SPEAKER ABSTRACTS

SATURDAY, APRIL 18, 2015
6:50 p.m. – 7:45 p.m.

EMIL STEINBERGER MEMORIAL LECTURE
GENOMIC IMPRINTING: MECHANISMS AND ENVIRONMENTAL SENSITIVITY
Marissa S. Bartolomei, PhD
University of Pennsylvania School of Medicine, Philadelphia, PA, USA

Imprinted genes are expressed from a single parental allele and most reside in clusters that are located throughout the mammalian genome. The clusters typically contain an imprinting control region (ICR), which harbors allele-specific methylation and governs the imprinting of the entire domain. Although most imprinted clusters use long non-coding RNAs to regulate imprinted gene expression, a few are regulated by CTCF and allele-specific insulator function. One such cluster harbors the H19 and Igf2 imprinted genes, and is controlled by an ICR that contains multiple CTCF binding sites. Gain of maternal methylation and loss of paternal hypermethylation of the H19/Igf2 ICR are associated with the human growth disorders Beckwith-Wiedemann Syndrome and Silver-Russell Syndrome, respectively. Using gene targeting and genome editing, we have generated ES cells, iPSC cell lines and mice to study the mechanisms of imprinting for these imprinted loci and to model the epigenetic mutations in human syndromes. We have also developed SNV-FISH to study the dynamics of allele-specific gene expression at the single cell level in cell lines and tissues with loss of imprinting. We have additionally studied imprinting in animal models of Assisted Reproductive Technologies (ART) and endocrine disrupting chemical exposures (EDCs). Both ART and EDCs are associated with increased loss of imprinting of various genes and with DNA methylation aberrations. The effects are especially pronounced in placenta, where regulation of multiple genes and DNA methylation of repetitive elements are perturbed, and where morphological changes are evident.

This work is supported by the NIH (GM051279, HD068157, ES023284, EB019767).

SUNDAY, APRIL 19, 2015
8:00 a.m. – 9:00 a.m.

AUA LECTURE
REPRODUCTIVE GENETICS AND THE AGING MALE
Paul Turek, MD, FACS, FRSM
The Turek Clinic

Objectives: To provide an overview of the known effects of advanced paternal age on sperm genetics and birth defects and disease in offspring


Results: Advanced paternal age (>40 years) is linked to changes in quality control associated with spermatogenesis and meiosis. The consequences of these changes include well-delineated anomalies in sperm chromosomes, both numerical and structural, and increased sperm DNA damage (3%/year) and single gene mutations (10-fold). Associated increases in offspring-related events have also been described, including miscarriage (2-fold) and fetal loss (2-fold). An increase in rare, single gene disorders (relative risk 1.3 to 12) and congenital anomalies (20%) among offspring also exists. Recent research suggests that rates of autism, schizophrenia and other forms of “psychiatric morbidity” increase in offspring (relative risk 1.5 to 5.7) with advanced paternal age, and genetic mechanisms related to sperm quality control in the single gene mutation pathway have been implicated as root causes.

Conclusions: Advanced paternal age is associated with increased genetic risk to offspring. However, the precise age at which risk develops and the magnitude of the risk are poorly defined or may have graduated effects. Currently, there are no screening or diagnostic panels that target disorders associated with advanced paternal age. Concerned couples or care providers should pursue or recommend genetic counseling and prenatal testing regarding specific disorders.

Funding: None

SUNDAY, APRIL 19, 2015
9:15 a.m. – 10:45 a.m.

SYMPOSIUM I – Basic Science – Stem Cells, Niches and Reproductive Function
HETEROGENEITY OF SPERMATOLOGIN AND ITS RELATIONSHIP TO THE STEM CELL NICHE – A REVIEW
Makoto Nagano, PhD, DVM
McGill University and The Research Institute of McGill University Health Centre, Montreal, Quebec, Canada

It has been just over 20 years since spermatogonial transplantation was reported. It functionally detects spermatogonial stem cells and is simple in its technical concept. However, this technique also revolutionized our approach to looking at the foundation of spermatogenesis. The heterogeneity in spermatogenesis appeared to be established by 1901 while the concept of spermatogonial stem cells emerged by 1885. Since then, a large amount of information has been accumulated and survived over time in the study of spermatogonia and spermatogenesis in general, mainly based on morphological observations. Spermatogonial transplantation, reported in 1994, was novel in part because it freed us from this restriction, cell morphology, and allowed us to ignore it to study spermatogonial stem cells, even though it detects only one type of male germ cells. While the research based on this technique opened new avenues in research of spermatogenesis, it also introduced confusions and conflicts. Here, I intend to discuss what we know about the heterogeneity of spermatogonia and how it could relate to the function of their environment, and potentially, the regulation of stem cell fate.
SPEAKER ABSTRACTS

SUNDAY, APRIL 19, 2015
9:15 a.m. – 10:45 a.m.

SYMPOSIUM I – Basic Science – Stem Cells, Niches and Reproductive Function

MICRORNA REGULATION OF SPERMATOGENIAL DEVELOPMENT*
Wei Yan, MD, PhD
University of Nevada School of Medicine, Reno, NV

Objectives: One miRNA can target many mRNAs and one mRNA is usually regulated by many miRNAs. This “one-to-many” relationship may reflect a “fail-safe” mechanism, implicating the importance of miRNA-mediated posttranscriptional regulation of gene expression. When a particular miRNA fails to function, other miRNAs that either belong to the same family (i.e., sharing the same seed sequences), or target the same sets of mRNAs would compensate. This may explain why almost all knockout mice deficient in single miRNAs show no spontaneous abnormalities during development or adulthood. Although many miRNAs have been identified to be expressed in spermatogonia, physiological evidence of the necessity of the spermatogonial miRNAs remains lacking. We recently generated a mouse line lacking two miRNA clusters (called herein dKO mice), which encode five miRNAs (miR-449a, 449b, 449c, 34b, and 34c) that share the same “seed sequence”. These mice display defects in all three phases of spermatogenesis. In particular, both prospermatogonia and spermatogonia display abnormalities in the dKO males, suggesting a critical role of these miRNAs in the control of spermatogonial development.

Methods: dKO mice were generated by crossing the miR-34b/c KO mice with miR-449a/b/c KO mice. Histological and molecular analyses were conducted using testes dissected from dKO and control (WT or single KO) male mice.

Results: ~50% of the dKO males died at P7. Those survived beyond P7 tended to be smaller in body size before week 7, and similar body weight could be achieved after week 8. However, all male dKO mice were sterile. Abnormalities in all three phases of spermatogenesis were observed in adult dKO males. The testicular histology in dKO males was characterized by very thin seminiferous epithelia consisting of almost all types of spermatogenic cells, but much less in number at the age of 10 weeks. Epididymal sperm counts were drastically reduced and the dKO sperm were all deformed. During the initial cycle of spermatogenesis, the number of spermatogenic cells and the thickness of the seminiferous epithelia were comparable between dKO and WT testes. However, the dKO testes displayed much thinner seminiferous epithelia from the second round of spermatogenesis onward. No enhanced germ cell apoptosis was observed in either developing and adult dKO testes, and the number of both spermatocyte and spermatids was drastically reduced, whereas the number of differentiated spermatogonia appeared to be increased in dKO seminiferous tubules compared to controls.

Conclusion: Our data suggest an enhanced spermatogonial differentiation and a reduced meiotic entry when spermatogonia lack the five miRNAs.

*Funding for this study was provided by the NIH (HD060858)
markers not only help identify genes involved in spermatogenesis, but also are being used to elucidate molecular and cellular mechanisms of testicular toxicity. A revolution in toxicity testing is underway, based on high throughput in vitro screening and computational toxicology approaches. It derives from public concern about Endocrine Disrupting Chemicals which, in turn, emerged from reports suggesting that sperm counts may be declining in the Western world, and testicular cancer increasing, potentially due to chemicals in our environment. In this context, basic knowledge about androgen receptor function has been applied to in vitro screens for chemicals that interfere with androgen action and thereby impact male reproductive development. The fungicide vinclozolin provides an illustrative example. Thus, as emphasis shifts from adult to fetal sensitivities, knowledge of how early life exposures may impact life-long fertility and cancer risk is expanding. Coming full circle, evidence for epigenetic reprogramming during development is extending concerns to risks of obesity, metabolic syndrome and cardio-vascular health in adult men, with ever broader public policy implications.

SUNDAY, APRIL 19, 2015
4:45 p.m. – 5:30 p.m.

LECTURE II
FERTILITY PRESERVATION IN THE MALE: A NEW CLINICAL PARADIGM
Robert E. Brannigan, MD
Professor, Department of Urology
Northwestern University, Feinberg School of Medicine

Cancer is a highly prevalent condition, as is evident in the fact that a male has an approximately 50% chance of developing cancer during the course of his lifetime. Over the past century, the focus of cancer therapy has predominately been on devising therapeutic regimens to increase patient survival. Over time, however, a paradigm shift has occurred. While the overriding aim of therapy is still survival, oncologic treatment planning has increasingly incorporated strategies to mitigate the co-morbidities that often result from cancer therapy. Primary among these co-morbidities is male factor infertility. In this presentation, I will overview the impact of cancer and cancer therapies on male reproductive health, and I will discuss fertility preservation options for males with cancer. Three unique patient groups will specifically be considered: the prepubertal male, the adolescent male, and the adult male.

MONDAY, APRIL 20, 2015
8:00 a.m. – 9:00 a.m.

WOMEN IN ANDROLOGY LECTURE
FATHER’S LASTING INFLUENCE: PATERNAL ENVIRONMENT AND THE HEALTH OF HIS FUTURE GENERATIONS
Janice L. Bailey, PhD1, Clotilde Maurice, PhD1, Serge McGraw, PhD2, Romain Lambrot, PhD2, Nancy Côté1, Arnaud Droit, PhD1, Jacquetta M. Trasler, MD, PhD2 and Sarah Kimmins, PhD2
1 Université Laval, Québec, Canada; 2McGill University, Montreal, Canada

Although chemicals are a part of our daily lives, society is concerned about the impact of environmental contaminants on human health. Our team is particularly interested in Persistent Organic Pollutants (POPs), including PCBs and some insecticides, which bioaccumulate and biomagnify in the food chain. POPs are restricted according to the Stockholm Convention; however, exemptions are made for DDT, which is widely used in malaria-endemic areas. Therefore, POPs remain ubiquitous worldwide and particularly accumulate in the Arctic. We and others have demonstrated that reproductive parameters are compromised in men who are exposed to high levels of POPs, although there is little evidence that fertility is affected. More recently, the “developmental origins of adult disease” model has been proposed to explain how exposure of the developing fetus to environmental stressors while still in the womb predisposes it to dysfunction later in life. Indeed, we have shown that prenatal exposure to a mixture of POPs, designed to mimic that which contaminates the Arctic, induces male reproductive dysfunction and subfertility in the rat. We further hypothesized that prenatal exposure to this environmentally-relevant POPs mixture affects the paternal sperm epigenome and the health of his unexposed future generations. We used the F1 prenatally-exposed sires to produce F2, F3 and F4 generations. Numerous health defects, including male subfertility were observed throughout these three generations. Moreover, >200 genes in the sperm from the F1 prenatally-exposed males were differentially methylated, including those in regions involved in embryo development. Pathologies linked to the altered genes include metabolic, neurological, psychological and cardiovascular disorders, cancers and reproductive dysfunction, many of which are either documented to be related to prenatal exposure to organochlorines or are of concern to Arctic populations.

In conclusion, our study indicates that prenatal paternal exposure to environmentally-relevant POPs induces reproductive dysfunction as well as developmental pathologies in his offspring and future generations, possibly due to epimutations in his sperm DNA.

Financed by FQRNT & CIHR.
of diabetics are on oral hypoglycemics with only 12% of diabetics being on medication to increase insulin sensitivity or insulin secretion. 58% of type 2 diabetes is not universally insulin replacement but either oral or insulin. The highly prevalent condition, type II diabetes, yet, the treatment of hypogonadism will require treatment for life, not unlike another highly prevalent condition, type II diabetes. Barring major lifestyle changes, men diagnosed with hypogonadism will require replacement therapy for life, not unlike another highly prevalent condition, type II diabetes. 

**Objective:** IMPRESS (Investigation for Maximal Peyronie’s Reduction Efficacy and Safety Studies) I and II investigated the clinical efficacy and safety of collagenase clostridium histolyticum (CCH) (Xiaflex®, Auxilium, Malvern, PA) intralesional injections in subjects with PD. Co-primary outcomes in 2 identical phase 3 randomized, double-blind, placebo-controlled studies included % change in the penile curvature and change in PD questionnaire symptom bother score from baseline to week 52.

**Materials and Methods:** IMPRESS I and II examined CCH intralesional injections in 417 and 415 subjects through a maximum of four treatment cycles (2 injections per cycle); each separated by 6 weeks. Men were stratified by baseline penile curvature (30-60 vs. 61-90 degrees) and randomized to CCH or placebo 2:1.

**Results:** IMPRESS I and II data analysis revealed that PD men treated with CCH showed a mean 34% improvement in penile curvature (mean ±SD −17 ± 14.8 degree change per subject) compared to a 18.2% improvement (−9.3 ± 13.6 degree change per subject) for placebo (p=0.0037). The mean change in PD bother score was also significantly improved in treated vs. placebo (−2.8 ± 3.8 vs 1.8 ± 3.5, p=0.0037). Three serious adverse effects (corporal rupture) were successfully surgically repaired.

**Conclusion:** IMPRESS I and II support the clinical efficacy and safety of CCH for the physical and psychological aspects of PD. Clinicians now have an effective FDA-approved intralesional product to offer their patients suffering with PD.

CC was originally designed for female infertility. Approved by the FDA in 1967 it has since become an inexpensive generic drug. It is a selective estrogen receptor modulator comprised of a 38%/62% racemic mixture of cis and trans isomers, zuclofen and clofeno. It has antagonistic effects on the estrogen receptors in the hypothalamus and the pituitary thereby increasing endogenous gonadotropin releasing hormones, LH and FSH. It ability to increase LH in men was recognized as early as 1968. As with all SERMs organ estrogen agonistic effects are also possible. In a study aimed at using CC challenges to diagnose hypogonadotropic hypogonadism, Paulsen demonstrated significant increases in LH, FSH and T in normal older men taking 50 mgs of CC twice a day. Sherins et al were able show the CC was able to block the LH and FSH suppression that occurs with exogenous T and estrogen administration, thus demonstrating estrogen agonistic effects are also possible. In a study aimed at using CC challenges to diagnose hypogonadotropic hypogonadism, Paulsen demonstrated significant increases in LH, FSH and T in normal older men taking 50 mgs of CC twice a day. Sherins et al were able show the CC was able to block the LH and FSH suppression that occurs with exogenous T and estrogen administration, thus demonstrating that estrogen was the primary inhibitory hormone on GnRH, LH and FSH. Over the ensuing decades, CC was used to increase male fertility with mixed results. Though an increase in T and estrogen level was consistently demonstrated, no consistent effect of seminal parameters or pregnancy rates was observed. A 6 month multicenter international placebo controlled study cast doubts on the efficacy of CC on idiopathic male infertility. It is important to realize that in the international study, the infertile population was eugonal and the mean baseline T levels of 481ng/dl. Well controlled studies in the hypogonadal infertile male are lacking, despite the high prevalence of secondary hypogonadism in this group of men.

Tenover et al looked at an 8 week trial of CC (50mg BID) in 5 healthy older and 5 young eugonadal men (mean age 73 vs 29; mean baseline T 518 vs 498) and demonstrated that older men both increased LH and FSH and T and E-2. Though levels of T were significantly lower in the older group, the levels achieved in both groups were at least comparable to those achieved with many current day exogenous treatments. Lim observed normalization of testosterone levels in 5 hypogonadal uremic men with uniform increase in libido, sexual potency, and a general sense of well-being using 100 mgs of CC daily for as long as 12 months. The normalization of T continued for 4-5 months after discontinuation of therapy. Plasma estradiol levels were elevated at baseline and did not change significantly from baseline. Guay el al challenged 21 older men with erectile dysfunction and secondary hypogonadism with 50 mg CC bid for 7 days and normalized their T, demonstrating that at that at least in the short term, the concept of testosterone restoration was possible in older men. He then expanded the concept with an eight week double blind placebo controlled crossover study in older men (mean age 62) with secondary hypogonadism and erectile dysfunction (documented with nocturnal penile tumescence scan (NPT)). Again, normalization of
serum testosterone was seen but no improvement was seen in NPT or sexual function questionnaires in the group as a whole. When the study population was split between younger and older groups (mean age 53 and 66 respectively) in a post hoc analysis, not surprisingly, the differences between the treatment groups with the sexual function questionnaires and NPT testing achieved statistical significance. The older men were more likely to have “end organ” disease refractory to hormonal manipulation. This was the first demonstration that CC could not only normalize T levels in SHGD but result in symptomatic improvement. Guay then began treating men in his practice with SHGD with CC (50 mgs) three times a week. He reported an observational series of 173 men with ED and SHGD treated for 4 months. The diagnosis of ED was based on self-report and not a validated questionnaire and a placebo arm was lacking. The outcome was measured as “responder” to treatment (successful intercourse >75% of the time), partial responder (successful intercourse 50-75% of the time) and non-responder. As in his previous studies, LH, FSH and free testosterone levels increased. Sexual function improved in 75% and did not change in 25%. Age and vascular co-morbidities negatively affected the response rates.

Taylor et al in an observational study compared the biochemical efficacy of CC to exogenous gel treatment (TRT) in 104 men (65 CC vs 39 on TRT). The groups were not strictly identical but demonstrated comparable increases in testosterone with a 182 $ monthly savings in the CC group. PSA levels and HCT did not significantly change in follow up (23 months). Moskovic demonstrated an excellent chemical response in a younger cohort of 29 men (mean age 44) followed for three years on CC 25 mgs every other day. In addition, despite an unusually high percentage of men with altered bone mineral density at baseline (75%) BMD normalized at one year in 25%. No improvement in BMD was observed after the first year. Though estradiol increased significantly no gynecomastia or breast tenderness occurred. No side effects were reported.

The efficacy of CC in relieving the symptoms of hypogonadism is often anecdotally reported as being inferior to exogenous therapy without the support of randomized double blind studies. Katz et al retrospectively looked at symptom relief with CC (25mg every other day) in 86 young (mean age 29) hypogonadal men, most of whom were presenting for infertility (57%) over a 4 year period at a Sloan Kettering andrology practice. The men were followed for a mean of 19 months. Surprisingly the median number of positive baseline responses on the androgen deficiency in aging males (ADAM) questionnaire was 5 that dropped to 2. These “generally very healthy” young men started at a mean T level of 192 ng/dl and increased their T to 485 (despite a target treatment level of 550 ng/dl). The symptoms that showed significant increases included “decreased libido, lack of energy, decreased life enjoyment, sad/grumpy, decreased sports performance”. The lack of a placebo arm weakens the strength of the study. Further support of the efficacy of CC in relieving hypogonadal symptoms comes from a retrospectively gathered observational comparative study from Baylor by Ramasamy. In examining the effect of CC vs replacement therapy on hypogonadal symptoms, no significant differences were seen in between T injections, T gels or CC. T levels were highest with injections (1104 ng/dl) vs CC (504 ng/dl) or the gels (412 ng/dl). The lack of a difference in symptom relief supports the concept that symptom relief may be tied to a threshold level that is achieved with TRES and TREP. Unfortunately pre-treatment quantitative ADAM scores (QADAM) were not reported and the QADAM has not been fully psychometrically validated.

Recently there has been interest in the trans isomer of CC (EC). Distinct differential pharmacokinetics of the two isomers have been demonstrated. Though the Cmax, and Tmax were comparable, the AUC for the isomers was dramatically different after a single dose administration of 50 mgs of CC in women with polycystic ovaries. At 456 hours, ZC was detected in 9/9 patients vs 1/9 for EC. The half-life of EC is 7-8 hrs. EC was evaluated in an early proof of concept randomized, open-label, fixed dose, active-control (7EC and 5 exogeous gel), two-center phase IIB study in 12 men with secondary hypogonadism treated previously with topical testosterone. After T discontinuation of exogenous T, T levels in both groups averaged 165ng/dl. After treatment T levels increased in both groups to over 540 ng/dl but decreased to baseline after cessation of treatment suggesting that the hypothalamic testicular axis reverts to its pretreatment state and continued therapy is necessitated. Whereas sperm counts were increased in all men on EC at 6 months only 2 of 5 of gel patients increased their sperm concentrations to over 20 million/cc. GTP increased only in the EC arm. In follow up clinical trials, safety and clinical efficacy were comparable to a gel preparation while preserving sperm counts. Sperm counts were decreased in the men treated with gels. Side effects were comparable to CC. The most significant adverse events were hot flushes (10%), visual disturbances headaches, nausea and vomiting. Aside from the hot flushes, all events occurred in less than 5% of the study population. The ease of use, low side effect profile, therapeutic efficacy and preservation of fertility, make EC, if approved, an attractive therapeutic alternative to standard TREP.

Summary

In view of the EMAS studies secondary hypogonadism accounts for over 85% of late onset hypogonadism. Ample evidence exists for a deficiency in GTP stimulation with the older men and the ability of the testes to respond to increased GTP production. We currently have generic medications that accomplish an increase in GTP and normalization of serum testosterone with a favorable side effect profile. Though shown to be efficacious and well tolerated in a number of trials, restorative strategies are not FDA approved and caution must be advised in their off label use. Hopefully future trials will be undertaken to establish the long term efficacy and safety these restorative therapies. Early clinical trials of the compound enclomiphene are encouraging and hopefully will lead to a change in paradigm from TREP to TRES.
DIVERSITY LECTURE
CURRENT TRENDS IN THE TREATMENT OF INFERTILITY IN MEN WITH SPINAL CORD INJURY
Nancy L. Brackett, PhD, HCLD
University of Miami Miller School of Medicine, Miami, FL

Objectives: This presentation will describe causes and treatments for infertility in men with spinal cord injury (SCI).

Methods: Evidence from published medical literature, as well as recent findings from my laboratory, will be presented.

Results: Most men with SCI are infertile due to a combination of erectile dysfunction, ejaculatory dysfunction, and semen abnormalities. Treatments that are effective for erectile dysfunction in the general population are also effective in men with SCI. In anejaculatory patients who wish to father children, semen retrieval is necessary. Penile vibratory stimulation (PVS) is recommended as the first line of treatment. Patients who fail PVS can be referred for electroejaculation. Surgical sperm retrieval should be considered as a last resort when other methods fail. Most men with SCI have a unique semen profile characterized by normal sperm concentration but abnormally low sperm motility. This problem does not seem to be due to lifestyle factors, such as elevated scrotal temperature from sitting in a wheelchair, infrequency of ejaculation, or methods of bladder management. Longitudinal and cross-sectional studies have found no progressive decline in semen quality with the ensuing years post-injury. Endocrinopathies may be present, but are not the sole cause of abnormal semen quality in these men. Evidence suggests that a toxic seminal plasma environment contributes to the problem. For example, the inflammasome may play a role in elevating semen cytokines in these men. Inactivating components of the inflammasome, and/or neutralizing elevated semen cytokines, improves sperm motility in this severely affected group of patients.

Conclusions: Despite sexual dysfunction and semen abnormalities, biologic fatherhood is possible men with SCI. Semen retrieval by PVS or electroejaculation often results in sufficient numbers of total motile sperm to consider intrauterine insemination or even intravaginal insemination in couples with SCI male partners. Surgical sperm retrieval combined with in vitro fertilization and intracytoplasmic sperm injection should be considered as a last resort for assisted conception. New therapies for abnormal semen quality in this population are on the horizon.
The definition of rejuvenation is the process of making someone look or feel younger. Aging men may not be hormone deficient and replacement in not indicated. Human growth hormone and androgens including dehydroepiandrosterone (DHEA) and testosterone (T) have been used in the rejuvenation community because these hormones decline with aging in men. Data on recombinant human growth hormone do not show consistent efficacy or evidence to justify its use in older men in the absence of demonstrated low GH levels and a definable congenital or acquired causal mechanism. DHEA is a weak androgen and supplements of DHEA are not useful in producing androgenic effects except as a precursor of T. Recent studies in several countries/continent showed that serum T levels decrease with age in older men over the age 60 to 70 years. The decrease in serum T levels is associated with symptoms of male hormone deficiency in about 2 to 6 % men between 40 to 70 years. Co-morbidities such as obesity, metabolic syndrome, diabetes and chronic illness are associated with low T levels. Life style modification for obesity and treatment of the medical conditions may sometimes restore serum T to the adult malerange. There is controversy regarding T replacement in older men because of relative benefits versus risks. The benefits of T substitution shown in small placebo controlled randomized clinical trials include: increased sexual desire/activity, bone mass, lean mass, improved erectile dysfunction, and decreased fat mass. Studies have not conclusively shown that T substitution improves quality of life, mood, vitality or cognition in older men. A larger scale randomized controlled trial using transdermal T in elderly men with unequivocal low T levels (The T Trial) has been completed but awaiting data analyses. The long term effects of T substitution in older men with low T levels on risks of prostate cancer or cardiovascular disease are unknown and controversial. Given the unknown long term adverse effects of T and inconclusive data on subjective improvement in symptoms, there is no justification of administration of T treatment for older men without consistently low T levels and symptoms consistent with a hypogonadal syndrome. In symptomatic men with persistently low T levels despite management of concurrent comorbidities, T substitution to achieve serum T level in the mid adult male range may be used. Under all circumstances analysis/consideration of benefit/risk of intervention is prudent.

There is very little quality data on using the high doses of testosterone and growth hormone, commonly used in the rejuvenation clinics or by athletes. Most available data is based on the use of growth hormone in men and women with documented hypopituitarism and/or growth hormone deficiency. In these situations, growth hormone has a role, but even there, the safety data emphasizes the importance of careful monitoring to prevent diabetes mellitus or acromegalic changes. Testosterone clearly has a role in hypogonadal men, but high doses or its use in eugonadal men will result in suppression of the hypothalamic-pituitary-gonadal axis. In supraphysiological doses, the increases in muscle mass could be disadvantageous. The use of DHEA will also be discussed.
A common treatment of prostate cancer is prostate removal or radical prostatectomy (RP). This cancer operation is associated with high rates of erectile dysfunction (ED). There has been major advances in the treatment of ED with the advent of phosphodiesterase type 5 (PDE5) inhibitors. However, post-RP ED is refractory to oral PDE5 inhibitor therapy. Thus new disease specific therapies are necessary. The CME goals of this lecture are to discuss the evidence for penile rehabilitation using PDE5 inhibitors, discuss novel disease-specific molecular based therapies including pharmacotherapies, small molecular inhibitors, and stem cell based therapies.

When it comes to nuclear integrity, spermatozoa and oocytes are not equal. The oocyte has DNA repair activities throughout its life while the mature spermatozoa is devoid of it. Therefore, immediately post-fertilization one of the most important tasks of the oocyte is to repair sperm nuclear DNA damage prior to initiating S-phase of the first mitotic division. If there are too many sperm DNA lesions, the repair mechanisms activated in the oocyte may be overwhelmed, leading to errors during the ensuing DNA replication. The most frequent sperm DNA alteration in natural as well as in assisted reproduction involves an oxidative attack, leading to the formation of oxidized base adducts such as 8-hydroxy-2'-deoxyguanosine. Via the use of mouse transgenic strains in which a post-testicular oxidative stress is established we show that not all the mouse sperm chromosomes are equally susceptible to post-testicular DNA oxidation. Some chromosomes or and chromosomal regions were found most sensitive to oxidative attack most likely because of their position in the compacted sperm nucleus. Bioinformatics analyses of sperm chromosomal regions and sequences susceptible to oxidation indicated noticeable sequence features.

Some chromosomes and regions were found most sensitive to oxidative attack most likely because of their position in the compacted sperm nucleus. Bioinformatics analyses of sperm chromosomal regions and sequences susceptible to oxidation indicated noticeable sequence features. Gamete nuclear integrity is the most critical parameter to ensure complete and harmonious embryonic development. It is also a major contributor to the health and wellbeing of the progeny. Oxidative alterations of the paternal genome that are not efficiently repaired by the oocyte or not compensated by the maternal genome emphasize the risk of trans-generational effects that may be increased when fertilization involves such spermatozoa showing high oxidative DNA damage. It also may partly explain the low efficacy of assisted reproductive technologies.

Funding provided by the French Ministère de l’Enseignement Supérieur et de la Recherche (MESR), the French Centre National de la Recherche Scientifique (CNRS) and the Institut National de la Santé et de la Recherche Médicale (INSERM).

The risk/benefit ratio associated with testosterone therapy may vary with the context of use. In young men with classical hypogonadism due to known diseases of the testis, pituitary and hypothalamus, testosterone improves many symptoms of hypogonadism and is associated with a low frequency of adverse events. In contrast, neither the risks nor the benefits of testosterone therapy have been demonstrated in older men with age-related decline in testosterone concentration or in elderly men with frailty, mobility limitations, or critical illness.

A number of pre-clinical and clinical observations support the biological plausibility of a relation between testosterone administration and risk of cardiovascular events. T increases hemoglobin and hematocrit. Testosterone administration reduces plasma HDL cholesterol, induces platelet aggregation, is associated with sodium and water retention. In pre-clinical models, and has been shown to promote smooth muscle proliferation and increased VCAM expression. Testosterone also has been shown to have potentially beneficial effects on the cardiovascular system. Testosterone acts as a vasodilator by inhibition of L-type calcium channels, resulting in increased coronary and penile blood flow. Testosterone consistently decreases whole body, subcutaneous and intra-abdominal fat. Testosterone has been reported to reduce vascular reactivity and improve endothelial function. Testosterone has been reported to increase both prothrombotic as well as anti-thrombotic factors.

A small number of epidemiologic studies have reported an inverse relationship between testosterone concentrations and common carotid artery intima-media thickness. The relationship of testosterone and coronary artery disease and CV events is inconsistent. A meta-analysis of 11 randomized trials by Araujo et al found that in aggregate, lower testosterone levels were associated with higher risk of all-cause mortality, especially cardiovascular mortality.

There are no published or ongoing trials that were specifically designed or powered to determine the effects of testosterone therapy on CV events. Retrospective analyses of data have yielded conflicting results. Some studies have reported an association of testosterone therapy and increased cardiovascular events, while others have not. TOM trial was a placebo-controlled randomized trial of testosterone in older men with mobility limitation. The trial was stopped early due to the increased frequency of cardiovascular-related events in men assigned to testosterone arm than in placebo arm. The divergence in cardiovascular events continued throughout the 6-month intervention duration, and abated after treatment was discontinued. The cardiovascular-events were small in number overall, and variable in their severity and significance. The TOM trial was not designed for cardiovascular events. Accordingly, cardiovascular events were not pre-specified, were not collected in a standardized manner, and cardiovascular events were not adjudicated prospectively.

Therefore, the available data from randomized clinical trials are insufficient to establish a causal link between testosterone therapy and cardiovascular events. To more clearly understand the risks of testosterone therapy in older men with low testosterone levels, we need larger randomized trials and prospective mechanisms for tracking of adverse events, particularly cardiovascular events and major cardiovascular events.
Objective: To examine the role of Testosterone (T) deficiency in contributing to cardiac relaxation abnormality (diastolic dysfunction) and if this dysfunction is potentially reversible by T replacement.

Methods and Results: Castrated and sham-operated male rats were randomized to placebo versus T replacement for 6 weeks. After 3 weeks of castration, invasive hemodynamic measurements revealed that rats developed evidence of diastolic dysfunction as evidenced by slower dp/dtmin (rate of relaxation) and longer tau (time constant of relaxation). There was also a non-significant trend towards increase in left ventricular end-diastolic pressure. All these variables were reversed and returned to normal range in the T-treated rats but remained abnormal in the placebo-treated group.

Conclusions: T deficiency contributes to left ventricular diastolic dysfunction while T replacement completely reverses these abnormalities. An ongoing clinical pilot study in hypogonadal men will help determine if clinical T effects on diastolic function are worthy of a larger randomized clinical trial.

In view of evidence showing that low endogenous bioavailable testosterone associates with higher rates of cardiovascular mortality, some have hypothesized that testosterone replacement therapy might have beneficial effects. In contrast to initial expectations, testosterone replacement therapy has been shown to increase the risk of cardiac morbidity. Interpretation of such paradoxical findings would be facilitated by a thorough understanding of the effects of testosterone on the heart, but information on this topic is still limited. In mice, we have compared two mouse strains harboring genetic differences that are restricted to only chrY, i.e. male C57BL/6J mice and their consomic C57.YA counterparts (i.e. the same mouse strain where the original chrY has been replaced by that from the A/J strain). We found that castration decreased the expression of more cardiac genes in male C57.YA mice at different times of the day. We found that strain-specific differences were found for only 3 circadian genes, i.e. Dbp, Tef and Hlf, which are all functionally redundant members of the PARbZip family. Previous studies in mice devoid of all three genes have shown that they collectively regulate left ventricular function. The effects of chrY variants on these three genes were seen at only one particular time point, i.e. 2PM, and were found only in intact (but not castrated) mice. Interestingly, we found that castration decreased the expression of contractility genes in hearts from C57.YA mice at 2 PM, but not at 10 AM, and did not affect the expression of these genes in C57BL/6J mice at either time, thus mirroring the strain-specific differences in circadian gene expression. The data indicate that, downstream of chromatin remodeling, differential regulation of circadian genes may constitute one of the mechanisms via which chrY genetic variants regulate the effects of testosterone on the heart.
INTERNATIONAL LECTURE

A PERSPECTIVE FROM DOWNUNDER: TGB SIGNALING IN TESTIS DEVELOPMENT AND SPERMATOGENESIS
Kate Lakoski Loveland, PhD
School of Clinical Sciences, Monash University and MIMR-PHI
Medical Research Institute, Clayton, Victoria Australia

A precise molecular dialogue between testicular somatic and germ cells is required for normal spermatogenesis. During fetal and postnatal life, this communication influences tightly controlled periods of germ cell reprogramming, proliferation and quiescence, migration and cell fate selection, events relevant to formation of testicular germ cell tumours (TGCTs) which are the most common solid tumour in young men. Transforming growth factor (TGF)-β-signaling pathway components exhibit gender-biased production in fetal gonads, with male gonads making activin A and female gonads making its potent inhibitor, follistatin. Through animal model studies, we and others discovered that activin beta A levels determine Sertoli and germ population cell size and maturation pace. Levels of key proteins such as Kit and Dmrt1 correlate with activin levels during development, providing clues to how activin impacts on male fertility. The restriction of activin signalling by the inhibitory inhibin alpha subunit is essential for normal Sertoli cell maturation, and the mechanisms by which Sertoli cells respond to activin alters as they develop. Exposure of juvenile mice to the phthalate DBP upregulates inhibin alpha subunit levels inappropriately, and this may be linked with reduced adult fertility. In humans, activin receptors present in Sertoli and germ cells indicate both are potential targets of activin and TGF-β ligands in normal and neoplastic adult testes. Exposure of the TCam-2 model human seminoma cell line to activin A, to the TGF-β ligand, BMP4, or to retinoic acid altered transcripts encoding activin receptors and Kit, and also impacted on cell survival or proliferation. Our recent analyses with testis tissue fragments from testicular cancer patients showed activin A reduced KIT mRNA and protein amounts in seminoma samples, reinforcing the understanding that regulated activin A bioactivity is important for male reproductive health. This talk will offer a framework for understanding how altered structure or function of TGF-β superfamily components, including inhibin alpha, NODAL, TGFBR3/betaglycan and BMP7, may be implicated in male infertility and in TGCTs.

SYMPOSIUM V: Novel Male Contraceptive Strategies

NA,K-ATPase 4 ISOFORM AS A TARGET FOR MALE CONTRACEPTION
Gladis Sánchez, MD, Jeff P. McDermott, PhD and Gustavo Blanco, MD, PhD
Department of Molecular and Integrative Physiology. University of Kansas Medical Center. Kansas City, KS 66160

Objectives: Na,K-ATPase comprises a group of plasma membrane enzymes that hydrolyze ATP to exchange cytoplasmic Na+ for extracellular K+. Na,K-ATPase is composed of different molecular forms of a catalytic and a glycosylated subunit. Na,K-ATPase 4 is a testis specific subunit, restricted to male germ cells and the sperm flagellum. We have explored the role of 4 in sperm physiology.

Methods: We used a pharmacological approach, taking advantage of the unique high sensitivity of 4 to the inhibitor ouabain and a genetic approach, deleting or over-expressing 4 in mice.

Results: Selective ouabain inhibition showed a role for 4 in sperm motility and hyperactivation. Knockout of Na,K-ATPase 4 in mice resulted in complete male, but not female infertility. Moreover, sperm from these mice were incapable of fertilizing oocytes in vitro, demonstrating the essential role that 4 plays in male fertility. Deletion of 4 resulted in severe loss of sperm motility and hypermotility. Sperm lacking 4 also exhibited several other defects, including a bend in the sperm flagellum and alterations in intracellular Na+, membrane potential and pH. Maintenance of these cell parameters represents the mechanisms by which 4 supports sperm flagellar beat, sperm capacitation and fertility. In contrast, 4 does not appear to be involved in sperm acrosomal reaction. On the other hand, overexpression of 4 in transgenic mice resulted in activation of total and multiple parameters of sperm motility, further demonstrating the crucial role that 4 plays in sperm flagellar beat.

Conclusion: Our results highlight the specificity of function of Na,K-ATPase 4 in sperm physiology and male fertility. This places Na,K-ATPase 4 as a potential marker for male fertility and an attractive candidate for male contraception.

[NIH grant U01HD080423].
**Retinoic Acid Receptor Antagonists for Male Contraception**

Debra J. Wolgemuth, PhD  
Columbia University Medical Center

**Objectives:** A combination of physiological, genetic, and pharmacological experimental approaches has revealed that all trans retinoic acid, a metabolite of vitamin A, and its receptor retinoic acid receptor alpha (RAR) are key players in the regulation of spermatogenesis. Following up on initial reports of ‘testicular toxicity’ in rats resulting from pan-RAR antagonists, we showed that in the mouse model, spermatogenesis could be inhibited by the antagonists, importantly in a reversible manner and without overt side effects. We now extend these observations to assess the lowest doses that can be effective, the length treatment that can be tolerated with restoration of fertility, the molecular targets of antagonist actions, and the possibility of developing RAR-specific antagonists.

**Methods:** Adult male mice were administered BMS189453/compound 9 or newly synthesized RAR-selective antagonists by oral gavage at various concentrations (mg/kg body weight) for varying lengths of dosing periods as described in our previous studies and expanded in the present study. Inhibition of fertility was assessed by testis weight, presence of sperm in the epididymis, mating studies, extensive morphological analysis of testicular histology, and ability of recovered males to sire offspring.

**Results:** Treatment with the pan-antagonist BMS189453/compound 9 at 5mg/kg/day for days was shown to inhibit spermatogenesis in a reversible manner. Extension of these regimens to lower doses and longer periods (as low as 1mg/kg/day for as long as 16 weeks) was shown to reversibly disrupt spermatogenesis. In fact, mating studies and morphological analyses suggested a more rapid recovery in mice subjected to these extended dosing periods. Recent preliminary studies suggest that modulation of various drug transporters may be involved in the shortened recovery time. Resulting progeny of recovered males after the 16-week dosing were healthy with normal fertility. An aspect of spermatogenesis that was most rapidly affected involved disruption of alignment of sperm at the lumen in stage VIII tubules and a failure of release to the lumen.

**Conclusions:** Our results suggest that spermatogenesis is exquisitely sensitive to disruption of retinoid signaling and that RAR-antagonists may represent new lead molecules in developing non-steroidal male contraceptives.

Funding provided by a grant from the NIH, U01 HD060479
The use of these agents in Men's Health.

Hormone has also been found to improve muscle mass and stimulate associated weight loss increasing well-being and muscle mass. Growth oxandrolone, an orally administered androgen, has been studied in HIV-syndrome, cardiovascular disease and diabetes mellitus. Indeed a associated with obesity and its related pathologies (i.e. metabolic syndrome, cardiovascular disease and diabetes mellitus). Indeed a role of testosterone in counteracting obesity is well known. Anavar, or oxandrolone, an orally administered androgen, has been studied in HIV-associated weight loss increasing well-being and muscle mass. Growth hormone has also been found to improve muscle mass and stimulate lipolysis in adipocytes. These agents thus represent novel adjuncts to the treatment of obesity in hypogonadal men with premature alopecia and joint pain.

A further role for TST lies in the treatment of hypogonadism associated with obesity and its related pathologies (i.e. metabolic syndrome, cardiovascular disease and diabetes mellitus). Indeed a role of testosterone in counteracting obesity is well known. Anavar, or oxandrolone, an orally administered androgen, has been studied in HIV-associated weight loss increasing well-being and muscle mass. Growth hormone has also been found to improve muscle mass and stimulate lipolysis in adipocytes. These agents thus represent novel adjuncts to the treatment of obesity in hypogonadal males.

This presentation will seek to provide a background and rationale for the use of these agents in Men’s Health.

Objectives: To review new and emerging concepts in role of sex steroids and their serum and paracrine carriers on cardiovascular risk. Method: Review of published literature and experimental data from own work.

Results: Cardiovascular disease and stroke remain major causes of mortality and morbidity in both sexes. Gender differences in prevalence of CAD and cardiovascular effects of estrogen manipulation in females with menopause and/or breast cancer have provided strong evidence that sex steroids modify risk factors for CAD in both sexes. Recent genome wide association studies (GWAS) and better insight into steroid signaling point out that it is the end organ and tissue specific action of sex steroids which impacts risks of CAD and not serum levels per se. Discovery of single nucleotide polymorphisms in SHBG, which change binding affinity of sex steroids to SHBG, illustrate role of genetic background in individual effects of sex steroids on vascular function. Estrogens (E) have a number of effects on cardiovascular function and disease. Among its many cardiovascular effects, E modulates vascular function, the inflammatory response, metabolism, insulin sensitivity, cardiac myocyte and stem cell survival, and the development of hypertrophy. Estrogen mediates these effects on the cardiovascular system by activation of estrogen receptors (ER), which can alter gene transcription in the nucleus or acutely activate kinase signaling in the cytosol. However non-genomic effects of E play dramatic role in fast changes in vascular tone and resistance. E2 regulates expression of nitric oxide synthase (NOS) and it is intriguing to notice that females have higher levels of NOS than males. However changes in intracellular amounts of tetrahydropterin (cofactor for NOS) can shift NOS activity from producing nitric oxide (NO) to generation of reactive oxygen species – thus same enzyme under control of E2 can either cause vasodilation or damage to endothelium and atherosclerosis. Northern Finland Birth Cohort study showed that men with higher SHBG levels had more favorable CAD profile independent of testosterone levels. Lower levels of testosterone and SHBG were associated with higher levels of triglycerides and insulin over six years in 24-45-year-old men participating the Cardiovascular Risk in Young Finns study.

Conclusions: Growing evidence from epidemiological, genetic, and translational studies indicate that cardiovascular risks depend on interplay between testosterone, estradiol, SHBG and local factors. Better understanding of complexity of sex steroids action on CAD is needed and will be critical for our field so we can provide effective and safe therapies for men with hypogonadism, infertility, and prostate cancer. Funding: Robert Dow Foundation
CONCURRENT SESSIONS – Oral Session I – Basic Science

Podium/Poster #1
MULTICELLULAR HUMAN TESTICULAR ORGANOID: A NOVEL IN VITRO GERM CELL AND TESTICULAR TOXICITY MODEL

Samuel Pendergraft, MS¹, Hooman Sadri-Ardekani, MD, PhD², Tanya Reid, BS³, Anthony Atala, MD² and Colin Bishop, PhD¹
¹(1) Molecular Medicine and Translational Science Graduate Program, Wake Forest University Health Sciences, Winston-Salem, NC; ²(1) Wake Forest Institute for Regenerative Medicine, Wake Forest School of Medicine, Winston-Salem, NC; ³(1) Wake Forest Institute for Regenerative Medicine, Wake Forest School of Medicine, Winston-Salem, NC; ²(1) Department of Urology, Wake Forest School of Medicine, Winston-Salem, NC; ³(1) Wake Forest Institute for Regenerative Medicine, Wake Forest School of Medicine, Winston-Salem, NC

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

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

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

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

Funding: AFIRM II, Award No. W81XWH–13–2–0052. NIH grant SU42RR006042 and Erret-Fisher Foundation grant GTS 3679.
and form corticosterone. Changes in the mRNA expression of Star and Cyp11a1 steroidogenic genes were observed to be increased in testes and adrenals of these mice, suggestive of adaptive changes. Moreover, expression of steroidogenic signaling receptors were divergent in the tissue of the Tspo cKO mice, with Lhcgr levels increased in testis, whereas adrenal Mc2r levels were unaffected.

**Conclusion:** The results of these genetic engineering experiments provide evidence that, in an in vivo setting, TSPO is required for reproductive development in male rats. We hypothesized that early-life paternal exposure to an environmentally-relevant OC mixture impairs expression of sperm proteins across multiple generations in a paternally-mediated manner.

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

**Results:** F1 males exposed to OC during early development had decreased sperm motility (P=0.04), lower daily sperm production per testis (P=0.006), and decreased epididymal sperm concentration (P=0.0001). Their F2 OC sons were subfertile (P=0.02) and their F3 OC grandsons had fewer pups per litter (P=0.0001). In generations F1, F2 and F3, respectively 7, 19 and 37 differentially expressed proteins were identified due to OC exposure of the F1 fathers.

**Conclusion:** Our results show that CX3CR1+ macrophages take up and process luminal DQ−OVA and CD11c+CD103+ DCs also contained processed DQ−OVA. Interestingly, imaging has revealed that CD103+ DCs are positioned at the base of the epithelium in close proximity to CX3CR1+ cells; however they are not observed interacting with the lumen, suggesting that luminal antigens are sampled by CX3CR1+ cells. This study, based on high-resolution imaging and flow cytometry (FC), assesses the ability of epididymal MPs (eMPs) to cross apical tight junctions and sample the luminal contents. Using CD11c−EYFP and CX3CR1−GFP reporter mice, we show that CD11c+ and CX3CR1+ cells are able to cross tight junctions (revealed by ZO−1 immunolabeling) in the initial segment of the epididymis, making direct contact with the lumen. Following the microinjection of fluorescent beads into the lumen, CX3CR1+ cells accumulated beads in projections that had reached the lumen (see figure), and within phagosomes in the basal region. In order to phenotypically characterize MPs involved in luminal antigen uptake and processing, the fluorogenic substrate DQ−OVA was administered into the lumen and FC analysis of MPs was performed after 4 hours.

**Results:** Our results show that CX3CR1+ macrophages take up and process luminal DQ−OVA and CD11c+CD103+ DCs also contained processed DQ−OVA. Interestingly, imaging has revealed that CD103+ DCs are positioned at the base of the epithelium in close proximity to CX3CR1+ cells; however they are not observed interacting with the lumen, suggesting that luminal antigens are sampled by CX3CR1+ cells and transferred to migratory DCs for presentation to T cells, a mechanism described in the small intestine mucosa.

**Conclusion:** Our results indicate that macrophages and DCs functionally cooperate to monitor the lumen of the mouse epididymis. Therefore, we can speculate that the epididymal mucosal immune system is a regulator of peripheral tolerance to sperm antigens. A better understanding of basic reproductive immunology is necessary to identify new targets for the treatment of male infertility and develop innovative strategies of immuno-contraception.

**Funding:** Work supported by NICHD grant R01HD069623.

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**ORAL ABSTRACTS**

**SUNDAY, APRIL 19, 2015**

2:30 p.m. – 2:45 p.m.

**CONCURRENT SESSIONS – Oral Session I – Basic Science**

**Podium/Poster #3**

**EARLY-LIFE EXPOSURE TO AN ENVIRONMENTAL ORGANOCHLORINE MIXTURE REDUCES THE FERTILITY OF MALE RATS AND INDUCES DIFFERENTIAL EXPRESSION OF SPERM PROTEINS ACROSS MULTIPLE GENERATIONS IN A PATERNALLY-MEDIATED MANNER**

Nancy Côté, PhD, Clotilde Maurice, PhD, Florence Roux-Dalvai, MSc, Arnaud Droit, PhD and Janice L. Bailey, PhD

Université Laval

(Presented By: Nancy Côté, PhD)

**Introduction:** Organochlorines (OC) are a family of persistent hydrocarbon compounds that were used for industrial and agricultural purposes in North America and Europe between 1930 and the mid-1980s. Due to their high lipophilicity and resistance to biodegradation, OC bioaccumulate in fatty tissues, are biomagnified through the food chain and have accumulated in Arctic populations. OC exposure is associated with decreased sperm quality in men and prenatal exposure to an environmentally-relevant OC mixture impairs reproductive development in male rats. We hypothesized that early-life paternal exposure to an environmentally-relevant OC mixture reduces fertility and changes the protein profile of sperm across multiple generations.

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

**Results:** F1 males exposed to OC during early development had decreased sperm motility (P=0.04), lower daily sperm production per testis (P=0.006), and decreased epididymal sperm concentration (P=0.0001). Their F2 OC sons were subfertile (P=0.02) and their F3 OC grandsons had fewer pups per litter (P=0.0001). In generations F1, F2 and F3, respectively 7, 19 and 37 differentially expressed proteins were identified due to OC exposure of the F1 fathers.

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

**SUNDAY, APRIL 19, 2015**

2:45 p.m. – 3:00 p.m.

**CONCURRENT SESSIONS – Oral Session I – Basic Science**

**Podium/Poster #4**

**MACROPHAGES AND DENDRITIC CELLS COOPERATE TO SURVEY THE EPIDIDYMAL LUMEN**

Tegan Smith, PhD, Gabriel Courties, PhD, Claire Barton, BA, Matthias Nahrendorf, MD, PhD and Nicolas Da Silva, PhD

Massachusetts General Hospital and Harvard Medical School

(Presented By: Tegan Smith, PhD)

**Introduction:** Male fertility relies on the immunological tolerance of millions of autoantigenic spermatozoa that are produced every day. During maturation and storage in the epididymis, male gametes are surrounded by various subsets of mononuclear phagocytes (MPs). In order to potentially regulate tolerance to sperm, these dendritic cells (DCs) and macrophages must acquire antigens located in the luminal compartment, i.e. across the blood–epididymis barrier.

**Methods:** This study, based on high-resolution imaging and flow cytometry (FC), assesses the ability of epididymal MPs (eMPs) to cross apical tight junctions and sample the luminal contents. Using CD11c−EYFP and CX3CR1−GFP reporter mice, we show that CD11c+ and CX3CR1+ cells are able to cross tight junctions (revealed by ZO−1 immunolabeling) in the initial segment of the epididymis, making direct contact with the lumen. Following the microinjection of fluorescent beads into the lumen, CX3CR1+ cells accumulated beads in projections that had reached the lumen (see figure), and within phagosomes in the basal region. In order to phenotypically characterize MPs involved in luminal antigen uptake and processing, the fluorogenic substrate DQ−OVA was administered into the lumen and FC analysis of MPs was performed after 4 hours.

**Results:** Our results show that CX3CR1+ macrophages take up and process luminal DQ−OVA and CD11c+CD103+ DCs also contained processed DQ−OVA. Interestingly, imaging has revealed that CD103+ DCs are positioned at the base of the epithelium in close proximity to CX3CR1+ cells; however they are not observed interacting with the lumen, suggesting that luminal antigens are sampled by CX3CR1+ cells and transferred to migratory DCs for presentation to T cells, a mechanism described in the small intestine mucosa.

**Conclusion:** Our results indicate that macrophages and DCs functionally cooperate to monitor the lumen of the mouse epididymis. Therefore, we can speculate that the epididymal mucosal immune system is a regulator of peripheral tolerance to sperm antigens. A better understanding of basic reproductive immunology is necessary to identify new targets for the treatment of male infertility and develop innovative strategies of immuno-contraception.

**Funding:** Work supported by NICHD grant R01HD069623.
THE SPlicing FACTOR RBM5 IS REQUIRED FOR SPERMATOGONIA DIFFERENTIATION

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

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

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

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

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

Michael Eisenberg, MD¹, Alexander W. Pastuszak, MD, PhD², Peter Langlois, PhD³, Karen Moffitt³, Dolores J. Lamb, PhD³ and Larry I. Lipshultz, MD³

¹Stanford University; ²Baylor College of Medicine; ³Texas Department of State Health Services

(Presented By: Alexander W. Pastuszak, MD, PhD)

**Introduction and Objectives:** Approximately 15% of couples have fertility problems, with a 50% male factor contribution. While assisted reproductive technologies (ART) have greatly enhanced the ability of couples with fertility difficulties to conceive, evidence suggests an increased risk for congenital defects in children conceived using ART. Both the technique of in vitro fertilization (IVF) as well as infertility itself are possible explanations. We sought to determine if the severity of male factor infertility, as assessed by sperm quality and mode of conception, was associated with birth defect rates.

**Methods:** Fathers with semen analysis data in the Baylor College of Medicine Semen Database (BCMSD) were linked with offspring in the Texas Birth Defects Registry (TBDFR) using data from 1999–2009. To determine the association between birth defects and semen parameters, we identified the subset of men with complete birth covariates. Semen parameters were stratified based on subfertile cutoffs defined by the WHO 5th edition.

**Results:** Initial linkage between the BCMSD and TBDFR yielded 6,087 men with linked data. No association between semen parameters and birth defects was observed. As a sensitivity analysis, a subset of 1,382 men who had been evaluated for infertility was identified. After the above correction, 109 infants with and 2,115 infants without birth defects were identified. No statistically significant association was observed between birth defect rates, semen parameters, and mode of conception before and after adjustment for paternal, maternal, and birth covariates. Semen parameters were stratified based on subfertile cutoffs defined by the WHO 5th edition.

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

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**ORAL ABSTRACTS**

**SUNDAY, APRIL 19, 2015**

**CONCURRENT SESSIONS – Oral Session II – Clinical**

**Podium/Poster #7**

**THE RISK OF CONGENITAL BIRTH DEFECTS IS NOT ASSOCIATED WITH SEMEN PARAMETERS OR MODE OF CONCEPTION IN OFFSPRING OF MEN VISITING A REPRODUCTIVE CLINIC**

**Introduction and Objectives:** Approximately 15% of couples have fertility problems, with a 50% male factor contribution. While assisted reproductive technologies (ART) have greatly enhanced the ability of couples with fertility difficulties to conceive, evidence suggests an increased risk for congenital defects in children conceived using ART. Both the technique of in vitro fertilization (IVF) as well as infertility itself are possible explanations. We sought to determine if the severity of male factor infertility, as assessed by sperm quality and mode of conception, was associated with birth defect rates.

**Methods:** Fathers with semen analysis data in the Baylor College of Medicine Semen Database (BCMSD) were linked with offspring in the Texas Birth Defects Registry (TBDFR) using data from 1999–2009. To determine the association between birth defects and semen parameters, we identified the subset of men with complete birth covariates. Semen parameters were stratified based on subfertile cutoffs defined by the WHO 5th edition.
(lower bound 95% CI = 80.3%) and 86.5% of obese hypogonadal men (lower bound 95% CI = 78.8%). Mean T Cave, 24h value was 498±200 ng/dL and 467±194 ng/dL, mean T Cmax value was 1288±557 ng/dL, and 1224±625 ng/dL for non-obese and obese men, respectively. No significant differences were observed between obese and non-obese hypogonadal men in terms of percent of subjects restored in the eugonadal range, mean T Cave, 24h and mean T Cmax (p>0.1) suggesting LPCN 1021 is effective in treating both non-obese and obese hypogonadal men.

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

SUNDAY, APRIL 19, 2015
2:30 p.m. – 2:45 p.m.

CONCURRENT SESSIONS – Oral Session II – Clinical
Podium/Poster #9
CIGARETTE SMOKING AND THE SPERM EPIGENOME
Timothy Jenkins, PhD, Kenneth Aston, PhD, James Hotaling, MD, MS and Douglas Carrell, PhD
University of Utah
(Presented By: Timothy Jenkins, PhD)

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

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

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

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

SUNDAY, APRIL 19, 2015
2:45 p.m. – 3:00 p.m.

CONCURRENT SESSIONS – Oral Session II – Clinical
Podium/Poster #10
WHOLE-EXOME SEQUENCING IDENTIFIES NOVEL HOMOZYGOUS MUTATION IN NPAS2 IN FAMILY WITH NONOБSTRUCTIVE AZOOSPERMIA
Ranjith Ramasamy¹, M. Emre Bakircioğlu, MD², Cenk Cengiz, BS¹, Ender Karaca, MD¹, Jason Scovell, BS¹, Matthew Bainbridge, PhD¹, James Lupski, PhD¹ and Dolores Lamb, PhD¹
¹Baylor College of Medicine; ²Bahçeci Fulya IVF Center, Istanbul, Turkey
(Presented By: Ranjith Ramasamy)

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

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

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

Conclusion: Using WES, we identified a novel homozygous mutation in NPAS2 as a likely disease-causing variant in a Turkish family diagnosed with NOA. Our data reinforce the clinical role of WES in the molecular diagnosis of highly heterogeneous genetic diseases where conventional genetic approaches have previously failed in achieving a proper diagnosis.
SUNDAY, APRIL 19, 2015
3:00 p.m. – 3:15 p.m.

CONCURRENT SESSIONS – Oral Session II – Clinical
Podium/Poster #11
ENCLOMID AND TOPICAL TESTOSTERONE ELEVATE TESTOSTERONE IN HYPOGONADAL MEN BUT ENCLOMID DOES NOT DECREASE TESTES SIZE
Ronald Wiehle, PhD, Gregory Fontenot, PhD, Martin Sandel, BS and Jaye Thompson, PhD
Repros Therapeutics
(Presented By: Ronald Wiehle, PhD)

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

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

Methods: Two trials (ZA−304 and ZA−305) were randomized, double blind, placebo- and active-control, multi-center phase III studies in 224 men with secondary hypogonadism between 25 and 60 years of age. Men received 12.5mg or 25mg of enclomis as a daily capsule and were provided with a placebo gel. Other men received AndroGel 1.6% and placebo capsules. Other men received placebo capsules and gels.

Results: To be enrolled subjects needed to have two baseline testosterone (T) values below 300ng/dL. The End of Study (EOS) was after 16 weeks of treatment. There was a statistically significant rise in T in men receiving either enclomiphene citrate or topical testosterone into the normal range (see table). Placebo subjects did not change. Enclovism did not decrease sperm counts unlike the topical gel. As we have seen before, enclomiphene citrate increased LH and FSH while men in the topical arm showed decreases (not shown). All men were similar at baseline in testes volume (p = 0.94, ANOVA) by orchidometry. In both studies, men on topical testosterone demonstrated decreases in mean testicular volume (−0.86 cm3) and a significant decrease overall compared to the enclomid (p < 0.05) or placebo (p < 0.05).

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

SUNDAY, APRIL 19, 2015
3:15 p.m. – 3:30 p.m.

CONCURRENT SESSIONS – Oral Session II – Clinical
Podium/Poster #12
CONCENTRATIONS OF THE RETINOIC ACID SYNTHESIZING ENZYME ALDH1A2 ARE SIGNIFICANTLY REDUCED IN MEN WITH OLIGOZOOSPERMIA
John Amory, MD, MPH, Margarett Shnorhaviorian, MD, MPH, Samuel Arnold, MD, Faith Stevison, BS, Nina Isoherranen, PhD, Thomas Walsh, MD, MPH and Charles Muller, PhD
University of Washington
(Presented By: John Amory, MD, MPH)

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

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

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

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

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