The theme of the 2012 ASA meeting, "Male reproductive health: This generation to the next," reflected some of the most pressing questions for the present and future of reproductive biology and health in the male. Scientists and clinicians face a fascinating and changing landscape for male reproductive health issues. With unprecedented changes in the environment in which we live, questions come up about how this environment affects male reproductive health, as well as the offspring that will be produced. With increases in life expectancy, more men are surviving to advanced age and also living longer in these later decades, producing new issues for healthcare related to male reproduction and general health. And finally, we looked with a keen eye for how basic science and translational discoveries will make advances in understanding and managing male reproductive health, in the near term and for future generations.

Named Lectures

EMIL STEINBERGER MEMORIAL LECTURE

BEDSIDE TO BENCH: DISCOVERY OF GENES THAT CONTROL REPRODUCTION IN MEN
William F. Crowley Jr., MD; Harvard Medical School

This Keynote Lecture provided perspective on the genetic insights into the CNS control of reproduction provided by various human models. These models include those in which gene mutations alter the normal physiology of GnRH secretion and hence the timing, onset, or pace of sexual development. Foremost among these are men and women with Kallmann Syndrome and normosmic Idiopathic Hypogonadotrophic Hypogonadism, both of which result in an isolated defect in GnRH secretion. Using these human models, 15 new genes crucial for normal functioning of the GnRH neuronal network have been discovered, which can be placed into three different groups based on their presumed site of action: (1) neurodevelopmental genes (e.g. KAL1, NELF, FGF8, FGFR1); (2) neuroendocrine genes (GnRH, GnRHR, TAC3, TAC3R, KISS1, KISS1R); and (3) ‘overlap’ genes that have clinical features of both groups (CHD7, FGFR1, PROK2, PROKR2). This work has demonstrated how various genes not only account for rare Mendelian disorders but also contribute to the more common reproductive failures.
AUA LECTURE

ANDROGEN RECEPTOR REGULATION IN PROSTATE CANCER AND OTHER CELLS
Donald J. Tindall, PhD; Mayo Clinic, Rochester, MN

This lecture addressed critical events of androgen (AR) signaling in the progression to castrate-resistant prostate cancer, as a molecular foundation to developing successful future therapies. Considerable evidence now supports the concept that development of CRPC is causally related to continued transactivation of the AR. The goal of Dr. Tindall's research is to understand the mechanisms by which the AR regulates this androgen-refractory phenotype. His research program has discovered novel AR variants discovered in castrate-resistant prostate cancer cells. Most of these AR Variants do not contain the Ligand-Binding Domain of the protein and are constitutively active, promoting the expression of endogenous ARdependent genes, as well as the proliferation of castrate-resistant prostate cancer cells in a ligand-independent manner. Some AR Variants also appear to have prognostic utility.

WOMEN IN ANDROLOGY LECTURE

PATERNAL OBESITY AFFECTS OFFSPRING DIABETES RISK
Margaret J. Morris, PhD; University of New South Wales, Sydney, Australia

Dr. Morris' laboratory is examining the contribution of parental obesity to offspring outcomes, using the rat as an experimental model. Genetic and environmental factors are recognized as important contributors to maternal transmission of obesity, and recent work of the Morris research group has studied the role of the father's environment in transmitting obesity to his offspring. In this project, male Sprague Dawley rats were fed high fat chow or control chow for 12 weeks before mating; the chronic high fat diet induced increased body weight, adiposity, impaired glucose tolerance and insulin sensitivity. The female offspring of obese sires showed reduced glucose tolerance and insulin secretion from 6 weeks of age. These females had reduced β-cell reserve as well as altered islet gene expression. The islet gene with the greatest alteration in expression, interleukin13 receptor a2 (Il13ra2), showed reduced DNA methylation, providing evidence of epigenetic alteration. These findings extend the concept of developmental and adaptive plasticity to include a paternal role in the early life origins of disease.

INTERNATIONAL LECTURE

ENVIRONMENTAL PERTURBATIONS AND VULNERABILITIES IN MALE REPRODUCTIVE HEALTH
Jorma Toppari, MD, PhD; University of Turku, Finland
This lecture covered disorders associated with Testicular Dysgenesis Syndrome, examining the fundamental question of whether in human are linked with environmental exposures to endocrine disrupters. Dr. Toppari discussed how this is a complex question still awaiting a firm answer, but he noted that (1) the incidence of testicular cancer has increased over two generations, (2) the birth rates of hypospadias and cryptorchidism are high, (3) data show a clear decline semen quality in Finland during the last 15 years. His group has have analyzed the association of cryptorchidism with exposure to several endocrine disrupters. While he noted that such a study is correlative, rather than proving causality, cryptorchidism was found to have a weak positive association with exposure to chlorinated pesticides and polybrominated diphenyl ethers. It is likely that a mixture of several chemicals can cause cryptorchidism in genetically susceptible individuals. Modern systems biological approaches are needed to deal with complex exposure scenarios and genetic variability. First attempts to such approaches have revealed new genes that are associated with TDS disorders.

**Additional Lectures**

**HORMONE REPLACEMENT THERAPY IN MEN OF REPRODUCTIVE AGE**

Edward D. Kim, M.D; University of Tennessee Graduate School of Medicine

Testosterone therapies have been increasingly utilized in aging men, as well as in men of reproductive age. With the recent introduction of several newer commercial testosterone preparations and an increased public awareness of androgen deficiency syndromes, use of hormone replacement therapies (HRT) is likely to grow. Dr Kim's talk emphasized how it was important for physicians to be aware of the importance of intrinsic testosterone for spermatogenesis and how intrinsic testosterone can be maintained, noting that many physicians are not aware that hormone replacement therapies (HRT) may suppress spermatogenesis. This biological fact has clear implications for therapeutic strategies for hormone replacement, and yet in a recent study, it was identified that ~30% of physicians have used exogenous testosterone to treat low testosterone levels associated with male infertility. A goal of this presentation was for clinician attendees to have increased ability to advise and recommend on a variety of therapeutic strategies, including hCG and selective estrogen modulators (SERMs).

**AGING AND BENIGN PROSTATIC HYPERPLASIA—WHAT’S THE CONNECTION?**

Jill A. Macoska, PhD; The University of Michigan

Dr. Macoska addressed an often underappreciated pathology that has serious effects on a man's quality of life -- benign prostatic hyperplasia (BPH), or non-cancerous enlargement of the prostate. This is a common condition associated with aging in men and is often synonymous with lower urinary
tract symptoms (LUTS). She presented her research group's work that provides evidence that chemokines can, and likely do, promote BPH/LUTS development and progression. A variety of chemokines consistent with a senescence associated proteome are actively secreted by the prostatic microenvironment consequent to the aging process. Her lab's studies in a mouse model engineered to over-express murine form of the CXCL8 chemokine demonstrated that CXCL8 overexpression is associated with physiological changes that mimic what is found in human BPH tissues (e.g., myofibroblast accumulation). Additionally, progressive declines in the production and tissue levels of testosterone occur with age, and this may exacerbate TGFβeta- and chemokine-mediated changes in prostate tissue proliferation and architecture associated with LUTS.

Dr. Leslie Heckert (University of Kansas Medical Center) was due to speak on "Crosstalk Between Sertoli and Germ Cells - How Does This Lead to Testicular Cell Differentiation?" but she regretfully had to cancel days before the meeting, due to a serious personal illness. She was replaced in the program by one of the top two scoring abstracts. This talk was presented by Dr. Kent Hamra (Assistant Professor, University of Texas Southwestern Medical Center):

A SPERM STEM CELL BASED FORWARD GENETIC SCREEN FOR REPRODUCTION PHENOTYPES IN THE LABORATORY RAT
Gerardo A. Medrano, BS, Jaideep Chaudhary, BS, Heather M. Powell, BS, Karen M. Chapman BS, and F. Kent Hamra, PhD; Univ. Texas Southwestern Medical Center

Introduction: We recently established reproductive technology to help advance biomedical research through use of "Mutant Sperm Stem Cell Libraries" generated by transposon−based gene−trap insertions into cultures of spermatogonia. The study provided proof−of−principle for using custom, mobile DNA elements to "decorate" the rat genome with highly versatile gene manipulation cassettes to facilitate genome−wide gene inactivation, gene activation, exchanging DNA elements within genes, proteome tagging and the ability to repair defective genes. We chose germline stem cells from the laboratory rat to construct mutant libraries because historically, other than humans themselves, rats have been the most widely applied species to model human health. Methods: A panel of mutant Sprague Dawley rats that harbor intronic gene−trap mutations at pre−defined loci within distinct RNA polymerase II transcribed genes was generated with the mutant sperm stem cell library. The mutant rats where then used to conduct a small−scale forward genetic screen for reproductive phenotypes. Females and males of all heterozygous mutant strains used in the screen were fertile. Results: Currently, homozygous mutations in 15 of 19 mutant strains have been analyzed in which reproduction/developmental defects were found linked to 79% of the gene mutations. Disrupting expression of genes encoding rat Ube2k, Pan3, Btrc and Spaca6 resulted in female and/or male infertility, whereas disrupting expression of the Cdk8, Dlg1, Gsg1l, Exoc6b, Slc1a3,
Slc35a3 and Txndc13 genes were each found to be embryonic lethal. Homozygous mutations predicted to disrupt expression of Pclo, Grik3, Abca13, Flsl5 and Tbc1d1 did not affect reproduction. The fertility of rats with mutations in Prkcbp1, Ube2q2 and Rgs22 is currently being analyzed. Conclusion: This pilot study demonstrates the feasibility of using recombinant sperm stem cell libraries as a user-friendly and cost-effective strategy to advance biological research via forward or reverse genetics in the laboratory rat. Dr. Harvey Florman (University of Massachusetts Medical School) was due to speak on, "How a Sperm Learns to be Fertile," but had to cancel days before the meeting, due to a medical emergency with a family member. He was replaced in the program by one of the top two scoring abstracts, which was presented by Dr. Wei Yan (Associate Professor, University of Nevada), who also was the 2012 winner of the ASA's Young Andrologist award.

**CYTOPLASMIC DROPLETS FUNCTION AS AN ENERGY SOURCE ESSENTIAL FOR NORMAL SPERM EPIDIDYMAL MATURATION**

Shuiqiao Yuan, MSc1, Hui Xu, MD2, Zhihong Zheng, MD, PhD2 and Wei Yan, MD, PhD1

1Department of Physiology and Cell Biology, University of Nevada School of Medicine, Reno, NV, USA; 2Department of Laboratory Animal Medicine, China Medical University, Shenyang, China

Although the cytoplasm of spermatids is removed at the end of spermiogenesis, a tiny portion is usually retained to the sperm flagellum, which has been termed the cytoplasmic droplet (CD). In mice, CDs are mostly present on epididymal sperm and are believed to play a role in volume regulation for sperm osmolarity adaption. We, however, observed that epididymal sperm without CDs never developed motility after being collected into the HTF medium, whereas sperm with CDs were generally motile, suggesting that the CD may have a role in sperm motility competence during epididymal maturation. By analyzing mutant mice with late spermiogenic disruptions, we also found that abnormal position and morphology of CDs in epididymal sperm were associated with abnormal spermiogenesis and also with abnormal motility. Our data suggest that CDs represent a transient organelle in epididymal sperm, which are reflective of normal spermiogenesis and are predictive of sperm motility. To further explore the function of CDs, we purified CDs (>90% purity) from epididymal sperm by discontinuous sucrose gradient centrifugation and protein contents of CDs were determined by mass spectrometry. A total of 105 proteins enriched in CDs were identified, among which 72 were enzymes involved in numerous metabolic pathways. We detected 27 glycolytic enzymes of high levels in CDs, which were involved in various steps of the glycolytic pathways, suggesting that CDs may be a site of active glycolysis and the major source of ATP production during sperm maturation in the epididymis. By investigating effects of CD removal on sperm mitochondrial activity and sperm ATP production before and after in vitro activation, we demonstrated that sperm without CDs failed to have their mitochondria activated and their
capability of ATP production is minimal upon ejaculation or upon activation in the HTF medium. Taken together, our data have revealed that CDs are enriched of glycolytic enzymes and substrates for glycolysis, and CDs are essential for mitochondrial activation and normal ATP production upon ejaculation or in vitro activation. Given that the mitochondrial activity of epididymal sperm is mostly suppressed, we propose that CDs function as a source of ATP production to facilitate all molecular and cellular events [e.g. mitochondrial activation (mitochondrial “priming”), tyrosine phosphorylation, correct localization of glycolytic enzymes to the flagellum, etc.] during sperm epididymal maturation

SYMPOSIUM I – Paternal Contributions to Embryo Quality and Reproductive Success

FATHER KNOWS BEST: PATERNAL IMPACTS ON EMBRYOGENESIS
Douglas T. Carrell, PhD, HCLD; University of Utah School of Medicine

PATERNAL REPROGRAMMING OF METABOLIC GENE EXPRESSION IN OFFSPRING
Oliver Rando, MD, PhD; University of Massachusetts

EFFECTS OF FIRST- AND SECOND-HAND TOBACCO SMOKE: HOW DOES IT AFFECT MALE REPRODUCTIVE FUNCTION?
Francesco Marchetti, PhD, ScD; Health Canada, Ottawa, ON, Canada

This symposium was designed with a special nod to the "next generation" part of the 2012 meeting theme, putting together a series of presentations that address recent findings on how a man's lifestyle and environmental exposure can impact his own reproductive fitness and the health of his offspring. Dr. Carrell's talk addressed the phenomenon that sperm epigenetic marks likely portend a functional role of the sperm genome in guiding transcription during embryogenesis. While most canonical histones are replaced during spermiogenesis with protamines, retained histones are enriched in the gene promoters of developmental genes, micro RNAs, and imprinted genes. Many developmentally-relevant gene promoters are demethylated and have a bivalent enrichment of histones (H3K4me3/H3K27me3) similar to stem cells. Interestingly, limited genome-wide studies of some infertility patients, including men with poor embryogenesis during IVF, have demonstrated altered histone enrichment and aberrant DNA methylation. Dr. Rando's talk covered phenomenon going back a generation from Dr. Carrell's talk – the effect of paternal diet on the offspring. He presented an expression profiling screen done by his group, looking for genes in the livers of mice that responded to paternal diet. Compared to relative the offspring of males fed a control diet, offspring of males fed a low-protein diet exhibited elevated hepatic expression of many genes involved in lipid and cholesterol biosynthesis and decreased levels of cholesterol esters, relative to the offspring of males fed a control diet. Epigenomic profiling of offspring livers revealed numerous modest (~20%)
changes in cytosine methylation depending on paternal diet. These data indicate that parental diet can affect cholesterol and lipid metabolism in offspring. Dr. Marchetti addressed the effects of paternal smoking on reproductive outcomes. Complementing evidence from human studies associating paternal cigarette smoking with a variety of reproductive defects (increased chromosomal abnormalities in sperm and increased risk for spontaneous abortions, birth defects and neonatal death), his research group performed studies of male mice exposed to two doses of mainstream tobacco smoke (MTS), the smoke inhaled by active smokers, or sidestream smoke (STS), the main component of second-hand smoke. This study revealed that both MTS and STS smoke increased sperm chromatin structure abnormalities and caused DNA mutations in sperm, whereas only MTS smoke induced DNA strand breaks in sperm, and only STS reduced sperm motility. These data provide further evidence that male exposure to second-hand smoke, as well as direct cigarette smoke, is likely to have reproductive consequences that go beyond the passive smoker.

**SYMPOSIUM II – Future Technologies and Targets for Improving Men’s Health**

**H2-GAMENDAZOLE, A NEW NON-HORMONAL ORAL MALE CONTRACEPTIVE: THE ROAD TO CLINICAL TRIALS**
Joseph S. Tash, PhD; University of Kansas Medical Center, Kansas City, KS;

**GENE THERAPY, STEM CELLS AND TISSUE REGENERATION FOR ERECTILE DYSFUNCTION: PAST, PRESENT AND FUTURE**
Trinity J. Bivalacqua, MD, PhD; Urology and Oncology, Johns Hopkins Medical Institutions

**CAVERNOUS NERVE REGENERATION AS AN ED THERAPY**
Carol A. Podlasek, PhD; Northwestern University

The overall goal of this symposium was to present a range of cutting-edge research with direct translational applications for men's health, focusing on advances in non-hormonal male contraception and treatments for erectile dysfunction (ED). Dr. Tash presented his group's recent work on gamendazole (GMZ), a non-hormonal contraceptive agent that, at the lowest single oral dose that gave 100% infertility in rats, showed 60% recovery of fertility, and that with single oral doses caused significant dose-related reductions in spermatid counts in mouse, rat, and rabbit. Pilot studies in Rhesus monkeys showed significant and reversible reductions in semen sperm count and spermatogenic index at single oral doses and no adverse side effects with the doses tested (0.6-2.0 mg/kg). Early studies using affinity pull-down suggested that HSP90 and eEF1A were direct binding targets of H2-GMZ, and H2-GMZ-eEF1A binding was confirmed and specifically inhibited the noncanonical F-actin bundling activity. Their current model holds that H2-GMZ’s contraceptive effect is based on transient
disruption of the apical ectoplasmic specializations that tether spermatids to Sertoli cells by causing structural disruption of the apical ectoplasmic specializations through unbundling of the associated F-actin filaments. Dr. Bivalacqua provided an excellent overview of gene therapy and stem cell based therapy strategies for erectile dysfunction. He noted that although all three selective type 5 phosphodiesterase (PDE5) inhibitors are effective in the majority of ED cases, PDE5 inhibitor therapy is less efficacious in some hard-to-treat populations (diabetics, post-radical prostatectomy). Over the last decade, a great deal has been learned about gene and stem cell based therapies in pre-clinical animal models, as well as the first erectile dysfunction gene therapy clinic trial in ED patients. Gene therapy approaches have focused on signaling pathways that are crucial for penile erection; these include nitric oxide/cyclic guanosine monophosphate, RhoA/Rho-kinase, growth factors, ion channels, peptides, and controlling oxidative stress. Various viral and nonviral vectors have been used to date for the transfer of genetic material to target cells or tissues with various degrees of success. With the advent of adult stem cell therapies for regeneration of new healthy tissue, in particular neuronal, endothelial and smooth muscle cells, the use of this form of therapy may be utilized to improve erectile function in the penile vascular bed. Dr. Podlasek's talk complementary to the talk of Dr. Bivalacqua, with information on ED associated with cavernous nerve (CN) damage, which can occur with prostatectomy surgery. Penile tissues innervated by the damaged CN undergo smooth muscle apoptosis and fibrosis, resulting in significantly reduced effectiveness of PDE-5 inhibitors. She proposes that the secreted protein sonic hedgehog (SHH), which plays a prominent role in nerve development, can be part of a novel therapy to facilitate CN regeneration. She presented her research group's data on use of local SHH protein treatment of the CN at the time of injury using novel peptide amphiphile (PA) nanofibers in a Sprague Dawley bilateral CN crush rat model. Their findings suggest that there is a window of opportunity immediately after nerve insult in which manipulation of the nerve microenvironment can affect long-term regeneration outcome.

**SYMPOSIUM III – Male Germ Cells: From Their Birth to Their Grave**

**HUMAN DELETED IN AZOOSPERMIA (DAZ) GENE FAMILY—600 MILLION YEARS IN THE MAKING**

Eugene Yujun Xu, PhD; Nanjing Medical University, Nanjing, China, and Northwestern University Feinberg School of Medicine, Chicago, IL

**THE RULES OF TRANSCRIPTIONAL REGULATION OF SPERMATOGENESIS: KEEP IT SIMPLE**

Prabhakara Reddi, PhD; University of Virginia Medical School

**SERTOLI CELLS: NOT JUST NURSE CELLS BUT UNDERTAKERS TOO**

Jeffrey J. Lysiak, PhD; University of Virginia, Charlottesville, Virginia.
This symposium consisted of a well-conceived theme and brought together three highly appropriate research areas within one session. In this “birth to grave” theme, the first presentation tracing the origin of the DAZ-gene family which is essential for mammalian spermatogenesis sparked the interest of the audience on the evolutionary roots of spermatogenesis. The third presentation highlighted the mechanisms and molecules involved in how the testis deals with the dead germ cells. Thus, the first and the last talks represented the “birth” and the “grave” parts of the symposium theme while the middle talk on how ordered expression of differentiation markers keeps spermatogenesis on its wheels covered what happens between birth and the death of germ cells. Dr. Xu’s presentation on traced the origins of Dazl, Boule, and DAZ (members of the DAZ gene family) to the dawn of animal evolution and concluded that Boule, the oldest member, arose 600 million years ago and gave rise to Dazl during vertebrate evolution. DAZ arose during recent primate evolution. Dr. Reddi shared the story of how the study of a candidate gene (acrv1) to understand transcriptional regulation of round spermatid-specific gene expression led to the findings that remodeling of nuclear lamina-chromatin interactions might be key for spermatid differentiation and also that the spatiotemporal gene expression within the seminiferous epithelium relies on the mechanism of RNA Pol II pausing at the proximal promoter region. Dr. Lysiak showed that Sertoli cells, in addition to nurturing the differentiating germ cells, also play the role of “undertakers” because they clear out the dead germ cells. Using elegant conditional gene knockout technologies his lab showed that the engulfment protein ELMO1 as well as the guanine nucleotide exchange factor RAC1 plays significant roles. The Sertoli cells require ELMO1 and RAC1 proteins for efficient removal of the germ cell corpses after the latter have undergone apoptosis in the normal course of spermatogenesis.

**SYMPOSIUM IV – Androgen Actions and Responsive Tissues**

**ANDROGEN AXIS DISRUPTION IN PROSTATE CANCER**
Dr. Karen E. Knudsen, Thomas Jefferson University

**APPLYING "-OMICS" TECHNOLOGY TO UNDERSTAND SPERM PRODUCTION**
Liza O'Donnell, PhD; Prince Henry's Institute of Medical Research, Australia

**SYMPOSIUM IV – Androgen Actions and Responsive Tissues**

**DISSECTING ANDROGEN ACTION: NEW CLUES FROM CONDITIONAL KNOCKOUT MICE**
Lee Smith, PhD
MRC Centre for Reproductive Health, University of Edinburgh, UK.

This symposium covered a variety of ways in which male reproductive function and health is regulated by androgens. Dr. Knudsen addressed a fundamental aspect of prostate cancer, that these cancers are exquisitely
dependent on the action of the androgen receptor (AR) for survival and proliferation. There is a significant need to develop new means for targeting recurrent AR activity in both locally advanced and castration-resistant prostate cancers. Dr. Knudsen's group demonstrated that the enzyme PARP1 promotes AR DNA occupancy; without PARP1, AR is removed from DNA. Indeed, her lab has shown that combination of AR inhibitor, radiation therapy, along with a PARP1 inhibitor significantly slows down growth of prostate cancer cells (cell line and primary tumor) compared to the currently used treatments (AR inhibitor + radiation). Her group is currently involved in 2 ongoing clinical trials for late stage prostate cancer patients. Therefore PARP1 represents a novel therapeutic target for the treatment of prostate cancer. Dr. O'Donnell's talk addressed ways that “omics” technologies have revealed multiple level of androgen regulation during sperm production. Androgens are essential for sperm production despite the fact that germ cells do not express the androgen receptor (AR). AR is rather expressed in neighboring Sertoli cells. In addition, synergy in sperm production between androgens and FSH suggest that FSH potentiates AR signaling in Sertoli cells. The challenge is to define the mechanisms by which androgens, together with FSH, modulate sperm production. This has direct implications for fertility induction as well as fertility suppression (male hormonal contraception). Dr. O'Donnell’s group used various “omics” approaches (mRNA, proteins, miRNA) on a unique system of isolated spermatogenic tubules to identify genes, proteins, and cellular processes that are modulated both in Sertoli cells and germ cells in the absence of androgens and FSH. This led to the identification several pathways, including novel ones, which are regulated by androgens. Dr. Smith’s work provides evidence for a complex androgen-dependent paracrine signaling pathway within the testis, with each AR-expressing cell type influencing others to ensure their correct development and function. Androgen signaling is important for male reproductive health during both development and adulthood. Furthermore, androgen manipulation is widely used as a clinical treatment. Our current knowledge of AR action is based on data from global gene deletion in mouse models or clinical data from naturally occurring mutations in humans. The precise molecular mechanisms of androgen action, however, remain poorly understood since AR is expressed in several cell types within the testis (Sertoli cells, Leydig cells, peritubular myoid cells, vascular smooth muscle cells, vascular endothelial cells). Dr. Smith’s group has generated several lines of mice lacking the AR in specific cells of the testis. This work has led to the identification of novel roles for each cell type in the promotion of androgen-mediated male reproductive function.

Symposium V - Genetic Foundations of Male Infertility

REPRODUCTIVE FITNESS OF THE HUMAN Y CHROMOSOME
Sjoerd Repping, PhD; University of Amsterdam

MUTATIONS IN X-LINKED GENES AS CAUSE OF INFERTILITY IN MEN
NEW INSIGHTS IN GENETICS OF OLIGOZOOSPERMIA IN INFERTILE MEN

Alexander Yatsenko, MD, PhD; Magee Womens Research Institute at University of Pittsburgh

This symposium included talks from three different researchers examining three different aspects of male infertility and underlying genetic causes. Dr. Repping provided a cohesive review of our current understanding of these Y chromosome aberrations, their molecular origins as well as their implication for clinical practice and future research implications. This presentation built on a growing body of evidence suggests that the structure of the modern day Y chromosome is the result of extensive rearrangements through the course of evolution. One of the driving forces behind the transmittance of these rearrangements is reproductive fitness, where some chromosomes are more likely to be transmitted due to their effect on semen quality. Dr. Yatsenko has examined patients with oligospermia, utilizing a novel high-throughput microarray technology and comparative genomic hybridization. This work identified protein ubiquitination and germ cell apoptotic pathways in post-meiotic spermatozoa leading to oligozoospermia. Through this they were able to corroborate previous animal and human studies that indicate the ubiquitin conjugating enzyme 2B (UBE2B) as an important enzyme in late stages of spermatogenesis affecting sperm density. Dr. Wang presented on his group's work on searches for single genic mutations in idiopathic infertile men; they also sought to validate these mutations as a cause of infertility in humans using mouse models. They found several different types of single mutations in two Xlinked germ cell specific genes (TEX11 and TAF7L) that lead to disruptions in sperm density, and validated these mutations by modeling these infertility causing mutations in mice. This finding is highly significant, given that a large number of genes specifically regulate fertility.

2012 International Travel Awards sponsored by the International Society of Andrology

Steven Mansell
IDENTIFICATION OF A VOLTAGE ACTIVATED K+ CHANNEL IN HUMAN SPERMATOZOA BY WHOLE CELL PATCH CLAMPING

Steven Mansell, BSc, Christopher L.R. Barratt, PhD and Stuart M. Wilson, PhD University of Dundee

Introduction: Ion channels are important in normal sperm physiology since they regulate membrane potential, intracellular pH and Ca2+ entry. Recently with the successful application of sperm whole cell patch clamping we are starting to explore the characteristics of these channels, and their role in
male fertility. The aim of this study was to use whole cell patch clamping to elucidate and characterise ion channels in human spermatozoa under quasi-physiological conditions. Methods: Semen samples were obtained from a cohort of healthy donors with no obvious fertility issues (Ethical approval number 09/s1402/6) and normal semen parameters (WHO 2010). Sperm isolated by swim up were incubated under capacitating conditions for 4 hours (25mM NaHCO3, 6% CO2 at 37°C). Currents recorded by ramp changes in holding potential (−80 to 80mV, Vhold = −80mV) were analysed from cells under voltage clamp in whole cell configuration over a 250ms period. Standard pipette and bath solutions were designed to mimic quasi-physiological ionic conditions ([Na+]o = 135mM [K+]i =113 mM, [K+]o = 4.7 mM pH 7.4. Results: The membrane potential (Vm) under resting conditions was −40.0 ± 4.0mV (n=10). Data also showed an outwardly rectifying current only active when depolarised past resting Vm (>−40mV). Raising [K+]o to 130mM depolarised Vm to −2.5 ± 5mV toward EK (n=9, p= 0.001). Moreover, replacing pipette K+ with Cs+ (n=8) consistently abolished the depolarization–induced current and shifted Vm to 2.9 ± 5mV. Cytoplasmic acidification from [pH]i 7.4 to [pH]i 6.8 caused a decrease in the depolarised outward current (~50%) suggesting an important link between [pH]i and channel activation. Channel inhibition by intracellular acidification had no effect on resting Vm. Conclusion: This is the first time resting membrane potential has been assessed using electrophysiology in sperm cells under quasi-physiological conditions. We have identified a voltage activated K+ channel in spermatozoa that is inactive at resting Vm, indicating other conductances are involved in regulating Vm, this was further confirmed by intracellular acidification. However, the role of these channels in male fertility remains to be explored.

**Venkatesh Sundararajan**  
*ANALYSIS OF GENETIC, MOLECULAR AND LIFE STYLE FACTORS IN IDIOPATHIC INFERTILE MEN*

Venkatesh Sundararajan, MPharm, PhD, Rajeev Kumar, MD and Rima Dada, MD, PhD

Introduction and Objectives: Male infertility is one of the major reproductive health problems among the married couples and majority of them are idiopathic. Therefore the aim of the present study was to analysis genetic, molecular and various life style factors in 200 consecutive men with idiopathic infertility. Materials and Methods: The study included cytogenetic, Y chromosome micro-deletion, sperm mitochondrial DNA mutation and sperm nuclear protein gene mutation analysis. Sperm DNA integrity and oxidative stress were also measured. A predesigned proforma were applied to record all their life style factors such as smoking, alcohol consumption, and mobile phone usage. Results: The study revealed 13% harbored cytogenetic abnormalities, and 9.5% harboured Azoospermia factor (AZF) microdeletions. Sperm nuclear protein gene mutation screening revealed no significant difference in the frequency between infertile and controls. Fifty
eight mtDNA variants were found to be common for both infertile men and control and five pathogenic mutations including ND1 (mt.3391G>A) and ND2 (mt.5186A>T) were found in 8 cases but not in controls by in silico analysis. Non−synonymous (NS) change was found to be significantly higher (p<0.05) in ATPase 6, ATPase 8, ND1 and CO II genes compared to ND2, CO III, ND3, ND4, and ND4L genes. Sperm DNA fragmentation index (DFI) and seminal oxidative stress in infertile men was found to be significantly higher (p<0.0001) compared to control men. A strong negative correlation(r=−0.260) between testosterone level and body mass index (BMI) was observed. Smoking, alcohol and cell phone usage had moderate effect on sperm parameters. Serum prolactin level in smokers and alcoholics was found to be significantly (p<0.01) lower compared to non smoker and non−alcoholic men. Infertile men using cell phone for longer duration had significantly (p<0.05) lower sperm motility compared to non−users or moderate users. Conclusion: Male infertility is a multi−factorial problem and our study showed more factors are involved. Cytogenetic, Y microdeletion and mtDNA variants among the genetic factors, sperm DNA fragmentation and oxidative stress and compromised life style factors play an important role in male infertility. Detailed work up of infertile men may provide a significant outcome in the treatment of male infertility.

Adesina Arikawe

Comparison of streptozotocin-induced diabetic and insulin resistant effects on spermatogenesis with proliferative cell nuclear antigen immunostaining of adult rat testis

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Introduction: The overall reproductive processes are well−defined, but the underlying molecular basis of each step, from the formation of germ cells and haploid gametes to the fertilization process, is less understood. Methods: Adult male Sprague−Dawley rats (120 – 140gm) were randomly divided into 7 groups. Group 1 > control group; fed on normal rat pellets. Group 2 > Type 1 diabetic untreated group; received a single IP injection of streptozotocin 45 mg/kg BW (Guneli et al. 2010) in Na+ citrate buffer pH 4.5. Group 3 > Type 1 diabetic treated group; received IP streptozotocin as in group 2; treated with 0.5 – 1IU isophane insulin. Group 4 > Type 1 diabetic treated group; received IP streptozotocin as in group 2; treated with 500mg/kg oral ginger daily. Group 5 > insulin resistant diabetic untreated group; fed ad libitum on a special diet containing 25% fructose weight/weight. Group 6 > insulin resistant diabetic treated group; fed ad libitum on special diet as in Group 5; treated with 15mg/kg oral Pioglitazone daily. Group 7 > insulin resistant diabetic treated group; fed ad libitum on special diet as in Group 5; treated with 500mg/kg oral ginger daily. Results:
Following hyperglycaemia confirmation, animals were perfused with 4% Paraformaldehyde (PFA). Testes were isolated, weighed and fixed in 4% PFA and embedded in paraffin. 5μm thick sections were made and mounted on poly–L–lysine coated slides. Immunohistochemistry was done using PCNA and spermatogenesis was studied at Stage VII (middle) of the spermatogenic cycle through light microscopy. Conclusion: Mean seminiferous tubular diameter, PCNA index & numerical density were significantly lower (P<0.05) in all the experimental groups compared to the control group. Streptozotocin–induced diabetic and insulin resistance impair meiotic division of both primary and secondary Spermatocytes into early spermatids. Germ cells proliferation rate is enhanced by insulin, pioglitazone and ginger administrations.