth and differentiation. In the lineage of rat Leydig cells, there is an increased expression of the α receptor (PDGFRA) during pubertal development. However, the mechanism of PDGF in the regulation of Leydig cell development is unclear.

Methods: In the present study, rat immature Leydig cells were isolated from the testes of 35−day−old Sprague Dawley rats, and treated with 1 and 10 ng/ml of PDGF−BB.

Results: After 24 hours of treatment, these cells were harvested for genomics profiling and the medium steroids were measured. 1 and 10 ng/ml PDGF−BB significantly increased androgen production by rat immature Leydig cells.

Conclusion: Genomics profiling analysis showed that the expression levels of steroidogenic acute regulatory protein (Star) were increased by 2−fold. Further analysis showed that Egr1 and Egr2 expression levels were increased 4.9 and 3.6 fold by 10 ng/ml PDGF−BB, respectively. In conclusion, PDGF−BB stimulated the differentiation of rat immature Leydig cells via regulating Star.

ABSTRACTS

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TESTOSTERONE AS PROGNOSTIC INDEX IN ACUTE EXACERBATION OF CHRONIC OBSTRUCTIVE PULMONARY DISEASE

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(Presented By: Antonio Mancini, MD)

Introduction: Today chronic obstructive pulmonary disease (COPD) is not considered only a lung disease, in fact, systemic comorbidities, like weight loss, have not secondary role in evolution of the disease. Exacerbation of COPD (AECOPD) negatively influenced the natural history of the illness and it has been found related to muscle dysfunction. In this pathway, hypogonadism could play a pivotal role.
Methods: Our study want to evaluate possible relationships among prognostic indexes of AECOPD (APACHE II Score), inflammation (serum amyloid A, SSA) and hormonal axes primarily involved in metabolic balance of COPD patients. 24 patients, aged 75 ± 13 yrs, 17 males, were studied. Descriptive statistical analysis shows reduced values of testosterone (T) (1.85 ± 2.28 ng/mL), free testostosterone (f−T) (0.028 ± 0.030 ng/mL), dihydrotestostrone (DHT) (0.18 ± 0.19 ng/mL) and IGF−1 (91.840 ± 74.19 pg/mL).
Results: Frequency distributions of Apache II score and SSA were calculated and, using tertile as cut off point, three categories were made and used in the analysis (SSA: = 8 mg/mL; 9−160 mg/mL; = 160 mg/mL); (APACHE II: = 10; 11−12; = 12). Using this classification, an inverse correlation between SAA and T (p: 0.01), f−T (0.01), DHT (0.001) and IGF−1 (p: 0.05) was found.
Conclusion: Data show the same inverse relationship between APACHE II tertiles on one hand and T (p: 0.01) and f−T (p: 0.02) on the other hand. Even if we cannot establish a causal relationship between hypogonadism and severity of disease, our data suggest systemic effects of AECOPD and a possible mechanism explaining wasting syndrome.

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INFLUENCE OF TESTOSTERONE DEPRIVATION ON OXIDATIVE STRESS INDUCED NEURONAL DAMAGE IN HIPPOCAMPUS OF ADULT RATS

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(Presented By: Prakash Seppan, PhD)

Introduction and Objective: Increasing evidence supports the role for androgens in brain function, through genomic and non−genomic mechanisms. Analyzes the testosterone action in hippocampus can lead towards identifying therapeutic targets for not only reproductive function, but also adult sexual behavior and cognition. Due to the functional and high energy demand in hippocampal neurons, increased reactive oxygen species is a common factor and hence requires very good anti−oxidant system, together the role of testosterone in these neuronal cells seem to be imperative in this process. To study the influence of testosterone deprivation induced oxidative stress and the cascade hippocampal cell damage, it's possible impact on memory and cognitive behavior.
Methods: Adult male Wistar albino rats were used as control, castrated and castrated + testosterone supplemented (5mg/Kg/day) groups. From 10th day after surgery, the animals were subjected to analysis of pituitary−testicular axis by estimating serum testosterone, FSH and LH. Assessment for memory using radial arm maze and affective behavior assessment was done by open−field test and elevated plus maze. By 18th day animals were sacrificed. Hippocampus processed for biochemical analyses of SOD, GPX, GR, Catalase, LPO, GST, Vit C and Vit E. Histology analyzed using H&E staining.
Results: Following castration, pituitary testicular axis was disrupted. Significant reduction of both enzymic and non−enzymic antioxidant levels were observed in castrated animal hippocampus. Memory acquisition was also significantly reduced in castrated animals. Anxiety and affective behavioral changes were more pronounced in castrated animals compared to the intact animals. Histological sections of hippocampus showed degenerative changes in the nuclear layer of CA3 and CA4 area of hippocampus. Above alteration were reverted to normal state as that of control animals in castrated +testosterone group. **Conclusion:** It was evident it is the testosterone deficiency that induces oxidative stress in the hippocampus and clearly affecting its physiological functions as well as its anatomical integrity. Physiological testosterone therapy is able to suppress oxidative stress probably mediated via the AR−independent or dependent pathway, indicating critical role of testosterone in neuro−biology. This requires further study to understand their complex relationship(s).

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EFFECTS OF FOUR CHEMOTHERAPEUTIC AGENTS, BLEOMYCIN, ETOPOSIDE, CISPLATIN AND CYCLOPHOSPHAMIDE, ON DNA DAMAGE AND TELOMERES IN A MOUSE SPERMATOGONIAL CELL LINE

Mingxi Liu, PhD, Barbara Hales, PhD, Bernard Robaire, PhD
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(Presented By: Mingxi Liu, PhD)

Introduction: Treatment with chemotherapeutics agents may induce persistent DNA damage in male germ cells with the possibility of long term consequences on fertility and progeny outcome. Telomeres, specialized structures at the physical ends of chromosomes, play an important role in the maintenance of genetic stability and in the response of somatic cells to anticancer drugs.

Methods: Our objective was to test the hypothesis that exposure to bleomycin, etoposide, or cisplatin (the drug regimen used to treat testicular cancer) or cyclophosphamide (a commonly used anticancer agent and immunosuppressant) targets telomeres in the male germ line. C18−4 spermatogonial cells (a gift from Dr. MC Hofmann) were exposed to bleomycin, etoposide, cisplatin or 4−hydroperoxycyclophosphamide (4−OOHCPA, a pre−activated analog) in vitro. DNA damage was assessed by γH2AX immunofluorescence. Telomeres were detected by fluorescence in situ hybridization (FISH) using a telomeric Cy3−conjugated peptide nucleic acid (PNA) probe. The extent to which DNA damage was localized in telomeres was analyzed with Imaris software. Telomere length (the ratio of telomere repeat copy number to single copy gene copy number) was assessed using q−PCR, telomerase activity was determined with the telomere repeat amplification protocol (TRAP) assay, and steady state concentrations of the mRNAs for telomerase enzyme components, Tert and Terc, by qRT−PCR analysis.
Results: All four anticancer drugs induced a significant increase in γH2AX immunofluorescence in C18−4 cells. Interestingly, the γH2AX signal was localized to telomeres after treatment with bleomycin, cisplatin, and 4−OOHCPA, but not etoposide. Mean telomere lengths, the intensity of the telomere FISH signal, telomerase activity, and the expression of Tert and Terc were reduced by exposure to cisplatin and 4−OOHCPA, but not by bleomycin or etoposide.
Conclusion: Thus, although all four anticancer drugs induce DNA damage in this spermatogonial cell line, only cisplatin and 4−OOHCPA, the two alkylating agents, induce telomere dysfunction. This telomere dysfunction may contribute to infertility and developmental defects in the offspring.
Supported by grant MOP−14851 from the Canadian Institutes of Health Research.

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EFFECT OF ROSMARINIC ACID ON SERTOLI CELLS APOPTOSIS AND SERUM ANTIOXIDANT LEVELS IN RATS AFTER EXPOSURE TO ELECTROMAGNETIC FIELDS

Arash Khaki, DVM, PhD
(Presented By: Arash Khaki, DVM, PhD)

Introduction and Objective: Rosmarinic acid belongs to the group of polyphenols; it has antioxidant, anti−inflammatory and antimicrobial activities and help to prevent cell damage caused by free radicals. The objective was to study the effect of Rosmarinic acid on sertolli cells apoptosis and serum antioxidant levels in rats after they were exposed to electromagnetic fields.
Methods: Male Wistar rats (n=40) were allocated into three groups: control group (n=10) that received 5cc normal saline (0.9% NaCl) daily by gavage method, Rosmarinic acid group that received 5mg/rat (gavage) (n=10), electromagnetic fields (EMF) group that had exposure with 50hz (n=20) which was subdivided to two groups of 10; EMF group and treatment group. Treatment group received 5mg/rat (gavage) Rosmarinic acid daily for 6weeks, respectively. However, the control group just received an equal volume of distilled water daily (gavage).
Results: On the 42nd day of research, 5cc blood was collected to measure testosterone hormones, total antioxidant capacity (TAC), levels from whole group's analysis. Level of malondialdehyde (MDA) levels and sertoli cells apoptosis significantly decreased in the group that received 5mg/rat of Rosmarinic acid ($P<0.05$) in comparison with experimental groups. Level of testosterone, total antioxidant capacity (TAC), significantly increased in groups that received Rosmarinic acid ($P<0.05$).
Conclusion: Since in our study 5mg/rat of Rosmarinic acid showed significantly preventive effect on cell damages especial sertoli cells apoptosis that caused with EMF, it seems that using Rosmarinic acid as food additive can be effective for supporting people living under EMF environmental pollution.
Keywords: Apoptosis, EMF, Rosmarinic acid, Sertoli cells, Testosterone.

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HUMAN SPERM BIOASSAY IN EVALUATING THE QUALITY OF BLOOD SERUM AND FOLLICULAR FLUID OF FEMALES UNDERGOING IN VITRO FERTILIZATION (IVF) BASED INFERTILITY TREATMENT

Amjad Hossain, PhD
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(Presented By: Amjad Hossain, PhD)

Objectives: Human sperm bioassay (HSB) is a convenient in−house quality control test in laboratories that are involved with assisted reproductive technology based fertility treatment. Proficiency test providers also take advantage of human sperm for developing proficiency test (PT). In ovarian stimulation with gonadotropins, gonadal secretions accumulate in follicular fluid (FF) and also in blood serum (BS). In this study, the ability of HSB in evaluating the variation in the quality of FF and BS of females undergoing IVF was assessed.
Methods: BS and FF were obtained from IVF patients. The samples were representative of 3 conditions: patient age (young vs old), ovarian response (normal, poor and high) and procedure outcome (pregnant vs non−pregnant). Embryo culture media (ECM) and supplement (serum albumin, SA) obtained from American Association of Bioanalysts (AAB) as PT sample was used to prepare control. ECM was also used as the base media in the experimental group. Conventional HSB was performed following the method provided by AAB. Briefly, 0.5 ml ECM was supplemented with SA, BS or FF at 5%. The culture medium was maintained in center well dish (Falcon) covered with 1 mL oil (Irvine). The sperm concentration was adjusted to 3x106/ ml in the culture and the culture dishes were kept in the incubator (37o C and 5.5% CO2). Sperm motility and motility grade were determined at 0 and 48 hour. In motility grade evaluation, only grades 3 and 4 were taken into consideration.
Results: By 48 hrs culture, the decline in motility grade was more stringent than that in motility (90% to 30% vs. 90% to 60%). Cultures supplemented with BS and FF exhibited a trend of higher motility compared to that of SA supplemented controls but no difference between them (SA 56+ 3%, BS 64+ 2 %, FF 64+ 3%). Similarly, as assessed by sperm motility, the cultures did not discriminate age (< 35 yrs 65+ 2 % vs > 35 yrs 64+ 2%), ovarian response (poor 64+ 3% vs normal 63+ 3% vs high 64+ 2%) and pregnancy potential (pregnant 64+ 3% vs non−pregnant 63+ 2%). Motility grade imitate the motility pattern in respective culture conditions.

Conclusion: There occurred increased deterioration in motility grade compared to motility. The sensitivity of the conventional human sperm bioassay was not strong enough in revealing the fine differences in the quality of the human body fluid such as BS and FF. The low sensitivity is probably attributable to the assay procedure.

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EFFECTS OF APIGENIN ON THE DEVELOPMENT AND FUNCTION OF RAT IMMATURE LEYDIG CELLS

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(Presented By: Qiqi Zhu, MA)

Introduction: Apigenin is a natural flavone. However, whether it interferes with the androgen production in Leydig cells is unclear. The object of the present study was to investigate the effects of apigenin on the development and function of rat immature Leydig cells.
Methods: Rat immature Leydig cells were incubated for 3 hours with 100 μM without (basal) or with 1 ng/ml luteinizing hormone (LH), 20 μM of the following chemicals: 8−bromoadenosine 3',5'−cyclic monophosphate (8BR), 22R−hydroxychloesterol (22R), pregnenolone (PREG), progesterone (P4), and androstenedione (D4). The medium level of 5α−Androstane−3α,17β−diol (DIOL), the primary androgen produced by rat immature Leydig cells, was measured.
Results: Apigenin significantly inhibited basal, 8BR, 22R, PREG, P4, and D4 stimulated DIOL production in rat immature Leydig cells. Further study showed that apigenin inhibited rat 3β−hydroxysteroid dehydrogenase, 17α−hydroxylase/17,20−lyase, and 17β−hydroxysteroid dehydrogenase 3 with IC50 values of 11.41± 0.7, 8.98± 0.10, and 9.37±0.07 μM, respectively. Apigenin inhibited human 3β−hydroxysteroid dehydrogenase and 17β−hydroxysteroid dehydrogenase 3 with IC50 values of 2.17 ± 0.04 and 1.31 ± 0.09 μM, respectively.
Conclusion: In conclusion, apigenin mainly inhibited rat and human steroidogenic enzymes.

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STIMULATION OF STEROIDOGENESIS IN RAT IMMATURE LEYDIG CELLS BY BROMINATED FLAME RETARDANT BDE−100

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(Presented By: Haiyun Deng, MD)

Introduction: Polybrominated diphenylether BDE−100 is considered as a potential endocrine disruptor. The objective of this study was to explore whether BDE−100 could affect androgen biosynthesis and metabolism in rat immature Leydig cells.
Methods: Rat immature Leydig cells (ILCs) were treated with 3×10−9 to 3×10−6 M BDE−100 in vitro for 3hr, the production of 5α−androstane−3α,17β−diol (DIOL), the primary androgen produced by rat immature Leydig cells and steroidogenic enzyme activities were determined.
Results: 3×10−6 M BED−100 significantly increased basal, LH−, 8bromo−cAMP−stimulated DIOL production by 2, 2, and 5 fold. At this concentration BED−100 did not affect 22R−OH−cholesterol and pregnenolone−stimulated DIOL production. Indeed, at this concentration BED−100 stimulated Scarb1 and Lhcgr expression levels of ILCs. However, it did not affect the expression levels of other Leydig cell genes, including Star, Tpso, Cyp11a1, Hsd3b1, Cyp17a1, Hsd17b3, Srd5a1 and Ark14c.
Conclusion: The results of this study indicate that environment−related level of BDE−100 in vitro increased DIOL production in a dose−dependent manner. The stimulated effects of BDE−100 on Scarb1 and Lhcgr might play key roles in BDE−100−mediated stimulation of DIOL production.

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MONONUCLEAR PHAGOCYTES FROM THE PROXIMAL MOUSE EPIDIDYMIS TAKE UP LUMINAL BACTERIA.

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Massachusetts General Hospital/Harvard Medical School
(Presented By: Tegan Smith, PhD)

Introduction: Our discovery of a dense and heterogeneous network of mononuclear phagocytes (MPs) in the murine epididymis raises questions regarding the function of these antigen−presenting cells, which express classic macrophage and dendritic cell markers including CD11c, F4/80 and CX3CR1. We hypothesize that one such function might be to contribute to the maintenance of a pathogen−free luminal environment, therefore the objective of this study was to assess the ability of epididymal MPs (eMPs) to take up Escherichia coli (E. coli), a bacterium known to induce inflammation in the human epididymis manifesting in epididymitis.
Methods: In this study, CX3CR1+ MPs isolated from the proximal mouse epididymis (initial segment and caput) were co−incubated with fluorescent E. coli bioparticles, fixed and assessed by fluorescence microscopy. After 2 hours co−incubation, CX3CR1+ cells were filled with E. coli particles, indicating that eMPs have very potent phagocytic capabilities in vitro. In order to determine the ability of eMPs to capture antigens in vivo, we have developed a micro−inoculation technique whereby soluble and insoluble materials can be administered into the lumen of the proximal epididymis via the efferent ducts, causing minimal damage and disruption to the epididymis.
Results: Four hours following injection of fluorescent E. coli, particles could be clearly visualized in the lumen of the proximal segments, and a number of E.coli particles were located within epithelial cells that did not express V−ATPase, indicating that principal cells phagocytosed E. coli particles. Very few particles were located within CD11c+ and CX3CR1+ MPs. However, 24 hours after micro−inoculation, E.coli particles were observed accumulating within CD11c+ and CX3CR1+ MPs located in the basal region of the epithelium.

Conclusion: Our results indicate that peritubular MPs from the proximal epididymis take up antigenic particles originated specifically from the luminal compartment. We are currently characterizing the uptake of other soluble and insoluble antigens, as well as the mechanisms that control antigen acquisition. The respective roles of epithelial cells and epithelium−associated mononuclear phagocytes in the epididymal mucosa remain to be elucidated.

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ROLE OF SPERM TRANSCRIPTS IN THE ETIOLOGY OF IDIOPATHIC RECURRENT EARLY PREGNANCY LOSS

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Molecular Reproduction and Genetics Lab, Department of Anatomy, All India Institute of Medical Sciences, New Delhi, India
(Presented By: Kranthi Vemparala, PhD)

Introduction: Recurrent spontaneous abortion (RSA) is defined as two or more consecutive pregnancy losses before the 20th week of gestation. Although multiple factors involved in the etiology of RSA, RSA is traditionally diagnosed from the maternal perspective and the role of paternal factors in recurrent abortion less understood. The relationship between sperm parameters and RSA is controversial and Molecular parameters like sperm DNA fragmentation index (DFI) are not sufficient in RSA diagnosis and still there is need to find other molecular factors which compliment DFI in better diagnosis. As paternal genome is has profound importance in fetal development, and our objective of this study is to understand the role of sperm gene expression in RSA.
Methods: Ejaculates were obtained from 24 fertile healthy volunteers and 24 male partners of couple experiencing idiopathic RSA. After routine Semen analysis, cDNA was synthesized using Total RNA extracted from separated Sperm cells and gene expression analyzed by qPCR. The genes TOMM7, RPS6, RBM9, RPL10A, EIF5A, AKAP4, FOXG1, Sox3, and STAT4 were selected for gene expression analysis based on previous literature and were validated in RSA patients.
Results: Out of 9 genes studied, Expression of 7 genes (TOMM7, RBM9, RPL10A, EIF5A, AKAP4, FOXG1, Sox3, and STAT4) was slightly upregulated, one gene (Sox3) was highly upregulated (3 fold) and for one gene no change in expression was observed compared to their counterparts. The Mean fold changes for TOMM7, RPS6, RBM9, RPL10A, EIF5A, FOXG1, Sox3, AKAP4, and STAT4 are 1.78, 1.05, 1.73, 1.91, 1.22, 2.16, 4.1, 1.66, and 2.34 respectively.
Conclusion: The genes which are regulators of protein synthesis, mitochondrial import, alternate splicing, Sperm fibrous sheath assembly, apoptosis and cell survival which are critical for normal embryo development are upregulated and Sox3 gene which is essential for normal Spermatogenesis is highly upregulated suggests the role of these genes in recurrent spontaneous abortion. But further functional studies would confirm and validate the usability of the expression profile of these genes in the molecular diagnosis of RSA to compliment already established indicators like DNA fragmentation index.

No	Gene Name	Mean fold change
1	TOMM7	1.775
2	RPS6	1.051
3	RBM9	1.727
4	RPL10A	1.912
5	EIF5A	1.221
6	FOXG1	2.156
7	SOX3	4.098
8	AKAP4	1.657
9	STAT4	2.338

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PRIMARY TESTICULAR FAILURE: GENOTYPE PHENOTYPE CORRELATION OF 140 CASES

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Introduction: Primary testicular failure (PTF) refers to conditions where testes fail to produce sperms despite adequate hormonal support. PTF is classified into four distinct subtypes viz., Sertoli Cell Only Syndrome (SCOS), Maturation Arrest (MA), Hypospermatogenesis (HS) and Tubular Fibrosis (TF). Despite efforts, causes of PTF in most cases are still unknown. This study is based on 140 apparent idiopathic PTF cases. Known causes viz., mumps orchitis, varicocele, torsion, trauma, cryptochidism, etc or treatment with chemotherapeutic drugs was excluded before inclusion into the study.
Methods: Study groups were comprised of 54 cases of MA, 52 cases of SCOS and 34 cases of HS. FISH with XY probes were carried out in addition to conventional chromosome analysis to find out sex chromosome aneuploidy. STS PCR analysis was carried out for Yq microdeletion studies. There were 50 normal fertile male served as control. For sertoli cell maturity status anti−mullerian hormone and for sertoli cell functional status inhibin B as well as seminal lactate were estimated by ELISA method. Serum heavy metals levels were evaluated in 90 cases. Later, in a subset of 37 idiopathic MA cases DNA microarray was carried out to find out any association with recurrent CNV/LOH.

Results: Underlying cause (Yq microdeletion and chromosomal abnormality) was detectable in 30 cases (21.4%) of PTF (13 sex chromosomal abnormality & 17 Yq microdeletions). When we dissected out in relation to subtypes we find different frequency of detectable causes. Detectable cause was found in 16 (11.4%) cases of SCOS, 8 (5.7%) cases of MA & 6 (4.3%) cases of HS. Heavy metal like manganese, lead and nickel were found consistently high (3−7X) in PTF than control (lead and nickel were 6−7X higher in MA than control). Microarray finding on idiopathic MA cases (37) showed recurrent CNVs of Yp11.31−p11.2 (15 cases with 3 copies), Yp11.2 (8 cases with 3 copies), Yq11.223 (6 cases with deletion), Yq11.23 (3 cases with deletion), Yq11.223−11.233 (3 cases with 3 copies), Xp11.23 (6 cases with 2 copies), Xq28 (4 cases with 3 copies), 14q32.33 (5 cases with 3 copies), 14q11.2 (3 cases with 3 copies), 7q11.1−11.21 (2 cases with 3 copies), 10q11.22 (2 cases with 3 copies), 16p11.2 (2 cases with 3 copies), 17p11.22 (2 cases with 3 copies) and 22q11.22 (2 cases with 3 copies).
Conclusion: Role of associated genes within CNVs in probable causation of maturation arrest will be discussed.

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SPERM TELOMERE LENGTH AND DNA INTEGRITY: ROLE IN IDIOPATHIC MALE INFERTILITY: IMPACTS OF LIFE STYLE INTERVENTIONS

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(Presented By: Swetasmita Mishra, MSc)

Introduction: Telomeres are highly conserved hexameric repeats which confer chromosome stability and maintain genomic integrity. Telomerase a reverse transcriptase maintain telomere length. PARP1 is a DNA repair enzyme recruited when there are double strand breaks in DNA. PARP−1 also plays a role in telomere maintenance. As telomeres are Guanine rich repeats, they are highly prone to oxidative damage. So, this study was planned to evaluate seminal oxidative stress, sperm DNA damage, sperm telomere length and telomerase activity in infertile men and also evaluate the effect of life style interventions (Yoga, Breathing exercises) on levels of telomere length and telomerase activity at (pre day 0, post yoga day 10 & 90).
Methods: The study included 33 infertile men and 30 controls. The average telomere length from the sperm DNA was measured using a quantitative Real Time PCR. Telomerase activity per cell was assessed by PCR ELISA. 8−Isoprostane and 8−Hydroxy−2−deoxy−Guaonosine levels were assessed by Cayman's ELISA kits. DFI was assessed by Sperm Chromatin Structure Assay (SCSA). DNA repair enzyme PARP1 expression was measured by q−PCR.
Results: The mean T/S ratio in patients was 0.742 ± 0.040 and controls 0.789 ± 0.066 (p=0.001). The mean Relative Telomerase activity per cell in patients was 25.48 ± 3.49 and controls 11.7 ± 1.71(p=0.148). 8−Isoprostane level in patients was 1154.14 ± 237.90 pg/ml and in controls 286.65 ± 80.35(p=0.004) pg/ml. The 8−OHdG level in patients was 48.36 ± 14.9 pg/ml and in controls 8.97 ± 4.64(p=0.136) pg/ml. The mean DNA Fragmentation Index (DFI %) in Patients was 38.86 ± 13.79 and in controls 27.4 ± 6.96 (p=0.044). PARP1 expression was significantly lower in patients compared to controls. With life style modifications telomere length showed no significant difference in 10 days (0.689±0.032), but significant increase after 3 months (0.851±0.051). Telomerase activity showed significant increase in 10 days (28.31± 1.150) and 90 days (34.31±1.067).
Conclusion: This study highlights oxidative DNA damage and preferentially telomere shortening in infertile men. Post life style interventions showed up regulation in telomere length and telomerase activity. Future of ART lies in selection of gametes with optimal telomere length and adoption of simple life style interventions may actually improve DNA health.

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INTEGRATIVE DNA METHYLATION AND GENE EXPRESSION ANALYSES IDENTIFIES DISCOIDIN DOMAIN RECEPTOR 1 (DDR1) ASSOCIATION WITH IDIOPATHIC NONOBSTRUCTIVE AZOOSPERMIA (NOA)

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Introduction: Spermatogenesis is a complex process that involves proliferation, differentiation, and cell adhesion. Spermatogenic failure or non−obstructive azoospermia (NOA) results from mechanisms involved are incompletely understood. DDR1 is a member of a small subfamily of receptor tyrosine kinases that is involved in adhesion, migration, proliferation, apoptosis, cell morphogenesis and differentiation. Since, DDR1 is expressed in human post−meiotic germ cells of testis, we hypothesized that abnormal DDR1 expression could be a possible mechanism that can compromise spermatogenesis in a subset of men with idiopathic NOA.
Methods: We used the high resolution Infinium 450K methylation array and compared fibrobalsts cultured from testicular biopsies of 19 NOA men and 4 fertile controls. Microarray data was analyzed using Minfi (R software package) utilizing subset−quantile within array normalization. We investigated the functional role of abnormal promoter DNA methylation for selected genes using mRNA expression by quantitative RT−real time PCR. Immunohistochemistry was used to confirm testicular expression and potential importance in spermatogenesis.

Results: Differentially methylated CpG sites (~20K) were identified using an F−Test ($p<0.05$) in the NOA samples. We identified 24 genes with the >30% difference in methylation within promoter region of men with NOA and fertile controls. Of the aberrantly methylated CpGs, 13 were hypomethylated and 11 were hypermethylated groups. From the top 11 hypermethylated genes, six genes (MRI1, DCAF12L1, TMEM95, CECR2, DDR1, NPHS2) were selected for validation since they were shown to be expressed in testis. Of the 6 genes validated with qPCR, DDR1 showed aberrant gene expression pattern. Four (21%) patients out of the 19 NOA men had lower expression levels (1.8x) of DDR1, whereas two (10.5%) men had higher expression levels (2.5x) of DDR1compared to fertile men ($p<0.05$). Immunohistochemical analysis suggests presence of DDR1 within cytoplasm of germ cells in fertile men and men with maturation arrest histology. DDR1 protein is absent in men with Sertoli−cell only or germ cell aplasia.
Conclusions: Aberrant expression of DDR1 is associated with NOA. The functional relevance of abnormal methylation of DDR1 to NOA warrants further investigation
Support: Funding supported by a Male Reproductive Health Research Career (MHRH) Development Physician Scientist Award (K12) (HD073917−01) from the Eunice Kennedy Shriver NICHD Program.

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AFFECT OF OXIDATIVE STRESS AND SPERM DNA DAMAGE ON EARLY EVENTS OF CONCEPTION, INDICES OF EMBRYO GROWTH AND EMBRYO QUALITY IN COUPLES OPTING FOR IVF

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(Presented By: Monis Bilal Shamsi, MSc, PhD)

Introduction: Sperm genome plays a key role in maintaining reproductive potential. Impact of altered paternal genome is as important as that of maternal genome. However, while role of oocyte is being increasingly recognized, influence of male germ cells on conception is still not clear. The study investigates the association of reactive oxygen species (ROS) and sperm DNA damage on fertilization rate, cleavage rate, embryo quality and on pregnancy outcome in couples opting for in vitro fertilization (IVF).
Methods: In 278 infertile males opting for IVF and 124 fertile controls, ROS levels in semen was analyzed by chemiluminescence and sperm DNA damage was quantified by comet assay. Standard IVF protocol was adopted. Fertilization and cleavage rate, embryo quality and pregnancy outcome were followed and documented.
Results: ROS levels (32.41 RLU/sec/million) in non conceived group was significantly higher ($p=0.0325$) as compared to conceived group (22.19 RLU/sec/million). However, fertile controls had significantly lower ($p=0.0001$) ROS levels (16.73 RLU/sec/million) as compared to conceived group (22.19 RLU/sec/million). Increase in ROS was associated with decreased fertilization rate, cleavage rate and embryo quality in the conceived and the non conceived group. Sperm DNA fragmentation index (DFI) in conceived group (24.58) was significantly lower ($p=0.0001$) than non conceived group (34.17). Though DFI in conceived group (24.58) was significantly higher ($p=0.0002$) as compared to controls (18.95). Fertilization rate, cleavage rate and embryo quality had a negative correlation with DFI in non conceived group and conceived group. ROS levels and sperm DFI had no correlation with pregnancy outcome in both conceived and non conceived group. No correlation of sperm parameters was observed with any of the investigated parameters.
Discussion: Though ROS and sperm DFI adversely affect fertilization rate, cleavage rate and embryo quality, but in our study, ROS and DFI had no association with the pregnancy outcome probably due to selection of best quality of embryo for implantation during the IVF procedures. Thus ROS and sperm DFI have better diagnostic and prognostic capability to discriminate between fertile and infertile men. Considering the risk of childhood cancers, leukemias, and/or autism in children conceived by assisted conception, ROS and sperm DNA damage assessment should be included in workup of infertile males opting for assisted conception.

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DETECTING SPERM DNA FRAGMENTATION TO DISCRIMINATE BETWEEN FERTILE AND INFERTILE MEN

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(Presented By: Marta Cambi)

Introduction: Sperm DNA Fragmentation (sDF) is an anomaly of sperm genome consisting in single and double stranded DNA breaks. The impact of sDF on reproductive outcomes remains elusive due to the conflicting results of clinical studies. The ability of tests detecting sDF to predict the outcomes of reproduction is affected by many variables, including the sperm population where the damage is revealed.
Methods: Using TUNEL/PI, coupling the detection of sDF to the nuclear staining with propidium iodide, PI, our group unveiled two flow cytometric sperm populations that differ for PI staining (termed PI brighter and PI dimmer populations), for the amount of sDF and for cell viability. Indeed, PI dimmer sperm are all DNA fragmented and not viable. Conversely, PI brighter sperm are both fragmented and not fragmented and both viable and not viable. Based on this finding we reasoned that PI dimmer sperm have no chance to participate in fertilization, that the fraction of sDF really impacting on reproduction is that of PI brighter sperm and, within it, that of viable gametes. To verify this hypothesis, we set up a method able to detect sDF in viable spermatozoa by using a LIVE/DEAD fixable stain that labels dead cells permanently, thus remaining even after processing samples by TUNEL for sDF detection. Then we compared the levels of sDF as measured in total, PI brighter and live spermatozoa in 23 fertile and 22 infertile men.

Results: As expected, we found that sDF resulted increased ($p<0.05$) in infertile respect to fertile subjects, both in total (44.6±18.8 vs 35.2±13.6%) and PI brighter (33.2±15.8 vs 24.3±10.8%) and live sperm (25.1±19.3 vs 13.6±6.2%). However, the percentage increase in infertile vs fertile subjects was much greater for viable sperm (84.4%) respect to PI brighter sperm (36.4%) and total population (26.5%).
Conclusion: In conclusion, the ability of sDF to discriminate between fertile and infertile men, ameliorates considering PI brighter and above all viable sperm, respect to total sperm population.

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INFERTILITY, RECURRENT SPONTANEOUS ABORTIONS, CONGENITAL MALFORMATIONS AND CANCER: POINTS OF COMMON CAUSALITY

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(Presented By: Swetasmita Mishra, MSc)

Introduction: Infertility, recurrent spontaneous abortions (RSA), congenital malformation (CM) & cancer may have common underlying etiology. RSA is a common complication of pregnancy & the role of sperm factor has not been evaluated in idiopathic cases. The prevalence of CM is 2−3% worldwide; but role of paternal factors beyond karyotyping has not been studied. OS preferentially damages nucleohistone compartment of sperm genome. Telomeric DNA attrition disrupts homologous recombination, results in segregation errors, structural rearrangement and loss of DNA integrity and may have a role in infertility, RSA, CM and cancer. So, this study was planned to investigate sperm factors in these conditions. Non familial cases of childhood cancer (Retinoblastoma (Rb)) were enrolled who developed cancer by 1 yr of age & their father's sperm DNA integrity & OS were analysed.
Methods: 500 cases of idiopathic infertility, 86 couples with idiopathic RSA, 17 cases with CM, 41 cases of fathers of children with non familial cancer were enrolled for the study. Semen analysis, Seminal ROS was measured by chemiluminescence assay. 8−Isoprostane and 8−Hydroxy−2−deoxy−Guaonosine levels were assayed by Cayman's ELISA kit. DNA damage was assessed by SCSA. T/S ratio of sperm telomere length quantified by Q−PCR. Telomerase activity/ cell assessed by PCR ELISA.
Results: ROS levels (RLU/sec/million) were found to be higher than the controls in all the groups (infertile 47; RSA 38; CM 24.1; Retinoblastoma 36.086, Leukemia 24.69, controls< 22). The DFI% was also higher in the study groups (Infertility 31%; RSA 24%; CM 25%; Rb 43.50%) as compared to the controls (<21%). Telomere length was found to be significantly shorter in male partner of RSA or infertility cases. Levels of PARP were found to be significantly lower in these cases as compared to controls & explain for persistence of DNA damage. 8−Hydroxy−2−deoxyguanosine levels in sperm DNA were significantly higher in cases (infertile− 48.3pg/ml, Rb−165pg/ml) as compared to controls (8.9pg/ml).
Conclusion: Loss of sperm DNA integrity, accumulation of mutagenic bases, telomere shortening, chromosome aberrations, genome hyper mutability may be the underlying etiology of these disorders. Oxidative stress damages both mitochondrial and nuclear DNA. Evaluation of paternal factors must be included in diagnostic workup of couples having children with non familial cancer, CM, RSA and idiopathic infertility.

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MITOCHONDRIAL COPY NUMBER VARIATION: NO CORRELATION WITH SPERM DEFECTS: IMPLICATIONS IN ART

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Introduction: Mitochondrial DNA (mtDNA), the powerhouse of cell is not only source of ATP synthesis but also produces free radicals as byproduct. The number of mitochondria per cell type is highly variable depending on the cell's energetic demand. Although oogenesis is associated with a strong amplification of mtDNA copy numbers, spermatogenesis is associated with a drastic reduction in mtDNA content with maturation of sperm. Mature mammalian sperm are known to contain ~22−75 mitochondria. Few contradictory reports are available on mtDNA copy number amplification in poor quality sperm (impaired motility & morphological abnormality) and raise the concern of paternal mtDNA transmission due to defective oocyte filter with advancing age of couple opting for ART. Point mutations, deletions and the presence of a specific mtDNA haplogroup have been associated with poor sperm quality, but little attention has been paid to mtDNA copy number. Therefore, this study was planned to analyse mtDNA copy number in sperm with single defect, more than one defect and normal sperm.
Methods: For quantifying mtDNA, sperm DNA were isolated from mature spermatozoa of infertile men and fertile controls. MtDNA copy number was analysed by real time PCR in infertile men (n=66) and fertile controls (n=28) in order to compare the mtDNA content of normal and abnormal sperm. Of these 12 had single defects & 54 had defects in morphology & motility. The mtDNA/β−globin gene ratio was determined by realtime quantitative PCR.
Results: The average mtDNA copy number ratio was 1.11±0.209 in normal sperm (fertile controls) and 1.37±0.162 in abnormal sperm (cases with single & double defects). The ratio of patients with 2 abnormal criterion were 1.5±0.301 & with single abnormal criterion 1.25±0.105
Conclusion: Sperm with normal morphology & motility had 1.11 mtDNA copy number. This means that the majority of sperm are almost totally devoid of mtDNA, and that mature sperm probably do not contain any mtDNA at all. The mtDNA copy number was 1.37 in infertile men which was almost equal to normal sperm samples. There was no significant difference between abnormal and normal sperm mtDNA copy number. Thus there is no amplification of mtDNA copy number in poor quality sperm, thus no fear of risk of transmission of paternal mtDNA in ART.

ABSTRACTS

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THE ANALYSIS OF PATERNAL AGE ON INTRACYTOPLASMIC SPERM INJECTION OUTCOME

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Objective: In a retrospective study, advanced paternal age, fertilization rates and pregnancy rates after ICSI were compared. **Methods:** There were two age groups of men studied. Couples with male partners aged 60 years and over (group A) (n=27) with a mean age of 64±3 years were compared to couples with younger age−group male partners (group B) (n=57) with a mean age of 35±2 years. The control group of younger men was selected so that the women's age matched between the two groups.
Results: There was no significant difference in fertilization rate between the two groups (75.3 versus 82.4%). There was a significantly higher pregnancy rate in younger men (P<0.01). However, the long−term outcome of these pregnancies needs further investigation. Semen analysis showed significantly lower semen volume, sperm concentration and sperm morphology in group A versus group B (P<0.05), but these did not affect the fertilization rate.
Conclusion: It appears that paternal age has an effect on the pregnancy rate after ICSI.

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ACONITI LATERALIS PREPARATA RADIX IMPROVES SPERM MOTILITY THROUGH UP−REGULATION OF THE CYCLIC AMP RESPONSE ELEMENT MODULATOR (CREM) PROTEIN IN CYCLOPHOSPHAMIDE−TREATED MALE MICE

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(Presented By: Seong Kyu Park, PhD)

Introduction: Male reproductive dysfunction associated with poor sperm motility and count is one of the important indicators for male infertility. Cyclic AMP response element modulator (CREM) plays a vital role for sperm development.
Methods: In this study, to examine the effect of Aconiti Lateralis Preparata Radix (ALR) on the sperm functions and the CREM expressions in mouse testis, C57BL/c male mice were divided into five groups; the normal group, cyclophosphamide(CP) only−treated group and ALR with CP (100, 500, 1000 mg/kg of ALR and 100 mg/kg of CP) treated group for five weeks. We performed real time PCR and western blot analysis for the examination of the CREM expression and analyzed sperm parameters. **Results:** In our results, sperm motility was markedly increased in 100, 500, 1000 mg/kg of ALR treated group than that of control group (15.11 ± 4.53, 13.07 ± 3.18 and 14.81 ± 2.16 vs. 3.63 ± 1.03%; $p < 0.001$, respectively). CERM expression levels were dose−dependently increased in ALR treated groups than that of control group.
Conclusion: In conclusion, our results suggest that ALR plays an important role in sperm motility and male infertility by up−regulation of the CREM expression.

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STUDY ON CONTRACEPTIVE EFFECT OF ETHANOL EXTRACTED JUSTICIA GENDARUSSA BURM.F. LEAVES IN FERTILE MEN: PHASE II CLINICAL TRIAL

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Introduction and Objectives: Previous laboratory and clinical research suggest that Gendarussa has a contraceptive effect by preventing fertilization without effecting sperm macroscopic and microscopic parameters. It may decrease human sperm hyaluronidase activity, and sperm proteins of weights 38 kDa and 41.5 kDa were missing in the treatment group. A similar pattern of missing proteins has been found in infertile males. We have carried out a phase 2 clinical trial with larger sample size and short duration of Gendarussa administration. To quantify the reduction of sperm hyaluronidase activity and the disappearance of proteins at 38 kDa and 41.5 kDa; to determine pregnancy rate; to monitor the safety and reversibility of Gendarussa.
Methods: 70% ethanol extract of alkaloid−free Justicia gendarussa leaves was used. The subjects were 350 healthy fertile men age 21−40 years, normozoospermia, had at least one child and fulfilled other inclusion criteria. Single blind non−randomized method was undertaken. Group one (186 men) took 450 mg of Gendarussa capsules daily for 30 days; group two (164 men) took placebos and were instructed to use condoms. Semen analysis, hyaluronidase activity, sperm protein profile were examined before, during and after treatment. Subjects and their spouses were told to discontinue any contraception except Gendarussa or condoms. Group one was directed to have sexual intercourse three times during ovulation phase, after taking 20 capsules. The Ovulation phase was assessed individually for each couple. Spouses were asked to return after intercourse for post coital testing the next morning. Men in group two (placebo) were told to continue using condoms.
Results: Reduction of hyaluronidase activity by 5.81% and 6.47% after 15 and 30 days respectively and disappearance of band 38kDa and 41,5kDa of sperm protein after 5 days treatment, in group one. One pregnancy was found (1/186 = 0.54%), with strong suspicion caused by Gendarussa failure or possibly by not taking the medicine as directed. Hyaluronidase activity and sperm protein band 38kDa and 41,5kDa were found to be normal 30 days after stopping medication.
Conclusions: 70% ethanolic extract of alkaloid−free Justicia gendarussa leaves is an effective male oral contraceptive method that is reversible and had no serious adverse effects. Further study is needed to determine its mechanism of action, but data strongly suggest that it acts as a contraceptive by preventing fertilization.

ABSTRACTS

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ADVERSE EFFECTS OF CLOMIPHENE CITRATE IN INFERTILE MEN

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(Presented By: Mary Samplaski, MD)

Introduction: Clomiphene citrate (CC) is a selective estrogen receptor modulator, which has been used for the empiric treatment of male infertility with mixed results. We sought to determine the adverse effects of CC use in infertile men.
Methods: 85 men presenting for fertility evaluation from 2008−2013 were started on empiric CC. Data were analyzed for semen and hormonal parameters prior to starting CC, and then at 1 and 3m. At follow up men were queried about side effects experienced on CC.
Results: The most common starting dose of CC was 25 mg PO daily. 7 men had aromatase inhibitors started for rising serum estradiol. Side effects were reported in 18 men (121%), including: Dizziness (2), increased aggressivity or temper (2), gynecomastia (1), increased libido (8), decreased libido (3), bad taste in mouth (1), and back pain (1). 40 men (47%) had no improvement in total motile count (TMC) after CC. Of these 13 (32.5%) were azoospermic at the start and end of treatment. The remaining 27 (67.5%) had worsening of their semen parameters. 12 men had a decrease in TMC at 1m: 18.9±21.9 M to 10.7±11.2 M; mean decrease of 8.2±11.8 M, range 0.1−41.2 M; 5 men had a decrease in TMC >5M. 6 men discontinued CC at 1m due to semen parameters. 22 men had a decrease in TMC at 3m: 13.2±21.4 M to 7.8±11.7 M; mean decrease of 5.4±16 M. 7 men had a decrease in TMC >5M. Of the 22 men that had a decrease in TMC at 3m, 7 had a decrease at 1m but chose to continue CC. There were 2 men who had a substantial decrease in TMC on CC. 1 had a decrease of 41.4M at 1m, but related a possible incomplete collection. 1 had a decrease of 65.2M at 3m, however the samples were collected at different labs and motility was the primary difference. For the 7 men with a decrease in TMC >5M at 3m, hormonal parameters were as follows: The mean baseline FSH was 4.1±2.3 IU/L, the mean increase in FSH at 1m was 4.5±1.9, and at 3m 4.5±2.3. The mean baseline testosterone (T) was 8.4±4.0 nmol/L, the mean increase in T at 1m was 16.9±9.3, and at 3m 17.9±11.6. These hormonal changes were not different from those in men with a positive response to CC.
Conclusions: CC is well tolerated in men, with the most common adverse effects being increased libido and mood changes. There was a group of men that had worsening of their semen parameters, although these decreases were usually small. There were no clear predictors for these men.

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COCAINE USE IN THE INFERTILE MALE POPULATION: EFFECTS ON SEMEN AND HORMONAL PARAMETERS

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Introduction: The United States is the world's largest consumer of cocaine. Cocaine is commonly used in upper−middle class communities, the same group of men who often present for male fertility evaluation. We sought to evaluate the incidence of cocaine use in the infertile male population and if cocaine use is associated with changes in semen parameters.
Methods: Men presenting for a fertility evaluation from 2008−2012 reporting using cocaine were identified via a prospectively collected database. Data were analyzed for semen parameters.
Results: 39/4400 (0.8%) men reported using cocaine at presentation. Concurrent reported drug use was reported in 90% of the men and included: marijuana (32), ecstacy (9), LSD (2), heroin (1) and anabolic steroids for bodybuilding (3). 4 men reported using cocaine monthly, the rest reported using cocaine every 3 months or less. 5 couples had prior children and 4 couples reported therapeutic abortions. One man was a longstanding diabetic, with retrograde ejaculation. After trying cocaine for the first time he had his first antegrade ejaculation in many years, with a total sperm count (TSC) of 131 M. There were a number of other clear causes for infertility including 5 men taking cocaine who were seen for vasectomy reversals, 4 men who had oncologic therapies rendering them azoospermic and 2 men who were seen for biopsy−proven early maturation arrest. After excluding these men and those using anabolic steroids, 16 men had semen analyses available for analyses. For these men, the mean semen parameters were: ejaculate volume 4.48 ± 2.64 mL; sperm concentration 13.37 ± 13.79 M/mL; motility 22 ± 15.7 %; TSC 109.83 ± 133.66 M.
Conclusions: There are very few reports on the use of cocaine among men presenting for a fertility investigation: this report indicates that cocaine use in our centre is rare among men presenting for an infertility investigation and does indicate that most of the infertile men on cocaine have relatively preserved semen parameters.

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ROLE OF NON−INVASIVE MARKERS IN PREDICTION OF SPERM RETRIEVAL IN NON−OBSTRUCTIVE AZOOSPERMIA

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(Presented By: Vasan Srini, DNB, Fellowship)

Objective: To predict the accuracy of sperm retrieval by using such non−invasive markers in order to avoid the morbidities and complications of surgery.
Methods: Prospective, non−randomized cohort study. Andrology unit in a Tertiary Fertility Centre, India. 100 consecutive patients diagnosed to have non obstructive azoospermia between January 2009 and December 2010 and undergoing testicular sperm extraction (TESE). Patients with azoospermia scheduled for TESE: Serum Inhibin B and epididymal head size were measured. The biopsy report after TESE was recorded. All results thus obtained were tabulated, and correlation of these markers with respect to sperm retrieval were analyzed.

Results: Out of 81 patients in whom serum Inhibin− B values was > 40 pg/ml, 67(82.7%) patients had sperms in TESE. Out of 79 patients in whom epididymal head size was > 6 mm, 64(81.0%) patients had sperms in TESE. Out of 69 Patients in whom epididymal head size was > 6 mm and serum Inhibin− B value was > 40 pg/ml, 62(89.9%) patients had sperms in TESE.
Conclusions: Serum inhibin− B level and epididymal head size are the best non−invasive markers which in combination further increases the predictive accuracy of sperm retrieval with non obstructive azoospermia.
Key Words: Non Obstructive Azoospermia, Testicular Sperm Extraction, Inhibin –B, Epididymal head size, Intra Cytoplasmic Sperm Injection

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INFLUENCE OF AN AROMATASE INHIBITOR ON SEXUAL FUNCTION IN MEN WITH NON−MOSAIC KLINEFELTER'S SYNDROME

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(Presented By: Sotirios Koukos)

Introduction: We evaluated the role of anastrazole, an aromatase inhibitor, in the sexual function of men with non−mosaic Klinefelter's syndrome.
Methods: Twenty one men with non−mosaic Klinefelter's syndrome were divided into two groups A and B. Men of group A (n=13) received daily anastrazole (1 mg daily) for 12 weeks. Men of group B (n=8) did not receive any pharmaceutical treatment for a period of 12 weeks. There was not significant difference in the mean age of the participants of group A and B. The IIEF−5 questionnaire (Int J Impot Res,199;11;319) was completed by each participant of groups A and B at the beginning of the study and 12 weeks later (end of the study). Peripheral serum levels of testosterone were recorded, within each group, at the beginning of the study and at the end of the study. Within each group, the mean value of IIEF−5 outcome or testosterone at the beginning of the study and at the end of the study were compared using Wilcoxon test for paired observations. A probability P smaller than 0.05 was considered to be statistically significant.
Results: Within group A, mean IIEF−5 outcome or mean testosterone value was significantly larger at the end of the study than in the beginning of the study. In contrast, within group B, there was not significant difference in the mean IIEF−5 outcome or in the mean testosterone value between the beginning of the study and the end of the study.
Conclusion: It appears that anastrazole treatment increasing serum testosterone profiles improves sexual function in men with non−mosaic Klinefelter's syndrome. Additionally the increase in serum testosterone in men of group A may improve each individual psychology and self confidence with an overall positive effect on sexual function.

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INHIBITORY PROPERTIES OF POMOGRANATE JUICE ON HUMAN CORPUS CAVERNOSUM: EXPRESSION OF NOS ISOFORMS AND PDE5A1 ENZYMES

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(Presented By: Serap Gur, PhD)

Introduction and Objectives: Pomegranate juice (POM Wonderful, Los Angeles, CA) may benefit the erectile process. Molecular characterization and in vitro confirmation of its effect are lacking. The present study evaluated the action of POM on human corpus cavernosum (HCC) smooth muscle.
Methods: HCC tissues from patients (age:47−75, n=9), undergoing prosthesis implantation were placed in organ baths. After phenylephrine (PE, 10 μM) contraction, the relaxant effect of POM with or without several inhibitory and stimulatory agents were evaluated. Ex vivo organ culture of HCC was performed and cells were maintained in Dulbecco's Modified Eagle Medium: Nutrient Mixture (DMEM)−F12 and kept at 37°C and 5% CO2. Cells from early passage (p 3−5) were treated with 10 μl/ml (v:v) of POM and mRNA was collected. The expression of neuronal NO synthase (nNOS), endothelial (e)NOS, and phosphodiesterase (PDE)−5A was assessed by RT−PCR analyses.
Results: Our study demonstrated that POM in HCC induced marked relaxation (maximum response: 97.0±3.1%), which was not inhibited by nitric oxide (NO) synthase inhibitor L−NAME (100μM) and the soluble guanylyl cyclase inhibitor ODQ (10μM). POM potentiated EFS, but not addition of ACh (10μM), sildenafil (10μM) or sodium nitroprusside (SNP 0,1μM). The expression of nNOS was 7.2 ± 3.2 fold higher in POM−treated cells compared to controls ($p<0.0121$). There was no significant change in eNOS ($p<0.2715$) and PDE−5A ($p<0.09$) compared to controls.
Conclusions: POM induces marked relaxation of HCC and its effect is not by activation of the NO/cGMP pathway. Data from RT−PCR indicates that nNOS is the most robust response. POM may synergize with the neuronal reflex activated by nNOS to signal downstream relaxation, by bypassing NO/cGMP and PDE5systems. Hence, this food additive may help men with ED who do not respond fully to oral PDE5 inhibitor.

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EVALUATION OF THE CHRONIC TREATMENT WITH RESVERATROL ON THE METABOLIC AND REPRODUCTIVE PARAMETERS OF YOUNG ADULT RATS WITH TYPE 1 DIABETES INDUCED BY STREPTOZOTOCIN IN THE PREPUBERTY

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(Presented By: Joana N.Simas)

ABSTRACTS

Introduction: Diabetes Mellitus is a metabolic disorder of multiple etiology and epidemic proportions. Its pathogenesis unleashes the progression of a variety of complications, among which reproductive alterations. Resveratrol (RES), a fitoalexin found in several plants, constitutes a powerful antioxidant that also presents antidiabetic activity. Recent report has suggested that RES can improve spermatogenic parameters that are altered due to testicular ischemia. Our goal is to assess the following trilogy: type 1 Diabetes (DM1), male reproduction and a possible benefit promoted by RES.
Methods: Eighty−four prepubertal male Wistar rats were used to compose 7 groups: absolute control (C); sham control (SC, treated with Carboximethilcelulose, which is RES vehicle); RES−treated (R); diabetic (D); diabetic insulin−treated (DI); diabetic RES−treated (DR), diabetic insulin− and RES−treated (DIR). DM1 was induced by a single intraperitoneal injection of streptozotocin (65 mg/kg) on the 30th day postpartum (dpp). Animals of DR, DIR and R groups received a daily dose of RES (150mg/day by gavage route) for 42 consecutive days (from the 33 dpp on). DI and DIR rats received daily subcutaneous injections of insulin (1U/100g bw) from the 5th day after the DM1 induction. An oral glucose solution was offered on the 1st (2.5%) and on the 2nd day (5.00 %) after the detection of DM1 to avoid abrupt hypoglycemia.
Results: The blood glucose measurement (BGM) of all rats was obtained at 4 different time−points: before the STZ treatment, on the 3rd day post treatment, at 45 dpp (peripuberty) and at 64 dpp (postpuberty). At 75 dpp (young adult phase) the rats were submitted to euthanasia for biometric and morphometric testicular analyses and spermatic evaluation. The BGM in the D group was significantly higher than in the DR, DI and DIR groups. The age of preputial separation was delayed in the induced−groups. The D group presented significantly reduced body weight when compared to the DR and DIR groups, as well as reduced relative testicular weight when compared to the DR and DI groups. Rats of the DR and DIR groups showed an increased frequency of morphologically normal sperms in the epididymal cauda and an improvement in the sperm mitochondrial activity when compared to the D and DI groups.
Conclusion: These results indicate that RES improve both glycemia and sperm quality parameters in diabetic rats. Additional metabolic analysis, sex hormone dosages and supplementary reproductive evaluations are being carried out.

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AN OBJECTIVE EVALUATION OF VIBERECT® (MALE VIBRATOR DEVICE) IN INDUCING FUNCTIONAL ERECTION IN COMPARISON TO INTRACAVERNOSAL VASOACTIVE INJECTION USING PENILE DUPLEX DOPPLER ULTRASOUND BLOOD FLOW ANALYSIS

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(Presented By: Suresh Sikka, PhD)

Introduction and Objective: Viberect® is a new FDA−cleared medical vibrator device that stimulates genital afferent nerves and induces penile erection. The degree and quality of penile rigidity induced by Viberect® has variable response and depends upon many factors. An objective evaluation of functionality of such device is needed. To compare erection rigidity and penile blood flow induced by Viberect® versus intracavernosal injection (ICI) of a vasoactive agent in patients undergoing color duplex Doppler ultrasound (CDDU) evaluation.
Methods: One hundred five ED/Peyronie's patients attending our Andrology & sexual dysfunction clinic during 2011−2013 consented to receive instructions and correctly use the Viberect® prior to undergoing penile CDDU. Viberect® stimulation was performed by patients at 70−100 Hz for 6−10 minutes and CDDU performed as per our standard protocol (JSM, 2013). After the penis becomes flaccid, an ICI (7−15mcg prostaglandin E1, PGE1) was administered and CDDU repeated by the same sonographer under similar environment and visual sexual stimulation (VSS) settings.
Results: Thirty three men (called "positive−responders"to Viberect®) showed >60% rigidity and 55 cm/sec mean peak systolic velocity (PSV) with Viberect® compared to >65% rigidity ($p>0.05$) and 70 cm/sec mean PSV (2−tailed paired t−test value of $p<0.05$) with PGE1. Forty five patients (called "borderline−responders") showed 36% mean rigidity and 44cm/sec PSV with Viberect® compared to 58% rigidity and 66cm/sec PSV with PGE1 ($p< 0.002$). Only 15 patients (called "non−responders") showed poor erection response with Viberect® (mean 15% rigidity and 29cm/sec PSV) compared to mean 56% rigidity and 59cm/sec PSV with PGE1 ($p<0.001$). Twelve patients could not complete Viberect® stimulation due to impending ejaculation. No complaints or adverse events were reported with Viberect®. Thus, Viberect® induced good blood flow and rigid erection response almost similar to ICI in "positive−responders". Many "borderline and negative responders"had high anxiety/environmental issues using this vibrator in clinical setting.
Conclusions: This study suggests that Viberect® that stimulates bulbocavernous and pudendo−cavernous reflex is safe, convenient, well−tolerated modality for inducing erection. Randomized prospective multicenter trials using standardized CDDU should be performed to further validate the concept of stimulating these reflexes with such vibrators for ED diagnosis and treatment.

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SURVEY OF THE RECOGNITION OF CIRCUMCISION

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(Presented By: JoonYong Kim)

Objective: Historically, circumcision is a very old surgical procedure but there are lots of debates and the regional, religious and cultural differences are shown regarding frequency, timing, reason, etc. Since the 1950s in Korea the frequency of circumcision has increased until recent years that it is stagnant or declining. We report awareness of Korean men about circumcision.
Method: 91 people at the age of 20 to 59 were participated in questionnaire with 16 questions about timing of surgery, medical professionals, motivation, surgical outcome, side effects, changes in sexual function, etc.

Result: The average age was 40.1 years old. The timing of surgery is 20s (46%), grades 1−3 in elementary school (14.6%), grades 4−6 in elementary school (12.4%) and preferred timing of surgery is grades 4−6 in elementary school (18.9%), high school (18.9%). Medical department is urology (42.7%), don't know (22.5%), army surgeon (6.7%). Surgical motivation is hygienic reason (33.7%), parents' recommendation (30.8%). Side effect is unobserved (86.6%) and complaints about surgery is insignificant (56.5%), not enough skin (19.6%). Sufficiency of penis skin at flaccid state after surgery is full exposure of glans (78.4%), coverage of partial glans (17%). Desired sufficiency of penis skin is fully exposed glans with folded skin (69%), partial (half) glans covered (19%). Change of Penile size is unobserved (44.4%), don't know (35.6%). Change in sensation is don't know (46%), unobserved (24.7%). Expectation after surgery is hygienic improvement (33.1%), prevention of sexual transmitted disease (24.1%). Necessity of circumcision is necessary (64.8%), highly necessary (14.8%) and appropriate medical department is urology (96.7%). Changes in sexual function after surgery is not observed (78.4%), improved (3.9%), worse (3.9%).
Conclusion: High incidence of circumcision in age 20s is because of the military service, etc. and the top motivation is hygienic reason but many chose to circumcise due to parents' recommendation and trend. This means that most decisions were passive and conventional. From the complaint that there is not enough penile skin after surgery and tightness at erection and the fact that many preferred sufficient penile skin, remaining enough skin after surgery should be considered. Expectation of surgical outcome is hygienic improvement as well as prevention of disease. The necessity and positive recognition of circumcision was relatively high and in reality.

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IMPACT OF LIFE STYLE INTERVENTIONS ON MARKERS OF CELLULAR AGING

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(Presented By: Shiv Basant Kumar, MSc)

Introduction: A hectic life style, psychological stress, increased fast food intake, increased electromagnetic radicals exposure leads to exposure to free radical. Hence this study was planned to evaluate role of life style intervention on various stress markers such as Cortisol, 8−hydroxy−2'deoxyguanosine (8−OHdG) and Reactive Oxygen Species (ROS) and inflammatory markers like Telomere length and Telomerase activity.
Objective: To evaluate effects of life style interventions (yoga) on markers of cellular aging and free radical levels. The telomerase activity and Telomere length which maintains chromosomal stability were assessed.
Methods: 50 healthy volunteers enrolled in IHC. Information was obtained about their lifestyle using a questionnaire about their life, such as food choices, habits and socioeconomic status. Venous blood samples were collected. Stress markers such as Plasma Cortisol, 8−OHdG and blood ROS levels were measured. We also assessed the telomerase level and telomere length.
Results: There was a significant reduction in various markers of oxidative stress in subjects and an increase in telomerase level at day 0 vs. day 10. Telomere length did not show any significant change. The mean Cortisol levels were significantly lower (P = 0.0072) in the subjects (pre yoga) (118.83 ± 30.58) ng/mL compared to 10days after practicing yoga (96.32 ± 36.06) ng/mL, while ROS level decreased from baseline to day 10 (1215.069 ± 0.88, 1020.81 ± 0.79 RLU/min/104 Neutrophils; p=0.024). Although 8−OHdG levels were reduced (10268.23± 3349.71 vs. 9367.57 ± 2709.58pg/mL) after yoga intervention, the difference was not statistically significant (p=0.459). Telomerase levels were elevated post intervention[(0.59 (0.114 − 2.043)IU/Cell Vs 2.40 (0.568 − 5.448)IU/Cell] but telomere length did not show any change.
Conclusions: This short time yoga−based lifestyle intervention reduced the markers of stress even in 10 days in the general population. We are following these cases up to 3 months but this study is ongoing. Decline in free radical levels may actively prevent several diseases in which oxidative stress is one of the chief causative factors. Telomerase level upregulation is key factor in maintenance of Telomere length which maintains genomic integrity. This yoga based life style interventions may be recommended as therapeutic in decreasing oxidative stress and oxidative DNA damage.

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EXCESSIVE EXTRACELLULAR ATP FORMATION BY MALIGNANT CELL−DERIVED PROSTASOMES DUE TO DOWN-REGULATED ATPASE ACTIVITY

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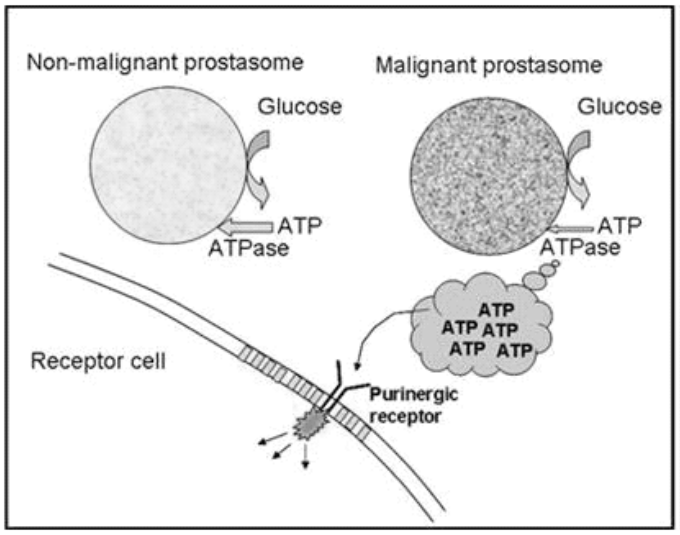
Dep. of Med. Science
(Presented By: K. Göran Ronquist, PhD)

Introduction and Objectives: Cancer with all its complexity means influences by not only intracellular genetic and epigenetic changes but also by stromal cells, local extracellular matrix and metabolic courses of events in the microenvironment. Prostasomes are small extracellular membrane vesicles with an endosomal origin that are released by prostate acinar cells into the extracellular environment. We wanted to investigate the overall energy metabolic capability of prostate cancer cell−derived prostasomes in comparison with their non−malignant counterparts in terms of net ATP gain after incubation with proper substrates.
Methods: Prostasomes were harvested from the growth medium of cancer metastatic PC3 cells and subjected to differential centrifugation steps including preparative ultracentrifugation, filtration through a 0.20 μm filter and sucrose gradient ultracentrifugation. Human seminal (non−malignant) prostasomes were subjected to a similar purification procedure where the filtration was replaced by gel chromatography. Prostasomes were incubated with and without glucose in presence of ADP and ATP was determined by a luciferin/luciferase method.
Results: PC3 cell−derived prostasomes displayed a 10−fold lower ATPase activity compared with seminal prostasomes. Both types of prostasomes were able to form ATP in about equal amounts by glycolysis in addition to adenylate kinase−catalyzed formation of ATP.
Conclusions: The net ATP gain of PC3 cell−derived prostasomes was high due to their low ATPase activity and this ATP may be at disposal of purinergic receptors and/or protein kinases in the cancer cell microenvironment.



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IMPACT OF SINGLE NUCLEOTIDE POLYMORPHISM (SNP) ON SUSCEPTIBILITY OF GALECTIN−3 TO CLEAVAGE BY PROSTATE SPECIFIC ANTIGEN (PSA)

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(Presented By: David Schoen, BS)

Introduction: Galectin−3 is a multivalent, carbohydrate−binding protein involved in cell adhesion, immunomodulation, and cancer progression, including prostate cancer. In the human male reproductive tract, galectin−3 function is regulated, in part, by proteolytic processing by PSA, which abrogates the ability of galectin−3 to oligomerize. Significantly, proteolytic cleavage of galectin−3 is associated with prostate cancer progression. The SNPs rs4644 and rs4652 generate proline (P)−to−histidine (H) and threonine (T)−to−P polymorphisms at amino acids 64 and 98, respectively, in galectin−3. Thus, these SNPs create four possible galectin−3 variants in humans (P64T98, P64P98, H64P98, H64T98).
Methods: To investigate the effects of galectin−3 allelic variation on susceptibility to PSA proteolytic cleavage, in vitro cleavage assays compared PSA proteolysis of each galectin−3 variant individually to emulate homozygous phenotypes and in pair−wise combination to emulate heterozygous phenotypes. Immunoblot analysis of galectin−3 cleavage products indicated that the galectin−3 H64 variants were up to 3.5−fold more susceptible to cleavage by PSA than were the P64 variants. The pair−wise combinations of galectin−3 P64T98/H64P98 and galectin−3 P64P98/H64P98 were cleaved by PSA with at least two−fold greater efficiency than was galectin−3 P64T98/P64P98. Moreover, the H64 variants exhibited an additional cleavage product not observed for the P64 variants, indicating that galectin−3 H64P98 and H64T98 contain a PSA cleavage site that is not present in galectin−3 P64T98 or P64P98.
Results: Immunoblot analysis identified a nearly identical galectin−3 cleavage pattern in prostate tumor lysates and PSA−cleaved galectin−3 variant samples, but not in matrix metalloprotease (MMP)−2 or MMP−9 cleaved galectin−3 samples. These results suggest that PSA is involved in cleaving galectin−3 in the prostate tumor microenvironment. Immediate future studies will identify the additional cleavage site in galectin−3 H64 variants, will determine whether there are any differences in secondary or tertiary structure between the four galectin−3 variants, and will evaluate the ability of the galectin−3 variants to form homo− and hetero−oligomers.
Conclusion: Overall, our results indicate that the galectin−3 genotype determines the susceptibility of galectin−3 to proteolytic cleavage by PSA and implicate galectin−3 genetic polymorphism as an etiological factor in prostate cancer progression.

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FLAGELLAR BIOGENESIS: A POTENTIAL LINK BETWEEN MFN2 AND MNS1

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(Presented By: Melissa Vadnais, VMD, PhD)

Introduction: MNS1 is a recently characterized protein that is abundantly expressed in post−meiotic spermatids and is required for proper flagellar formation. To explore the possible functions of MNS1, we performed a BLAST search and identified the conserved domain pfam13868, exemplified by trichoplein. This protein interacts with mitofusin 2 (MFN2), a protein that participates in regulating mitochondrial associations to subcellular organelles. We hypothesized that an association between MFN2 and MNS1 in the sperm is involved in flagellar biogenesis and function.
Methods: In the studies reported here, MFN2 was found in murine reproductive and somatic tissues high in ciliary content, and MNS1 was present as two closely migrating bands in reproductive tissues. Similar to Mns1, Mfn2 was expressed in the testis as detected by RT−PCR. In addition, Mfn2 and Mns1 decreased in expression from pachytene spermatocytes to condensing spermatids as assessed by quantitative RT−PCR. Co−immunoprecipitation demonstrated an association between MFN2 and MNS1 in spermatogenic cells. Indirect immunofluorescence indicated that MFN2 and MNS1 co−localized to the sperm flagellum in freshly collected cauda epididymal sperm. MFN2 associated with the midpiece while MNS1 was present throughout the sperm tail in caput and cauda epididymal sperm.
Results: In spermatogenic cells, MFN2 was seen in the mitochondria, and MNS1 was present throughout the cytoplasm. MFN2 and MNS1 were present in detergent−resistant structures of the sperm.
Conclusion: These results demonstrate that these proteins are present in spermatogenic cells and are an integral part of the sperm flagellum, indicating they may play a role in flagellar biogenesis and/or function.
Supported in part by NIH HD−051999, HD−057194, ES−013508.

ABSTRACTS

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ADENYLATE KINASE 8, ADENINE NUCLEOTIDE METABOLISM, AND A ROLE FOR AMP IN MODULATING FLAGELLAR WAVEFORMS IN MOUSE SPERM

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(Presented By: Melissa Vadnais, VMD, PhD)

Introduction: While most ATP, the main energy source driving sperm motility, is derived from glycolysis and oxidative phosphorylation, the metabolic demands of the cell require the efficient use of power stored in high−energy phosphate bonds. In times of high energy consumption, adenylate kinase (AK) scavenges one ATP molecule by transphosphorylation of two molecules of ADP, simultaneously yielding one molecule of AMP as a byproduct.
Methods: We previously demonstrated that AK1 and AK2 are present in outer dense fibers and mitochondrial sheath of the mouse sperm tail. Here we show that another AK, AK8, is present in third flagellar compartment, the axoneme. As a functional test of AK, either ATP or ADP supported motility in detergent−modeled mouse cauda epididymal sperm. While ATP or ADP fueled motility, the resultant flagellar waveforms were qualitatively different. Motility driven by ATP was rapid but restricted to the distal region of the sperm tail whereas ADP produced slower and more fluid waves that propagated down the full flagellum.
Results: Characterization of wave patterns by tracing and superimposing the images of the flagella, quantifying the differences using digital image analysis, and computer−assisted sperm analysis revealed differences in the amplitude, the periodicity, and propagation of the waves between detergent−modeled sperm treated with either ATP or ADP.
Conclusion: Surprisingly, addition of AMP to the incubation medium containing ATP resulted in a pattern of sperm motility similar to that supported by ADP alone. These results extended the known regulators of sperm motility to include AMP, which may be operating through an AMP−activated protein kinase. Grant support: NIH grants R01HD051999, R01HD057144, T32HD007305, and P30ES013508

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VARICOCELECTOMY: CLINICAL IMPLICATIONS AND PROGNOSIS IN MANAGEMENT OF INFERTILITY.

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(Presented By: Monis Bilal Shamsi, MSc, PhD)

Introduction: Varicocele is implicated as a major cause of testicular dysfunction in large number of infertile men. The varicocele results in decline of testicular function due to testicular hypoxia and hyperthermia which at molecular level alters normal production and maintenance of sperm. Increased levels of reactive oxygen species (ROS) and reduced total antioxidant capacity (TAC) in men with varicocele suggested that sperm dysfunction may be in part related to the sperm DNA damage induced by oxidative stress. The study was planned to assess the efficacy of varicocelectomy by comparing the OS and sperm DNA damage pre varicocelectomy and one and six months post varicocelectomy.
Methods: Forty three patient with clinical varicocele and 34 normozoospermic healthy controls were enrolled in study. Sperm DNA damage was assessed by Comet assay and ROS by luminol induced chemiluminescence. TAC was quantified by commercially available kit. Analysis was done pre varicocelectomy and one and six months post varicocelectomy.
Results: A remarked improvement in sperm DNA quality and reduced oxidative stress levels was observed 6 months post varicocelectomy (Table 1).
Conclusion: Varicocele is commonest surgically reversible cause of male infertility. Antioxidant supplementation and varicocelectomy are most common thereupatic approach in treatment of varicocele. Varicocele patients have high ROS levels in semen than fertile controls. Prolonged exposure to ROS would lead to irreversible sperm DNA damage consequently resulting in decreased fertility. The high ROS and low TAC levels showed significant improvement one month post varicocelectomy but DNA integrity improved significantly only after 6 months. Therefore we emphasize that though oxidative stress may significantly decline immediately following varicocelectomy, DNA damage takes longer to revert to normal, since genomic integrity is an important prerequisite for fertilization and embryogenesis and birth of healthy offspring. Such men with varicocele should attempt pregnancy only after 6 month of varicocelectomy.

	Healthy Control	Patients		
		Pre Varicocelectomy	Post Varicocelectomy (One month)	Post Varicocelectomy (Six months)
ROS (RLU/sec/million)	15.37 ± 4.98	159.42 ± 27.18	98.87 ± 73.45	25.27 ± 9.36
TAC (mM)	6.7 ± 2.9	1.7 ± 0.5	2.9 ± 1.5	4.3 ± 0.8
% sperm with DNA damage	14.2 ± 7.36	38.29 ± 10.43	32.43 ± 9.54	20.56 ± 6.43

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THE CATSPER CALCIUM CHANNEL IN HUMAN SPERMATOZOA: RELATION WITH MOTILITY AND INVOLVEMENT IN PROGESTERONE−INDUCED ACROSOME REACTION

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University of Florence
(Presented By: Lara Tamburrino, PhD)

Introduction: KO mice for any of the CatSper family genes, fail to acquire hyperactivated motility (HA) and are infertile. Less clear is the role of CatSper in human sperm HA/activated motility and in asthenospermia. Few men with CatSper mutations have been described but sperm motility and the ability to achieve HA has not been well established. CatSper has been shown to mediate progesterone (P)−induced Ca2+ influx in human sperm but whether it is involved in the acrosome reaction (AR) inducing effect of the steroid has not been established.
Methods: We evaluated the effects of two Catsper inhibitors, NNC55−0396 (NNC, 10 and 20 μM) and Mibefreadil (Mib, 30 and 40 μM), on human sperm motility parameters and P−induced AR. Catsper1 protein expression was evaluated in unselected and swim up selected sperm samples and in spermatozoa of normo− and astheno−spermic subjects. Semen sample kinematic parameters were analyzed by CASA. A fluorescent labelled lectin was used to evaluate P−induced AR in live spermatozoa. CatSper1 protein expression was determined by western blot and by flow cytometry. Intracellular calcium concentrations ([Ca2+]i) were evaluated by a spectrofluorimetric method following sperm loading with the calcium sensitive probe fura 2/AM.

Results: CatSper1 protein was localized in the tail and its expression was found highly increased after swim up selection both by western blot and by evaluation of the percentage of spermatozoa expressing the protein by flow cytometry (27.2±9.0% in unselected vs 52.7±15.8% in selected, n=7, p<0.01). Basal and P−stimulated [Ca2+]i were significantly higher in swim up selected sperm respect to 40% PureSperm selected (n=8, p<0.05). Basal [Ca2+]i evaluated in 40% PureSperm selected spermatozoa was significantly related to progressive motility of the samples (r=0.71, p=0.04, p=0.01, n=8). CatSper1 expression was decreased in astheno− (n=10) respect to normo−spermic (n=9) men (p<0.01) and was positively related the percentage of sperm with progressive motility (r=0.59, n=19, p=0.007).
Conclusion: NNC and Mib significantly reduced sperm progressive motility and several kinematic parameters but did not affect the HA. Mib showed a significant effect on sperm viability. P−stimulated AR was significantly reduced by both inhibitors (p<0.05). Our results indicate that, in human spermatozoa, CatSper channel expression and function are associated to progressive motility and may be involved in the pathogenesis of asthenozoospermia and in the AR process.

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SPERM'S MEMBRANE CHARGE: AN INTERESTING BIOMARKER FOR NON−INVASIVE METHOD OF SPERM SELECTION

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(Presented By: Luke Simon, PhD)

Introduction and Objective: The electrostatic property of sperm was first introduced in 1991, since then only a few research groups have used this concept for the selection of better sperm. The sperm head is covered by a negatively charged coating (20−60 nm thick), to facilitate the interaction with its extracellular environment. Mature sperm possess an electric charge of −16 to –20 mV. The negatively charged glycocalyx adjacent to the sperm plasma membrane helps to prevent sperm from self−agglutination and non−specific binding with the genital tract epithelium during its transport and storage. In a normal and matured sperm, the membrane glycocalyx are rich with sialic acid residues. High levels of sialic acid residue in the sperm's membrane increases its net negative charge, for its role during capacitation, and its possible participation in the formation of binding bridges between sperm membrane and ovum. The aim of this study is to determine the association between sperm's membrane charge and ART outcomes.
Methods: Under the electric field, the percentage of sperm with positively (PCS), negatively (NCS) and neutrally charged sperm were determined in the ejaculate of 81 patients undergoing IVF treatment and associated with their ART outcomes.
Results: The percentage of NCS in the ejaculate was positively associated with fertilization rate (r2 = 0.381, p = 0.050), embryos that developed to blastocyst (r2 = 0.315, p = 0.010) and inversely associated with the percentage of arrested embryos (r2 = −0.264, p = 0.032). However, there was no significant correlation between the sperm's charge and ICSI fertilization rate. Implantation rate was higher in patient group having greater than 15% negatively charged sperm in their ejaculate (63/103 = 61.17%; n = 51) compared with patient group less than 15% negatively charged sperm (3/38 = 7.89%; n = 19) in their ejaculate. Couples achieving clinical pregnancy (n = 41) had a higher percentage of negatively charged sperm population in their ejaculate (56.63 ± 4.91 vs. 26.34 ± 6.31, p < 0.001) and lower percentage of positively charged sperm population (41.61 ± 4.87 vs. 69.66 ± 6.58, p = 0.001), than couples who did not achieve clinical pregnancy.
Conclusions: There is a statistically significant association between sperm's charge and clinical outcomes. Hypothetically, selection of negatively charged sperm to be used for assisted treatment has a potential to improve ART success.

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HUMAN BINDER OF SPERM PROTEIN HOMOLOG 1 (BSPH1) CAN PROMOTE SPERM CAPACITATION.

Genevieve Plante, Isabelle Therien, PhD[1], Catherine Lachance, PhD[2], Pierre Leclerc, PhD[3] and Puttaswamy Manjunath, PhD[4]
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(Presented By: Genevieve Plante)

Introduction: Binder of SPerm (BSP) proteins are a family of proteins expressed exclusively in the male reproductive tract of several mammalian species and are known to promote capacitation. Our recent studies have shown that in human, the Binder of SPerm Homolog 1 (BSPH1) is expressed specifically in epididymal tissues. Up until now, no studies had ever been done on the role of human BSPH1 in sperm functions.
Methods: To test this, a recombinant BSPH1 (rec−BSPH1) was produced, purified and refolded on−column using a decreasing urea gradient. First, the effect of rec−BSPH1 on sperm capacitation and tyrosine phosphorylation was evaluated. Results obtained showed that human rec−BSPH1 was able to promote sperm capacitation of ejaculated sperm and that this effect was dose−dependent. However, it had no effect on tyrosine phosphorylation. We then tested the effect of rec−BSPH1 on sperm motility using a Sperm Class Analyzer system. The protein was found to have no effect on any parameters of sperm motility tested (total motility, progressive motility or hyperactivation). Binding pattern of BSPH1 on ejaculated sperm was also tested by immunofluorescence microscopy.
Results: It was found to be localized on the equatorial segment, post−acrosomal segment and neck of the sperm.
Conclusion: These results show that the human epididymal BSPH1 shares functional characteristics with BSP proteins secreted by seminal vesicles of ungulates. (Supported by NSERC, CIHR and FESP of university of Montréal)

ABSTRACTS

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QUANTITATIVE PHOSPHOPROTEOMIC ANALYSIS OF SPERM CAPACITATION REVEALS A KEY ROLE OF IGF1R TYROSINE KINASE IN HUMAN

Jing Wang, PhD Candidate[1], Lin Qi, PhD Candidate[2], Tao Zhou, PhD Candidate[2], Yueshuai Guo, PhD Candidate[2], Gaigai Wang, Master[2], Zuomin Zhou, PhD[2], Xuejiang Guo, PhD[2] and Jiahao Sha, PhD[2]
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(Presented By: Jing Wang, PhD Candidate)

Introduction and Objectives: Spermatozoa must reside in the female genital tract for a specific period of time acquire the ability of fertilizing eggs, and named 'Capacitation'. Several biochemical changes occur at specific time during sperm capacitation, such as cholesterol efflux, membrane ion infiltrative increases, and enhancement of tyrosine phosphorylation. Therein, one of the most important change is the enhancement of tyrosine phosphorylation. However, what the role of protein tyrosine phosphorylation in sperm capacitation is not clear. Aim is to discover new phosphorylation modification proteins and the key tyrosine phosphorylated kinases during sperm capacitation.
Methods: Here we empolyed labelfree quantitative phosphoproteomics to investigate the overall phosphorylation events during sperm capacitation. Totally, 3350 phosphorylated sites corresponding to 1017 phosphorylated proteins were identified (FDR<1%) using IMAC−TiO2 phosphopeptide continuous enrichment methods by LC−MS/MS.
Results: In capacitated spermatozoa, 86 phosphorylation proteins and 16 tyrosine phosphorylation proteins were up−regulated (median normalized ratio >2). The NetworKIN algorithm predicted the tyrosine phosphorylation kinases IGF1R and INSR involved in sperm capacitation. These results suggested that IGF1R and INSR may be important tyrosine phosphorylation kinases during sperm capacitation. Analysis of spermatozoa hyperactivation associated motility by CASA showed that GSK1904529A (inhibits IGF1R and IR) treatment either in containing IGF1 factor sperm or in containing insulin factor sperm caused a significant reduction of the motility parameter in a time−dependent manner. Simultaneously, IGF1 factor enhanced spermatozoa hyperactivation associated motility, but insulin factor didn't. Moreover, NVP−ADW742 (inhibits IGF1R specifically) treatment merely caused a significant reduction of spermatozoa hyperactivation associated motility parameter in containing IGF1 factor sperm. These results suggested IGF1R tyrosine kinases might be play a critical role during sperm capacitation. Western Blotting further confirmed these results.
Conclusion: IGF1R mediated tyrosine phosphorylation regulation pathways has played a key role and affected sperm hyperactivation associated motility during human sperm capacitation. Futhermore, it provide a candidate molecular target for clinical diagnosis and treatment of male contraception and male infertility.

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INSIGHTS INTO THE LYSINE ACETYLATION OF PROTEINS IN CAPACITATED HUMAN SPERM

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(Presented By: Xuejiang Guo, PhD)

Introduction: Protein lysine acetylation is a dynamic and revisable post−modification that is known to play diverse functions in eukaryotes. Nevertheless, the composition and function of non−histone lysine acetylation in gametes remain unknown.
Methods: In the present study, we found complex lysine acetylated proteins in human sperm. In human, only capacitated sperm have the capacity to fertilize an egg. After immunopurification enrichment of acetylpeptides with anti−acetyllysine antibody and high−throughout liquid chromatography−tandem mass spectrometry (LC−MS/MS) identification, we characterized 1206 lysine acetylated sites, corresponding to 576 lysine acetylated proteins in human capacitated sperm.
Results: Subcellular localization analysis showed that they mainly localize on mitochondrion (153 genes), microtubule (33 genes), flagellum (21 genes), nucleoplasm (25 genes), nucleosome (9 genes), cytosol (13 genes) and plasma membrane (8 genes). Most subunits of protein complexes such as respiratory chain complex I, proton−transporting ATP synthase complex and proteasome complex are acetylated. These identified acetylated proteins are associated with sperm functions, including motility, capacitation, acrosome reaction and sperm−egg interaction. Indirect immunofluorescence analysis of capacitated human sperm and mouse sperm revealed similar distribution of positive signals, with the strongest signals in the midpiece and principle piece. In vitro fertilization inhibition assay by anti−acetyllysine antibody showed essential functions of lysine acetylation in mouse sperm fertilization. And HDAC inhibitors, TSA and NAM, can significantly suppress sperm motility.
Conclusion: Lysine acetylation is expected to be an important regulator in sperm functions. And our characterization of lysine acetylproteome can be a rich resource for the studies of male fertility.

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SPERM MOTILITY LOSS AND ACTIVATION OF THE CAMP−PKA PATHWAY CAUSED BY THE STAT3 INHIBITORY COMPOUND V RESULT FROM EXCESSIVE REACTIVE OXYGEN SPECIES PRODUCTION.

Catherine Lachance, PhD, Serge Goupil, BSc, Roland R. Tremblay, DSc, MD, PhD, Pierre Leclerc, PhD
Université Laval
(Presented By: Catherine Lachance, PhD)

ABSTRACTS

Introduction: We previously showed that the Stat3 inhibitory compound (Stattic V) alters human sperm motility and mitochondrial activity. Higher levels of reactive oxygen species (ROS) were measured when sperm were incubated with the Stattic V, in agreement with the well−known increased production of ROS caused by mitochondrial dysfunction. Moreover, we recently observed that the negative effect of Stattic V on sperm motility was more pronounced when activators of the PKA pathway are present in the incubation medium. As the stimulation of the PKA pathway is also known to elevated ROS production in sperm, we hypothesized that the effect of Stattic V on sperm motility was caused, at least in part, by the elevated ROS production.
Methods: To address the role of elevated intracellular ROS on the different sperm functions affected by the Stattic V, a membrane permeable antioxidant, N−acetyl−L−cysteine (NAC), was added to the incubation medium. Following sperm incubation in different conditions, motility was evaluated visually, sperm acrosomal integrity was determined by FITC conjugated Pisum sativum agglutinin (PSA−FITC) staining and tyrosine phosphorylation of total proteins as well as serine/threonine phosphorylation of PKA substrates were evaluated by western blot.
Results: The effects of Stattic V on motility and the percentage of A23187−induced acrosome reaction were neutralized by the presence of NAC in the incubation medium. The phosphotyrosine content and the phosphorylation level of PKA substrates were also similar to those observed in the control condition when NAC was present with Stattic V in the incubation medium. We also observed that after one hour of pre−incubation with the Stattic V, the addition of NAC was not sufficient to prevent the gradual motility loss. Similarly, the phosphorylation level of PKA substrates depended on the length of exposition to Stattic V before the addition of the antioxidant.
Conclusion: Those results indicate that the effects of Stattic V on different sperm functions result directly or indirectly from excessive ROS production and that the motility loss and PKA activation caused by Stattic V are irreversible. Those results also suggest that the motility loss caused by Stattic V is PKA−independent and that the more pronounced effects of Stattic V on sperm motility observed when sperm were treated with PKA activators could results from a positive amplification loop of ROS production.

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ROBUST AUTOMATIC SPERM TRACKING

Leonardo Urbano, MSEE[1], Matthew D. VerMilyea, PhD[2], Puneet Masson, MD[2] and Moshe Kam, PhD[1]
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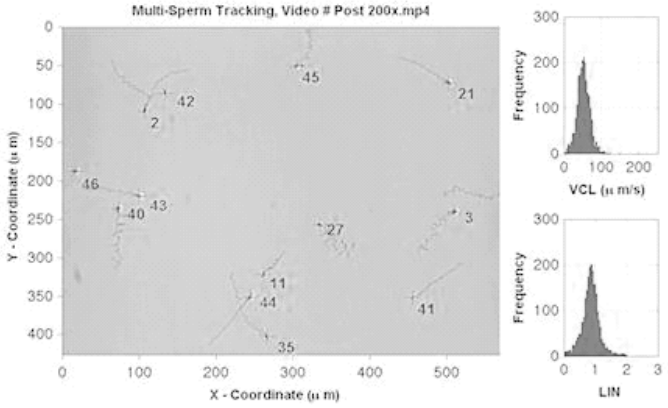
(Presented By: Leonardo Urbano, MSEE)

Objective: Our objective is to develop a fully−automated, robust, multi−sperm tracking algorithm capable of measuring sperm motility parameters accurately with minimal operator intervention. This effort is informed by progress in signal processing and target tracking technologies over the last three decades. A vast majority of sperm motility analysis is performed by technicians using subjective visual measurement−taking. Sometimes computer−assisted semen analysis (CASA) technology is used. However, most CASA systems are prohibitively expensive and require significant user intervention to track sperm whose paths have collided or are in close proximity. Target tracking algorithms developed originally for radar applications and video processing have addressed similar problems in other domains successfully and their methodologies can be used for sperm tracking and analysis.
Methods: Videos of washed sperm samples were recorded and digitized at 100x, 200x, and 400x magnification at 30 frames per second. A custom−made MATLAB algorithm was developed to automatically detect sperm in recorded video frames and perform multi−sperm tracking. A joint probabilistic data association (JPDA) filter − representing a mature technology employed in air traffic control systems − was used to reconcile sperm track−measurement association conflicts. This approach enabled accurate tracking of dozens of sperm simultaneously through collisions. In addition, tracks are automatically initiated and deleted as sperm enter and exit the video frame.
Results: Our algorithm is capable of tracking simultaneously every sperm in every video frame studied without any human intervention. Numbered sperm tracks were overlaid on the original video frames accompanied by an animated histogram of the curvilinear velocity (VCL) and path linearity (LIN) calculated for every sperm tracked. Our animated VCL and LIN histograms are useful for differentiating between samples of sperm based on motility.
Conclusions: The JPDA algorithm was effective at tracking simultaneously dozens of sperm through collisions while calculating VCL and LIN. To our knowledge, these results represent the first time JPDA has been applied to sperm tracking.



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JUSTICIA GANDARUSSA BURM.F. AS HYALURONIDASE HUMAN SPERMATOZOA INHIBITOR ACTIVITY

Bambang Prajogo
(Presented By: Bambang Prajogo)

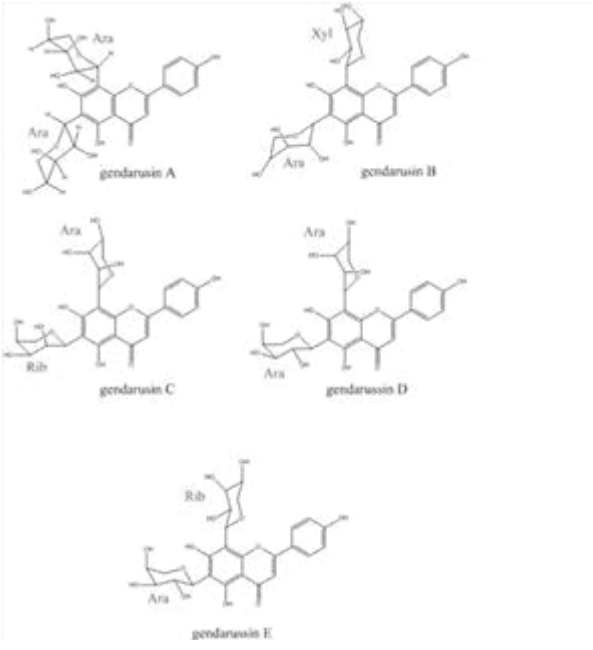
Introduction and Objective: Flavonoid glycoside is known as a hyaluronidase inhibitor which is an enzyme that has a role in human fertilization process. This enzyme present on the spermatozoa acrosomes digest hyaluronic acid substrate on the layer of the ovum. Justicia gendarusa Burm.f. leaf contains 12 components of flavonoid glycosides with the same molecular weight (MW 535). Gendarusin A is the major compound on it. In the preliminary research, isolate and extract showed the reversible competitive inhibitor activity in vitro. In the same activity also showed the decreasing of spermatozoa hyaluronidase on mice and human. Objective is to determine the decreasing of hyaluronidase human sperm activity which is inhibits the fertilization process.

Method: Research about anti−fertility which use capsule of 70% ethanol extract of J. gandarusa has been done. The dose of the capsule for 18 subjects was 450 mg/70 kg BW once a day for 30 days. The measurement used microplate with 96 wells to determine the catalytic and specific activity of the enzyme by spectrophotometer at Î» 595 nm. The subjects administered the capsules for 30 days. Assay of hyaluronidase activity was performed at the day 0, 15, 30 and 60.

Result: The study showed that the catalytic activity of hyaluronidase before taking the capsule was 1.5506.10−6 unit/million of spermatozoa. After taking the capsule for 15 days, the hyaluronidase activity was 1.4600.10−6 unit/million of spermatozoa and 1.48889.10−6 unit/million of spermatozoa at day 30. At day 60, i.e. 30 days after stopping the treatment, the activity was 2.7994.10−6 unit/million of spermatozoa. While the specific activity of hyaluronidase before taking capsule was 9.6672.10−8 unit/mg, after taking the capsule, on day 15th was 9.4911.10−8 unit/mg and on day 30th was 8.9350.10−8 unit/mg. At day 60, i.e. 30 days after stopping the treatment, the activity was 9.8056.10−8 unit/mg.

Conclusion: In conclusion, activity of hyaluronidase enzyme decreased after consuming the capsule. It was known that after stopping the capsule administration for 30 days, hyaluronidase enzyme was back to normal.

Keywords: Justicia gendarussa Burm.f., hyaluronidase, human spermatozoa, anti−fertility, gendarusin



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REGULATION OF ACROSOME REACTION BY LIPRIN A3, LAR AND ITS LIGANDS IN MOUSE SPERM

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National Institute for Research in Reproductive Health
(Presented By: Chetanchandra Joshi, Msc)

Introduction and Objectives: Zona pellucida (ZP) based induction of acrosome reaction (AR) is a popular and well accepted hypothesis. However, this hypothesis is being challenged in recent years and it has been proposed that the cumulus cells might be the site of AR. In the present study we demonstrate the Liprin α3 interaction with RIM and LAR and show the importance of interaction of Liprin α3 and LAR in acrosome reaction. The present study was designed to understand the role of Liprin α3 and its interacting proteins LAR, RIM in regulation of AR.

Methods: 1. Western blot analysis & Indirect Immunofluorescence (IIF) of LAR was carried out on tissue and sperm. 2. Co−localization of LAR, Rab Interacting Molecule (RIM) with Liprin α3 was carried out. The extent of overlap was calculated. 3. Mouse cumulus cells were analysed for the presence of Syndecan−1 with Anti Syndecan−1 antibody 4. To check the effect of LAR ligands i.e. Syndecans and nidogens and LAR wedge peptide capacitated sperm were spiked with different concentrations of recombinant ligands, wedge peptide & anti Liprin α3. Acrosome exocytosis index was then calculated and effect was considered significant at $p<0.05$

Results: It is observed that the presence of anti−Liprin α3 antibody inhibits the process of acrosome reaction. Co−localization experiments demonstrate the co−existence LAR (Leucocyte Antigen Related), Rab Interacting Molecule (RIM) and Liprin α3 on sperm acrosome thereby completing the identification of all the members of RIM/MUNC/Rab3A/liprinα complex required for membrane fusion. Our study demonstrates an increase in AR in presence of LAR ligands such as Syndecans, Nidogens and LAR wedge domain peptide on acrosome reaction. Based on these data we speculate that in presence of ligands or wedge peptide, LAR undergoes dimerization leading an increase in AR.

Conclusions: Overall this study demonstrates that sperm acrosome reaction is driven by common set of proteins like Liprin α3, LAR, RIM shown to be responsible for membrane fusion at synapse. We could also demonstrate that the ligands and wedge peptide can induce LAR dimerization and could be one of the mechanisms of stimulating acrosomal exocytosis. The observations support the hypothesis that cumulus could be another site of acrosome reaction.

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ASSESSMENT OF SPERM DNA FRAGMENTATION AFTER MICROSCOPIC SUBINGUINAL VARICOCELECTOMY
INTRODUCTION:

Brooke Harnisch, MD and Jay Sandlow, MD
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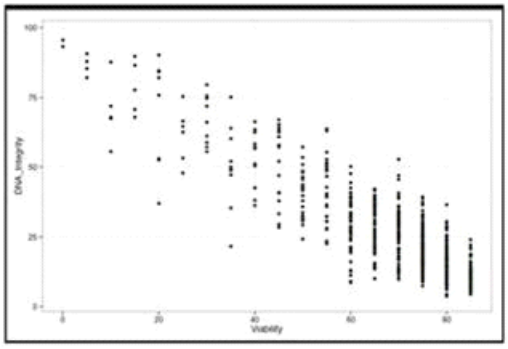
(Presented By: Brooke Harnisch, MD)

Objective: To evaluate DNA fragmentation in male infertility patients before and after microscopic subinguinal varicocelectomy.

Methods: An institutional review board (IRB) approved retrospective study was conducted on infertile men with palpable varicoceles who underwent microscopic subinguinal varicocelectomy between September 2012 and June 2013. Exclusion criteria included: adolescents and patients undergoing surgery for pain. Demographic, clinical and laboratory data was collected. DNA fragmentation was determined by Halosperm® diagnostic kit.

Results: A total of eight patients were identified who had complete pre and post op information. Mean total sperm count and sperm concentration significantly improved after varicocelectomy from 13.7 x106 to 29.5x106 and 4.4x106/ml to 8.6x106/ml ($p<0.05$). Total progressively motile sperm per ejaculate trended to significance from 2.7 x106 to 7.9x106 ($p=0.07$). Overall, there was no significant change in sperm DNA fragmentation after surgery. On subgroup analysis, patients with a DNA fragmentation <20% and a DNA fragmentation >20% had no significant improvement post−operatively. However, despite having similar pre−operative mean sperm count and concentration, patients with DNA fragmentation <20% had a significantly higher post−operative sperm count than patients with a DNA fragmentation >20% ($p=0.03$).
Conclusions: Varicocelectomy significantly improves semen parameters but does not decrease DNA fragmentation levels. Randomized controlled trials are needed before impaired sperm DNA integrity may be considered as an alternative indication for varicocele repair.

Figure 1: Sperm DNA fragmentation versus sperm viability



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THE RELATIONSHIP BETWEEN SPERM VIABILITY AND DNA FRAGMENTATION RATES

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(Presented By: Mary Samplaski, MD)

Introduction: We sought to determine the relationship between sperm viability and DNA fragmentation index (DFI). Specifically we evaluated the relationship between viability and DFI > 30%, since a DFI > 30% has been associated with the need for intracytoplasmic sperm injection.
Methods: Men having semen analyses with both vitality and DFI testing were identified. Viability was measured by the eosin−nigrosin assay. DNA fragmentation was measured using a sperm chromatin structure assay with flow cytometry. The relationship between DFI and viability was assessed by univariate analysis.
Results: A strong inverse relationship ($p<0.001$) was seen between viability and DNA fragmentation rates, with Pearson correlation coefficient $r=-0.87$ (Figure 1). A total of 3050 men had both DFI and viability assays. If viability was very high (≥ 80%, n=1104) then DFI was consistently ≤ 30% (100% sensitivity to predict DFI ≤ 30%). If viability was ≥ 75% (n=1736), then the DFI was ≤ 30% for 95% of the patients. For samples with very low viability (viability ≤ 35%, n=91) then DFI was always ≥ 30%. If viability was ≤ 50% then DFI was ≥ 30% for 95% of the samples (n=310).
Conclusions: Sperm viability correlates strongly with DNA fragmentation rates. In men with sperm viability ≤ 50%, 95% of the time the DFI is ≥ 30%; Conversely, if sperm viability ≥ 75%, 95% of the time the DFI is ≤ 30%.

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CHARACTERIZATION OF MEMBRANE OCCUPATION AND RECOGNITION NEXUS REPEAT CONTAINING 3, A MEIG1 BINDING PARTNER, IN MOUSE MALE GERM CELLS

Ling Zhang, Hongfei Li, MD, Yuqin Shi, PhD, Maria Teves, PhD, Zhiqiong Wang, MD, Gaofeng Jiang, PhD, Shizhen Song, PhD and Zhibing Zhang, PhD
(Presented By: Ling Zhang)

Introduction: Mammalian spermatogenesis is a well−organized process of cell development and differentiation; the morphogenesis of spermatozoa is the final step of spermatogenesis. During this process, haploid round spermatids differentiate into spermatozoa, with dramatic morphological changes. Meiosis, expressed gene 1 (MEIG1), plays an essential role in the regulation of this step.
Methods: To explore potential mechanisms of MEIG1 in the regulation of spermiogenesis, a yeast two−hybrid screen was conducted and several potential binding partners were identified; one of them was membrane occupation and recognition nexus repeat containing 3 (MORN3). The interaction between MORN3 and MEIG1 was confirmed by co−immunoprecipitation in cultured mammalian cells over−expressing the two proteins. Morn3 mRNA is only abundant in mouse testis. In the testis, Morn3 mRNA is highly expressed in the spermiogenesis stage. Specific anti−MORN3 polyclonal antibody was generated against N−terminus of the full length MORN3 protein, and MORN3 expression and localization was examined in vitro and in vivo. In transfected CHO cells, the antibody specifically crossed−reacted the full length MORN3 protein, and immunofluorescence staining revealed that MORN3 was localized throughout the cytoplasm.
Results: Among multiple mouse tissues, an about 25 kDa protein, but not the full length 28 kDa MORN3 protein was identified only in the testis. The protein was highly expressed after day 20 of birth. Immunofluorescence staining on mixed germ cells isolated from adult wild−type mice demonstrated that MORN3 was not present in spermacytes, but expressed in the acrosome in germ cells throughout spermiogenesis. The protein was also present in the manchette of elongating spermatids. The total MORN3 expression and acrosome localization were not changed in the Meig1−deficient mice. However, its expression in manchette was dramatically reduced in the mutant mice.

Conclusion: Our studies suggest that MORN3 might be another regulator for spermatogenesis, probably together with MEIG1.

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A MEIG1/PACRG COMPLEX IN THE MANCHETTE IS ESSENTIAL FOR THE TRANSPORT OF STRUCTURAL PROTEINS REQUIRED FOR CONSTRUCTION OF THE SPERM FLAGELLA

Maria Teves, PhD, David Nagarkatti−Gude, Kellie Archer, Waixin Tang, Darrell Peterson, Jerome Strauss, Zhibing Zhang
(Presented By: Zhibing Zhang)

Introduction: One of the hallmarks of spermiogenesis is the formation of flagella, which enables sperm to reach eggs for fertilization. The molecular mechanism of flagellogenesis is poorly understood. Meiosis−expressed gene 1 product (MEIG1) is a key regulator of spermiogenesis. Meig1−deficient male mice are sterile as a result of impaired spermiogenesis. Dynamic analysis of testicular histology revealed that the testes from Meig1−deficient mice have abnormal morphological after 28 days of birth, the time when germ cells enter the stage of elongation/condensation. Except Meig1, DNA microarray assays did not identify other genes whose expression in the testes was significantly changed at both 22 and 28 days after birth in the mutant mice. We previously discovered that Parkin co−regulated gene (PACRG) was the major binding partner of MEIG1.
Methods: Using PACRG as bait, MEIG1 was also identified to be its major binding partner. Male mice deficient in PACRG display a similar reproductive phenotype to that of Meig1−deficient mice. In spermatocytes of wild type mice, MEIG1 is expressed in the whole cell bodies, but it migrates to the manchette in the elongating spermatids. PACRG protein appears during the transition of round spermatids into elongating spermatids, which is much later than the appearance of Pacrg transcript, suggestive of translational or posttranslational control of expression of this gene.
Results: In the elongating spermatids of wild−type mice, PACRG co−localizes with α−tubulin, a marker for manchette, this localization was not changed in the remaining elongating spermatids of Meig1−deficient mice. However, MEIG1 is no longer localized in the manchette in the remaining elongating spermatids of Pacrg−deficient mice, indicating that PACRG recruits MEIG1 to the manchette. PACRG is not stable in either bacteria or mammalian cells, but can be stabilized by MEIG1. Besides PACRG, MEIG1 also associates with SPAG16L, a sperm axonemal central apparatus protein. SPAG16L is present in the spermatocyte cytoplasm of wild−type mice, and in the manchette of elongating spermatids, but in the Meig1−deficient mice, SPAG16L is no longer localized in the manchette. However, MEIG1 is still present in the manchette of Spag16L−deficient mice, suggesting that SPAG16L is a downstream partner of MEIG1.
Conclusion: Our studies demonstrate that MEIG1 and PACRG form a complex in the manchette, and that this complex is essential to transport sperm flagellar proteins, like SPAG16L, to build the sperm flagella.

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COMBINED ADMINISTRATION OF CURCUMIN AND GALLIC ACID INHIBITS GALLIC ACID−INDUCED SUPPRESSION OF STEROIDOGENESIS, SPERM OUTPUT, ANTIOXIDANT DEFENSES AND INFLAMMATORY RESPONSIVE GENES

Sunny Abarikwu, PhD, Mojisola Durojaiye, BSc, Adenike Alabi, BSc, Oghenetega Akiri, BSc
Redeemer's University, Nigeria
(Presented By: Sunny Abarikwu, PhD)

Introduction: In this study, we investigated the effects of administration of gallic acid (Gal) with or without curcumin (Cur) on the sperm output, steroid level and antioxidant defenses in rat testis in vivo and the expression of inflammatory responsive genes in vitro.
Methods: Male Wistar rats were divided randomly into four groups and given oral Gal (100 mg/kg/day) and Cur (100 mg/kg/day) alone or in combination for four weeks. The sperm quality was impaired following Gal treatment, while Cur prevented this and also improved the sperm count as well as the efficiency of sperm production (DSP/gm testis). The inhibitory effects of Gal on plasma testosterone level, glutathione levels, activities of glutathione peroxidase, catalase, superoxide dismutase and steroidogenic enzymes, 3β−hydroxysteroid dehydrogenase (3β−HSD) and 17β−HSD in the rat testis was blocked by Cur.
Results: Interestingly, the level of testosterone and the activities of the steroidogenic enzymes were significantly increased after treatment with Cur alone. Malondialdehyde concentration was unchanged following Gal treatment, while a significant decrease in malondialdehyde level was observed following treatment with Cur alone or in combination with Gal. We further analyzed the effects of Cur and Gal (25−100 μM) on the 93RS2 Sertoli cell−lines and observed that Cur blocked the Gal−induced suppression of inflammatory mediators such as TNF−α and IL−6, while Gal blocked the suppressive effect of Cur on IL−1α expression. Furthermore, the stimulatory or inhibitory effects of Gal on the expressions Tgf−β1 and CD−14 was concentration−dependent and could be blocked by Cur. When cultures of primary Sertoli cells were exposed to both Cur and Gal for 24 h, p−JNK/SAPK expression remain stable, whereas Gal−induced p−p65 (NF−ÎºB) expression and IÎºBα degradation was seen to be blocked by Cur but not Gal−induced expression of pERK1/2.
Conclusion: Overall, Cur has stimulatory reproductive effects and could protect the testis from the toxic effects of Gal by mechanisms that could not be explained by its effects on the expressions of inflammatory cytokines but by its anti−oxidant properties.

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THE TRANSCRIPTION FACTOR MEF2 IS A NOVEL REGULATOR OF GSTA1 EXPRESSION IN MA−10 LEYDIG CELLS

Mickael Di−Luoffo, MSc, Catherine Brousseau, MSc, Francis Bergeron, MSc and Jacques J. Tremblay, PhD
CRCHUQ−Universite Laval
(Presented By: Mickael Di−Luoffo, MSc)

Introduction: Testosterone is essential for spermatogenesis and for the development of primary and secondary male sexual characteristics. Steroidogenesis, however, produces a significant amount of reactive oxygen species (ROS), which in turn disrupt testosterone production.

Methods: Our lab has identified members of the Myocyte Enhancer Factor 2 (MEF2) family of transcription factors in the mouse testis. MEF2 factors are important regulators of organogenesis and cell differentiation in various tissues. In the testis, MEF2 is present in Sertoli and Leydig cells throughout fetal and adult life suggesting a role for this factor in somatic cell differentiation and function. Supporting this, we found that MEF2 regulates the expression of genes involved in steroidogenesis. Furthermore, analysis of the transcriptome of MEF2−deficient (siRNA knockdown) MA−10 Leydig cells revealed a significant decrease in the expression of Gsta family members (glutathione−S−transferase) that encode ROS inactivating enzymes. The aim of the present study was to determine the role of MEF2 in Gsta1 expression in Leydig cells.
Results: By qPCR, we confirmed that Gsta1 mRNA level was decreased by 74% in MEF2−deficient MA−10 Leydig cells. Conversely, overexpression of MEF2 in these cells lead to a 1.5 fold increase in endogenous Gsta1 mRNA levels. In silico analyses of the Gsta1 promoter revealed the presence of a consensus MEF2 binding site (YTAWWWWTAR) at −506 bp. MEF2 recruitment to the proximal Gsta1 promoter was confirmed by ChIP whereas no significant recruitment was observed on a distal Gsta1 promoter region lacking MEF2 element or when an IgG was used. Next a 2 kb fragment of the mouse Gsta1 promoter was isolated and fused to luciferase for functional studies. Mutation of the MEF2 element at −506 bp led to a 68% decrease in Gsta1 promoter activity. In addition, transfection of MEF2 in MA−10 cells led to a 2.2 fold activation of the Gsta1 promoter, which was lost when the MEF2 element was deleted or mutated. These data indicate that the MEF2 element at −506 bp is essential for MEF2 responsiveness. Since MEF2 can be activated by CAMKI (which is present in Leydig cells), MEF2 and CAMKI were co−transfected in MA−10 cells and this resulted in a 5.7 fold activation of the Gsta1 promoter.
Conclusion: In conclusion, our results identify a novel role for MEF2 in the regulation of genes involved in Leydig cell detoxification, a process essential for the maintenance of testosterone production. Supported by CIHR.

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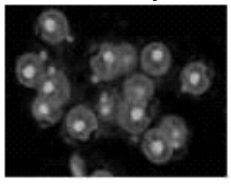
DETECTION OF STRONGLY REPRESSED AND HIGHLY ACTIVE MRNAS IN THE CHROMATOID BODY OF ROUND SPERMATIDS WITH A SIMPLE AND SENSITIVE FLUORESCENT IN SITU HYBRIDIZATION TECHNIQUE.

Danielle Cullinane, Graduate Student and Ken Kleene, PhD
Umass Boston
(Presented By: Danielle Cullinane, Graduate Student)

Introduction and Objectives: Many mRNAs are stored as translationally inactive free−mRNPs in round spermatids and actively translated in elongating and elongated spermatids. A popular idea is that free−mRNPs are repressed by storage in the chromatoid body, a cytoplasmic mRNP−granule in round spermatids that is devoid of ribosomes, and is thought to coordinate mRNA translation and degradation. A notable gap in this model is the paucity of evidence that mRNAs are even present in the chromatoid body. The objectives of this study are to develop reliable fluorescent in situ hybridization (FISH) techniques to detect mRNAs in the chromatoid body and to compare the localization of mRNAs that are strongly repressed and actively translated in round spermatids.
Methods: Dried down preparations of stage II−VI seminiferous tubules were analyzed with FISH using tiled fluorescently labeled antisense oligo probes for four mRNAs: the sperm mitochondria−associated cysteine−rich protein (Smcp) mRNA and a Smcp−Gfp transgenic mRNA, both of which are both strongly repressed in round spermatids, and the lactate dehydrogenase C (Ldhc) mRNA and another Smcp−Gfp transgenic mRNA, both of which are highly active in round spermatids. FISH was detected with conventional and confocal fluorescence microscopy, and correlated with immunological markers for the chromatoid body (DDX4/MVH) and free−mRNPs (Y−box protein 2, YBX2/MSY2).
Results: All four mRNAs exhibit intense FISH in a small irregular, perinuclear spot in round spermatids which overlaps DDX4. In contrast, YBX2 is present throughout the cytosol with a small amount in the chromatoid body. Interestingly, DDX4, YBX2 and mRNAs are differentially localized within the chromatoid body.
Conclusions: We suggest a counterintuitive interpretation of these findings. The strong FISH signal of all four mRNAs in the chromatoid body represents a high concentration of a small number of mRNA molecules in a very small volume, while the weak signal in the cytosol represents a low concentration of a larger number of mRNA molecules in free−mRNPs and polysomes in a much larger volume. Conceivably, mRNPs are transiently stored and remodeled in multiple compartments in the chromatoid body.



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A−SINGLE SPERMATOGONIA HETEROGENEITY AND CELL CYCLE SYNCHRONIZE WITH A SPECIFIC RAT SEMINIFEROUS EPITHELIAL STAGE

Shadaan N. Abid, PhD, Timothy E. Richardson, MD, PhD, Heather M. Powell, MS, Priscilla Jaichander, PhD, Jaideep Chaudhary, BS, Karen M. Chapman, BS and F. Kent Hamra, PhD
UT Southwestern Medical Center in Dallas
(Presented By: F. Kent Hamra, PhD)

Introduction: In mammalian testes, type A−single spermatogonia function as stem cells that sustain sperm production for fertilizing eggs. Currently, it is not understood how cellular niches regulate the developmental fate of A−single spermatogonia.
Method: Here, anatomical maps and immunolabeling studies in rat testes define a novel population of ERBB3+ germ cells as ~5% of total SNAP91+ A−single spermatogonia along a spermatogenic wave.
Results: As a function of time, ERBB3+ A−single spermatogonia are transiently detected during a 1−2 day period each 12.9 day sperm cycle, representing 35−40% of SNAP91+ A−single spermatogonia in stage VIII seminiferous tubules. ERBB3+ spermatogonia also synchronize their cell cycle during this epithelial stage where they form physical associations with preleptotene spermatocytes transiting the blood−testis−barrier, and Sertoli cells undergoing sperm release.
Conclusion: Thus, induction of stem cell heterogeneity within this specific, short−lived and re−occurring microenvironment highlights novel theories on how cellular niches could integrate with testicular physiology to orchestrate sperm development in mammals.

ABSTRACTS

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LINKING SPERMATID RNA BINDING PROTEIN DIVERSITY TO REPRODUCTIVE SUCCESS

Karen M. Chapman, BS, Jaideep Chaudhary, BS, Timothy E. Richardson, MD, PhD and F. Kent Hamra, PhD
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(Presented By: F. Kent Hamra, PhD)

Introduction: Spermiogenesis is a postmeiotic process that drives development of round spermatids into fully elongated spermatozoa. Spermatid elongation is largely controlled post−transcriptionally after global silencing of mRNA synthesis from the haploid genome.
Methods: Here, rats that differentially express EGFP from a lentiviral transgene during early and late steps of spermiogenesis were used to flow sort fractions of round and elongating spermatids. Mass−spectral analysis of 2D gel protein spots enriched >3−fold in each fraction revealed a heterogeneous RNA binding proteome (hnRNPA2/b1, hnRNPA3, hnRPDL, hnRNPK, hnRNPL, hnRNPM, PABPC1, PABPC4, PCBP1, PCBP3, PTBP2, PSIP1, RGSL1, RUVBL2, SARNP2, TDRD6, TDRD7) abundantly expressed in round spermatids prior to their elongation.
Results: Notably, each protein within this ontology cluster regulates alternative splicing, subcellular transport, degradation and/or translational repression of mRNAs. In contrast, elongating spermatid fractions were enriched with glycolytic enzymes, redox enzymes and protein synthesis factors. Retrogene−encoded proteins were over−represented among the most abundant elongating spermatid factors identified.
Methods: Consistent with these biochemical activities, plus corresponding histological profiles, the identified RNA processing factors are predicted to collectively drive post−transcriptional expression of an alternative exome that fuels finishing steps of sperm maturation and fitness.

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EFFECTS OF ALLII TUBEROSI SEMEN ON THE CYCLIC AMP RESPONSE ELEMENT MODULATOR (CREM) EXPRESSION DURING SPERMATOGENESIS

Jin hyoung Cho, MS, Sung Won Jee, PhD, Do Rim Kim, PhD, Ha Young Kim, MS, Eun Bit Ko, MS, Ho Jin Lee, MS, Mun Seog Chang, PhD, Seong Kyu Park, PhD
Department of Prescriptionology, College of Korean Medicine, Kyung Hee University
(Presented By: Seong Kyu Park, PhD)

Introduction: The cyclic AMP response element modulator (CREM) is a transcription factor highly expressed in the post−meiotic germ cells of the testis. CREM is a key factor in spermatogenesis and a causal factor of round spermatid maturation arrest in idiopathically infertile men.
Methods: In order to investigate the effects of Allii tuberosi Semen (AS) on CREM expression, real−time PCR and Western blotting assays were performed in this study. C57BL/c mice were divided into four groups, the normal group and AS treated groups (100, 500, 1000 mg/kg of AS) for five weeks.
Results: In our results, sperm count and motility were increased in 100, 1000 mg/kg of AS treated group than that of normal group (178.56 ± 23.90, 225.42 ± 51.00 ×106 vs. 166.82 ± 37.22 and 64.75 ± 3.64, 68.87 ± 4.02 vs. 53.22 ± 1.74%, respectively.).
Conclusion: CERM expression level was significantly increased in 100, 1000 mg/kg of AS treated group than that of normal group. In conclusion, our results suggest that AS can promote spermatogenesis and increases sperm motility through the induction of CREM transcription factor.

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PEROXISOME PROLIFERATOR−ACTIVATED RECEPTOR−B/D (PPARB/D) REGULATES SPERMATOGENESIS BY ALTERING CELL−CYCLE REGULATORS IN MICE

Pei−Li Yao, LiPing Chen, Frank Gonzalez, Jeffrey Peters
(Presented By: Pei−Li Yao)

Introduction: Peroxisome proliferator−activated receptors (PPARs) are nuclear hormone receptors which control a variety of biological processes, including cell differentiation and embryo development. Although Pparβ/Î´−/− mice are fertile, they display a significantly smaller litter size compared to Pparβ/Î´+/+ mice.
Methods: Here, we showed that Pparβ/Î´−/− mice exhibit multi−nucleated giant germ cells, cell cycle arrest, germ cell depletion, vacuolization in Sertoli cells, and mixed−stages of spermatogenesis in the seminiferous tubule compared to Pparβ/Î´+/+ mice. This indicates that PPARβ/Î´ has a critical role in the functional spermatogenesis during testis development.
Results: The overall incidence of atrophic testes and testis degeneration in Pparβ/Î´−/− mice is significantly higher than that in Pparβ/Î´+/+ mice. At both peri−pubertal and adult ages, testicular CYCLIN D1 expression is limited in spermatogonia and is higher in Pparβ/Î´−/− mice than in Pparβ/Î´+/+ mice. Sertoli cells in Pparβ/Î´−/− mice express less p27 and the average number of Sertoli cells in seminiferous tubules of Pparβ/Î´−/− mice is higher than that in Pparβ/Î´+/+ mice. The expression of carcinoma in situ marker, placental alkaline phosphatase (PLAP), is stronger in Pparβ/Î´−/− mice testes than in Pparβ/Î´+/+ mice testes. The testicular cKIT expression is also higher in Pparβ/Î´−/− mice than in Pparβ/Î´+/+ mice.
Conclusion: Combined, these novel data suggest that PPARβ/Î´ regulates spermatogenesis by maintaining the homeostasis between the developing germ cells and the matured Sertoli cells in the seminiferous epithelium and may play a role in preventing the occurrence of carcinoma in situ.

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EFFECT OF IRRADIATION ON THE LEVELS OF VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) IN MOUSE TESTIS

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Ben−Gurion University of the Negev
(Presented By: Mahmoud Huleihel, PhD)

Introduction and Objectives: Vascular endothelial growth factor (VEGF) is a protein produced by a wide range of cells. It promotes vasculogenesis and angiogenesis. VEGF also causes proliferation of endothelial cells and increase the permeability of the wall of blood vessels. Although the function of VEGF in the testis is unknown, this factor is attributed with survival and development of testicular germ cells as well as determining fertility in mice. VEGF was detected in Sertoli, Leydig and some testicular germ cells. Irradiation affects dividing cells. In the testes of adults, the main affected cells are the developing germ cells. Irradiation was also shown to affect some functions of Sertoli and Leydig cells. However, the effect of irradiation on testicular VEGF was not yet examined. Objective is to evaluate the effect of irradiation on mouse testicular VEGF levels and cellular localization.
Methods: Mice (BALB/c; 8 weeks−old) were exposed (total body irradiation) once (at the beginning of the experiment) to different doses of irradiation [control group; (CT), 0.5, 2.5 and 10 Gy). After 1−10 weeks of irradiation, mice were sacrificed, and testes were weighted and collected to be evaluated: 1) Histologically by using hematoxylin−eosin staining; 2) For the levels of VEGF in the testicular tissue by ELISA; 3) For cellular localization by Immunohistochemical staining using specific anti mouse VEGF antibodies.
Results: Our results show that irradiation damages the normal structure of the seminiferous tubules and that the strongest effect of high irradiation doses on testicular weight and seminiferous tubules was detected 3−4 weeks post−irradiation, after that there was a recovery. Irradiation significantly increased the levels of VEGF in testicular homogenates. The effect of the different doses of irradiation (low and high) on VEGF levels was expressed in different time points post−irradiation. In addition, we showed that VEGF levels in testes of normal mice decreased with age increase. The main increase of VEGF was detected in interstitial cells and spermatocytes.
Conclusions: Our results support the suggestion that VEGF could be involved in the regulation of spermatogenesis, under normal and pathological conditions, through regulation Leydig cell activities and germ cell niches which may affect their growth, proliferation and/or differentiation.

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INHIBITION OF MTOR SIGNALING DECREASES STRA8 EXPRESSION IN ADULT MOUSE TESTIS

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[1]Akdeniz University Medical Faculty Department of Histology and Embryology; [2]Near East University Medical Faculty Department of Histology and Embryology
(Presented By: Pinar Sahin, MSc)

Introduction: Mammalian target of rapamycin (mTOR) signaling serves as a regulator of growth and proliferation. Several studies have emphasized destructive impact of mTOR inhibitor, rapamycin, on male gonadal function in men. Recently, we showed that mTOR pathway components are localized in spermatogonia and preleptoten spermatocytes suggesting that mTOR pathway may have a role during proliferation and meiotic initiation of spermatogonia. Thus; we aimed to investigate the effect of mTOR inhibition to Stra8 expression utilizing seminiferous tubule culture system.
Methods: First, distribution of mTOR signaling molecules were evaluated in testes of adult mice by immunohistochemistry. Then, to evaluate the effect of mTOR inhibition on spermatogenic cells using seminiferous tubule culture experiments, 4 groups were established; control, 24 hour culture, rapamycin treated, and ethanol treated as vehicle. Up to five seminiferous tubule fragments were cultured in 30µl hanging drops and afterwards effects of rapamycin were examined using western blot analysis for p−p70S6K, PCNA, Stra8 and VASA. Cell viability assay and TUNEL was also performed in all groups.
Results: Firstly; our immunohistochemistry results showed that mTOR, p−mTOR, p−p70S6K, p−4EBP−1 proteins were localized in spermatogonial cells and preleptoten spermatocytes in adult mice testis. Secondly; seminiferous tubule culture experiments showed that cell viability was similar between the groups. Expression of p−p70S6K decreased significantly in rapamycin treated group indicating that mTOR signaling has been inhibited successfully. Furthermore, PCNA and Stra8 expressions decreased significantly in rapamycin treated group. No differences were observed for VASA expression between the groups. For all groups, the number of TUNEL positive cells was similar.
Conclusions: Our seminiferous tubule culture studies indicated that mTOR signaling may regulate spermatogonial stem cells by not only controlling their proliferative capacity but may also regulate their differentiation by controlling the expression of meiosis initiation molecule Stra8. Regulation of meiosis by this pathway is a novel finding and extensively under investigation in our laboratory utilizing in vitro and in vivo approaches. This study is supported by TUBITAK with the project numbers: 110S309 and 113S490, and Akdeniz University Scientific Research Projects with the project number 2010.02.0122.009.

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FUNCTIONAL CHARACTERIZATION OF ION CHANNELS IN SINGLE SPERMATOGONIA IN VITRO AND IN SITU.

David Fleck, MSc[1], Sophie Veitinger, PhD[2], Thomas Veitinger, PhD[1], Patricia Almeida Machado, BSc[1], Susanne Lipartowski[1], Corinna Engelhardt[1], Jennifer Spehr, PhD[1] and Marc Spehr, PhD[1]
[1]Department of Chemosensation, Institute for Biology II; [2]Institute for Cytobiology, Philipps−University Marburg
(Presented By: David Fleck, MSc)

Introduction: Spermatogenesis is a fundamental and highly complex biological process that ensures male fertility. Spermatogonia are the precursors of all male germ cell stages. Their differentiation assures the lifelong production of mature sperm. However, few physiological details are known about testicular cell communication during spermatogenesis. Since we and others have previously shown that Sertoli cells are able to communicate via ATP, we hypothesize a general role for purinergic signaling in the testis.
Methods: Using wildtype C57BL/6 mouse pups, we first developed a coculture of Sertoli cells and spermatogonia. Next, we investigated ATP−dependent signaling by whole−cell patch−clamp recordings from cultured spermatogonia. Pharmacological profiling and gene expression knockdown allowed identification of involved ion channels.

Results: Here, we report that cultured spermatogonia respond to extracellular ATP (1 − 100 μM). ATP−induced currents show fast activation and moderate desensitization. The current−voltage relationship reveals strong inward rectification. Current potentiation by ivermectin and inhibition by an acidic extracellular pH (6.3) and extracellular copper (100μM) indicate a functional role of P2X4 receptors. Accordingly, knockdown of P2X4R expression by RNA interference significantly reduced currents activated by ATP concentrations ≤ 300 μM. Interestingly, an increased ATP concentration (>300 μM) activated an additional current with different kinetics. A similar current could be activated by 300 μM 3'−O−(4−Benzoyl)benzoyl ATP (BzATP). Knockdown of P2X7R expression decreased the current activated by higher ATP concentrations (>300 μM). Combined with molecular evidence, our results indicate that at least two different of P2X receptor subunits (P2X7R and P2X4R) are functionally expressed in spermatogonia of young prepubescent mice. Downstream of P2X receptor activation, we found a slowly activating calcium−dependent potassium current functionally antagonizing the depolarizing P2XR−mediated current.
Conclusion: To confirm these results in situ, we established a new experimental approach. Using acute tissue slices of prepubescent mouse testis we electrophysiologically analyzed spermatogonia and found ATP−induced currents with similar characteristics. Together, these data represent a first important step towards a deeper understanding of cellular purinergic communication during spermatogenesis.

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FURTHER CONFIRMATION OF SEVERAL IMPORTANT TARGETS OF SUMOYLATION IN TESTICULAR CELLS

Yuxuan Xiao, PhD[1], Daniel Pollack, BsC[1], Avi Levy[1], Miriam Andrusier[1] and Margarita Vigodner, PhD[2]
[1]Yeshiva University; [2]Yeshiva University and AECOM, New York.
(Presented By: Margarita Vigodner, PhD)

Introduction: Sumoylation (a covalent modification by Small Ubiquitin−like Modifiers or SUMO proteins) has emerged as a critical regulatory event in cell function and has been implicated in various diseases; however, its role in reproduction is largely unknown.
Methods: In a previous study in our laboratory, using the STAPUT separation technique based on a gravity sedimentation followed by immunoprecipitation with SUMO antibody and mass spectrometry analysis, multiple SUMO targets were identified in meiotic spermatocytes and round spermatids. The identified targets of sumoylation included proteins involved in regulation of transcription, metabolism and stress response. Several specific targets with an important role in germ cells were chosen for further characterization.
Results: Co−Immunoprecipitation analysis confirmed sumoylation of CDC2 and CDC5l, the large subunit of RNA Polymerase II, Piwil2, MDC1 and several other proteins with an important role in regulating spermatogenesis.
Conclusion: Bioinformatic analysis revealed the presence of one or several consensus sequences for sumoylation in the majority of the studied targets.

Monday, April 7, 2014
11:00 a.m. - 12:30 p.m.

Poster Session II*
**Not CME Accredited*
Location: Venetian

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THE TRANSCRIPTION FACTOR SOX9 IS A NOVEL REGULATOR OF STEROIDOGENIC GENES EXPRESSION IN MA−10 LEYDIG CELLS

David Landry, BSc and Luc J. Martin, PhD
Université de Moncton
(Presented By: David Landry, BSc)

Introduction and Objectives: Sox genes encode a family of transcription factors characterized by a HMG box, which can bind and bend DNA through the consensus sequence (A/T)(A/T)CAA(A/T)G. Two members, Sry and Sox9, play important roles in male sex determination and differentiation in mammals. Leydig cells are essential for testosterone production in the testis. In these cells, the StAR protein allows cholesterol to enter the mitochondria and be converted to pregnenolone by the first steroidogenic enzyme Cyp11a1. Of the 20 Sox family members identified in vertebrates, several are expressed in gonads, including adult Leydig cells. Sox9 is expressed in steroidogenic cell lines, including MA−10 and R2C Leydig and Y1 adrenal cells. Interestingly, potential DNA regulatory elements for Sox members are present in promoter regions of steroidogenic genes, supporting that Sox9 might be involved in the regulation of steroidogenesis in Leydig cells. Our objective was to determine whether Sox9 regulates StAR and Cyp11a1 in Leydig cells and to better define its mechanism of action.
Methods: Mouse MA−10 Leydig cells were used in transfection and were harvested for total protein and total mRNA extractions. Protein quantifications were done by Western blot, whereas mRNA levels were determined by qPCR. Characterizations of Sox−dependent promoter activities of steroidogenic genes were done by transient transfections of MA−10 cells with StAR or Cyp11a1 promoter constructs and electrophoretic mobility shift assays (EMSA).
Results: Multiple potential Sox−dependent regulatory elements have been found in −1kb promoter regions for StAR and Cyp11a1, and these promoter constructs were activated 3 and 14 folds, respectively, by Sox9. Interestingly, PKA−dependent phosphorylation of Sox9 consistently reduced its transcriptional activity, as shown using transfection of a constitutively active PKA expression plasmid or 8Bromo−cAMP stimulations. Using 5' progressive deletion constructs for StAR (−843, −680, −515, −355, −72 bp) and Cyp11a1 (−888, −633, −427, −262 bp) promoters, regions important for Sox9−dependent activations were located between −680 and −515 bp for StAR and −88 and −633 bp for Cyp11a1.
Conclusion: Thus, our data identify Sox9 as a new regulator of steroidogenic genes expressions in Leydig cells. Future work will focus on post−translational modifications and protein−protein interactions involved in modulation of the transcriptional activity of Sox9 in Leydig cells.

