SPEAKER ABSTRACTS

SATURDAY, APRIL 6, 2014
6:30 p.m. – 7:30 p.m.

EMIL STEINBERGER MEMORIAL LECTURE

iPS Cell Technology, Gene Editing and Disease Research: Issues to be Resolved

Rudolf Jaenisch
Whitehead Institute for Biomedical Research and Department of Biology, MIT, Cambridge, MA 02124, USA

The recent demonstration of in vitro reprogramming using transduction of 4 transcription factors by Yamanaka and colleagues represents a major advance in the field. However, major questions regarding the mechanism of in vitro reprogramming need to be understood and will be one focus of the talk. A major impediment in realizing the potential of ES and iPS cells to study human diseases is the inefficiency of gene targeting. Methods based on Zn finger or TALEN mediated genome editing have allowed to overcome the inefficiency of homologous recombination in human pluripotent cells. Using this genome editing approaches we have established efficient protocols to target expressed and silent genes in human ES and iPS cells. The most recent advance comes from the use of the CRISPR/Cas9 system to engineer ES cells and mice. This technology allows the simultaneous editing of multiple genes and will facilitate establishing relevant models to study human disease.
We have used this technology to generate isogenic pairs of cells that differ exclusively at a disease causing mutation. The talk will describe the use or isogenic pairs of mutant and control iPS cells to establish in vitro systems for the study of diseases such as Parkinson's and Rett syndrome.

SUNDAY, APRIL 6, 2014
8:00 a.m. – 9:00 a.m.

AUA LECTURE

CURRENT STANDARDS AND CONTROVERSIES REGARDING VASECTOMY AND VASECTOMY REVERSAL

Jay Sandlow, MD
Professor and Vice-Chair, Department of Urology, Medical College of Wisconsin, Milwaukee, Wisconsin

Introduction: Vasectomy is a safe and effective method of permanent contraception. In the United States, it is employed by nearly 11% of all married couples and performed on approximately one-half a million men per year, which is more than any other urologic surgical procedure. However, far fewer vasectomies are performed than female sterilizations by tubal ligation, both in the US and worldwide, despite the fact that vasectomy is less expensive and associated with less morbidity and mortality than tubal ligation. Recently, the American Urological Association (AUA) formed a panel of experts to develop evidence-based guidelines on vasectomy. This has been published and has been met with great acceptance; however, questions still exist regarding several aspects of vasectomy which will be addressed in this talk. Conversely, vasectomy reversal is much less common than vasectomy. Approximately 4-6% of men who have had a vasectomy ultimately request a reversal. Reasons vary, from desiring children with a new partner to a couple's desire for more children together, and rarely, due to perceived changes after vasectomy. The main controversies regarding vasectomy reversal is patient selection and cost-effectiveness compared to in vitro fertilization (IVF). The following presentation will review the standards of both procedures (using evidence-based literature when possible), as well as discuss some of the controversies that exist.
Methods: The recent AUA Guidelines were developed using an evidence-based approach. A systematic review of the literature using the MEDLINE and POPLINE databases with search dates January 1949-August 2011 was conducted to identify peer-reviewed relevant publications. The search identified almost 2,000 titles and abstracts. Application of inclusion/exclusion criteria yielded an evidence base of 275 articles. Only a small subset of these articles is referenced in this summary. A complete list of references and a full explanation of AUA guideline methodology can be found in the unabridged text of Vasectomy: AUA Guideline (2012), which is available online at http://www.auanet.org/content/media/vasectomy.pdf. Although there is not a similar document for vasectomy reversal, a group of experts are currently in the process of assembling a literature search to develop similar Best Practice guidelines.
Results: The AUA Guideline on Vasectomy became available in print and on-line in 2012. The document reviews the entire procedure, from counseling to follow up, including best practice for performing the procedure, complications and future areas for research. Literature regarding vasectomy reversal outcomes has demonstrated an overall high success rate for reversal, dependent upon various factors, including time from vasectomy, surgeon training and experience, and most importantly, female partner factors. Multiple studies have reported on cost-effectiveness in comparison to IVF, with most showing lower costs and similar outcomes for reversals. Other studies have examined the role of vasectomy reversal for post vasectomy pain, with good efficacy in carefully chosen patients.
Conclusion: Vasectomy is a highly efficacious, minimally invasive form of permanent contraception. Evidence-based literature has been used to develop guidelines for vasectomy, addressing many of the controversies using published studies. Vasectomy reversal, although somewhat uncommon, provides couples with a cost-effective method for having children after previous vasectomy. Outcomes are dependent upon several factors which should be addressed on an individual basis.

SUNDAY, APRIL 6, 2014
9:15 a.m. – 10:45 a.m.

SYMPOSIUM I – Stem Cells in the Male Reproductive Tract

UNRAVELING SIGNALING PATHWAYS CONTROLLING GONOCYTE DIFFERENTIATION

Martine Culty, PhD
The Research Institute of the McGill University Health Centre and the Department of Medicine, McGill University, Montreal, Quebec, Canada

Spermatogenesis depends on the formation of a pool of spermatogonial stem cells shortly after birth, constituting a life-long reservoir of cells that will self-renew or form progenitor cells destined to enter the spermatogenic cycle. Spermatogonia arise from the differentiation of neonatal/transitional gonocytes (pre/pro-spermatogonia). Thus, the establishment of an adequate germline stem cell pool, and subsequently male fertility, rely on the ability of gonocytes to proliferate and undergo differentiation. It is our goal to identify the mechanisms regulating gonocyte differentiation, to understand how spermatogonial stem cells are formed and to gain insights into the origins of testicular cancer and infertility.
Neonatal gonocyte differentiation is a tightly regulated process occurring within two to three days in rat, following gonocyte proliferation and migration to the basement membrane of the seminiferous cords. To identify genes that might play a role in gonocyte differentiation, we compared the gene expression profiles of rat neonatal gonocytes and spermatogonia, reflecting in vivo differentiation. Among genes differentially expressed between gonocytes and spermatogonia and during in

vitro gonocyte differentiation were several related to the ubiquitin proteasome system. Indeed, active proteasomal degradation was required for differentiation. Next, we examined the mechanisms involved in in vitro retinoic acid-induced gonocyte differentiation, and identified two signaling pathways crosstalking with the retinoic acid pathway, src and JAK/STAT. We previously reported that gonocyte proliferation also involves signaling crosstalk between PDGF and estradiol, mediated by Erk1/2 activation. Thus, gonocyte functions appear to require the coordinated activation and interaction of several signaling pathways.
These findings suggest that more than one signal transduction pathways are necessary to maintain a tight control of germ cell function within the very dynamic environment of the developing testis, which is flooded by factors regulating somatic cell proliferation and/or differentiation.
Funding provided by NSERC, CIHR, CSR and RQR grants and awards.

SUNDAY, APRIL 6, 2014
9:15 a.m. – 10:45 a.m.

SYMPOSIUM I – Stem Cells in the Male Reproductive Tract
REGULATION OF SPERMATOGONIAL STEM CELLS IN THE ADULT TESTIS
William W. Wright, PhD
Division of Reproductive Biology, Department of Biochemistry and Molecular Biology, Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland

Spermatogonial stem cells (SSCs), a subset of the undifferentiated A single (A_s) spermatogonia, are the foundation of spermatogenesis. SSCs give rise to non-stem A_s spermatogonia, which along with the A paired (A_{pr}) and the A aligned (A_{al}) spermatogonia, constitute the transit-amplifying progenitor spermatogonia. It is know that the Sertoli cell product, glial cell line-derived neurotrophic factor (GDNF), is essential for maintaining numbers of these cells, but it is unknown whether the specific cells that this growth factor affects are A_s, A_{pr} and/or A_{al} spermatogonia, or whether SSCs and progenitor spermatogonia are equally responsive to changes in GDNF signaling. To examine these issues we used a chemical-genetic approach to inhibit GDNF signaling in the adult, and identified cells by their expression of GFR□1 and their connection to one or more cells. Results showed that inhibition of GDNF signaling for two days suppressed replication of A_s, A_{pr} and A_{al} spermatogonia. Furthermore, their replication was stimulated when GDNF was added to cultured seminiferous tubules for two days. We next asked whether *in vivo* GDNF suppressed a late step in spermatogonial differentiation, expression of Kit. Results show that *in vivo,* inhibition of GDNF signaling for three or seven days progressively increased the percentages of A_s, A_{pr} and A_{al} spermatogonia expressing this marker of differentiation. Finally, we report that SSCs are lost more slowly than progenitor spermatogonia when GDNF signaling is inhibited. This suggests that SSCs are less responsive to changes in GDNF signaling than are progenitor spermatogonia.

SUNDAY, APRIL 6, 2014
9:15 a.m. – 10:45 a.m.

SYMPOSIUM I – Stem Cells in the Male Reproductive Tract
HUMAN AND NON-HUMAN PRIMATE STEM CELLS
Kyle E. Orwig, PhD
Department of Obstetrics, Gynecology and Reproductive Sciences, Magee-Womens Research Institute, University of Pittsburgh School of Medicine, Pittsburgh, PA 15213

Spermatogonial stem cells (SSCs) are at the foundation of spermatogenesis and may have application for treating some cases of male infertility. This lecture will review similarities and species-specific differences in the stem cell pool and spermatogenic lineage development between mice, monkeys and humans. These comparisons have implications for the experimental tools that can be used to study SSCs in each species as well as the interpretation of data generated using those tools. SSC transplantation is a valuable bioassay of SSC activity and may be used to regenerate spermatogenesis in infertile men. Our results in a preclinical nonhuman primate model of chemotherapy-induced infertility suggest that SSC transplantation can be used to regenerate spermatogenesis in men. The lecture will conclude with a discussion of current challenges of translating the SSC transplantation technology to the clinic.

Funding by the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development grants HD055475, HD008610 and HD061289; Magee-Womens Research Institute and Foundation; the Richard King Mellon Foundation and the United States-Israel Binational Science Foundation.

SUNDAY, APRIL 6, 2014
4:00 p.m. – 4:45 p.m.

LECTURE I
WHAT'S GOOD FOR THE SPERMATOGONIAL STEM CELL MAY BE BAD FOR THE OFFSPRING: ADVANTAGEOUS MUTATIONS THAT INCREASE THE INCIDENCE OF HUMAN DISEASE
Norman Arnheim and Peter Calabrese
Molecular and Computational Biology, University of Southern California, Los Angeles, CA USA

Besides disease mutations already present in families, new mutations occur in the germline each generation and may be inherited. These *de novo* mutations cause many well-known genetic diseases.
We studied newly arising mutations that cause Apert syndrome, achondroplasia, multiple endocrine neoplasia 2B and Noonan syndrome in the testes of normal men. These conditions arise sporadically each generation at frequencies ranging from 1/2,000 to 1/100,000 live births and share common features. 1) The frequency of new cases due to spontaneous mutation is 100-1,000 fold higher than the highest known genome-average mutation rate. 2) A single mutated gene copy inherited by a child can cause the disease. 3) New mutations always arise in the unaffected father. 4) Older fathers are at greater risk for having affected children (paternal age effect). We introduced the term RAMP for diseases with these features, which is an acronym for Recurrent mutations, Autosomal dominant inheritance, Male-biased mutation and Paternal age effect.
The unexpectedly high frequency of offspring with a new disease mutation might result from the DNA site being more susceptible to mutation (a hot spot). The frequency of spermatogonial stem cells (SSC) that

acquire a new mutation over a man's life would increase explaining the paternal age effect. We studied the above RAMP mutations in the testes of unaffected men using a testis dissection and mutation detection approach. We rejected the hot spot hypothesis for each disease mutation. Instead our data were consistent with normal SSC rarely undergoing any one of these RAMP mutations but, when they do, they acquire a proliferative advantage over the non-mutated SSC. This advantage increases the frequency of sperm carrying the mutated allele and the risk that a father will have an affected child as he ages. It is surprising that some mutations that have a selective advantage in the testis might reduce the fitness of those individuals who inherit it.

I will discuss the evidence to support these assertions. I will also suggest plausible molecular mechanisms that might explain the selective advantage of the mutated spermatogonial stem cells based on what is known about mouse and human spermatogenesis.

SUNDAY, APRIL 6, 2014
4:45 p.m. – 5:30 p.m.

LECTURE II

NOVEL SPERMATOGENIC PATHWAYS AND MALE CONTRACEPTION

Martin M. Matzuk, MD, PhD, Denise Archambeault, PhD, Julio Castaneda, PhD, Zhifeng Yu, PhD, Mary Titus, Ryan Matzuk, Julio Agno, Ramiro Ramirez-Solis, PhD, James Bradner, MD and Masahito Ikawa, PhD

Baylor College of Medicine, The Wellcome Trust Sanger Institute, Dana Farber Cancer Institute and Harvard Medical School, and Osaka University

Objectives: Over the last two decades, our research program has focused on the identification and functional analysis of genes and pathways involved in mammalian reproduction. In the process, we have identified novel genes involved in germ cell intercellular bridge formation (*e.g.,* TEX14) and the piRNA pathway (*e.g.,* GASZ). Infertility in male mice lacking a specific gene would indicate that the gene product would be a novel target for contraception in men. Our goals are to identify and characterize germ-cell specific genes required for fertility and determine if these proteins are druggable targets for contraception.

Methods: We have taken a discovery-based approach to uncover male fertility required genes and small molecules that target these essential proteins for a reversible contraceptive effect.

Results: We have already published papers on some of the novel testis-specific gene products, and in 2012, we showed that JQ1 can target BRDT for a reversible contraceptive effect in mice.

Conclusions: Our discovery-based approach has opened up new avenues of research in our laboratory and the fields of infertility and contraception. We believe that germline-specific proteins are excellent targets for reversible contraception in men.

Funding for our research has been provided by the Eunice Kennedy Shriver National Institute of Child Health and Human Development.

MONDAY, APRIL 7, 2014
8:00 a.m. – 9:00 a.m.

WOMEN IN ANDROLOGY LECTURE

HORMONE SIGNALING AND REPROGRAMMING IN HUMAN PROSTATE STEM CELLS

Gail S. Prins, PhD
Department of Urology, University of Illinois at Chicago

Early-life exposures to estrogens reprograms the rat prostate gland structure and epigenome leading to differentiation defects and increased susceptibility to cancerous lesions with aging (1). We hypothesize that developmental estrogenization of the prostate occurs, in part, through stem and progenitor cell reprogramming that permits long-term memory of this exposure throughout life. To address whether this occurs in humans, we developed *in vitro* and *in vivo* models utilizing cells from organ donors as well as human embryonic stem cells (hESC). Stem and progenitor cells were isolated from primary prostate epithelial cells (PrEC) of young, disease-free donors using FACS and 3-D prostasphere (PS) culture. Studies confirmed both cell populations as ERα$^+$ and ERβ$^+$ and showed that estradiol-17□ (E_2) significantly increased their proliferation. The estrogenic endocrine disruptor, bisphenol A (BPA), likewise increased stem-progenitor cell self-renewal and stem-related gene expression. Further, findings identify reprogrammed genes and sncRNAs in prostate progenitor cells with E_2 and low-dose BPA exposure suggesting epigenetic reprogramming. While E_2 initiated genomic ERE signaling, both E_2 and BPA activated membrane ERs with rapid induction of p-Akt and p-Erk. Additional studies identified distinct roles for ERs with ERα driving stem cell self-renewal and ERβ promoting stem cell entry into a differentiation pathway.

An *in vivo* model to assess carcinogenicity was developed using human PS cells mixed with rat UGM and grown as renal grafts in nude mice, forming normal human prostate epithelium at one month. Exposure to E_2+T for 2 – 4 months led to PIN or PCa at low incidence (13%). Developmental BPA exposure was modeled by daily feeding of hosts for two weeks after grafting (0.39-1.35 ng free-BPA/ml serum). Upon E_2+T for 2 – 4 months, the PIN/PCa incidence increased ($P<0.01$) to 33-36%. Similar modeling utilizing hESC reveals that BPA can augment prostate stem-cell self-renewal and is sufficient drive prostate pathology in the mature human prostate epithelium. Together these findings indicate that early stage progenitor and stem cells in the human prostate are direct E_2 and BPA targets and that developmental BPA exposure reprograms the human prostate epithelium leading to elevated PCa susceptibility.

1. Prins GS and Ho SM: Early life estrogens and prostate cancer in an animal model. *Journal of Developmental Origins of Health and Disease*, 1 (6): 365-370, 2010.

SPEAKER ABSTRACTS

MONDAY, APRIL 7, 2014
9:15 a.m. – 10:15 a.m.

SYMPOSIUM II – Would You Give This Many Testosterone? Case Based Discussion

J. Lisa Tenover, MD, PhD and Peter N. Schlegel, MD[1]
Stanford University, Stanford, CA; [1]Weill Cornell Medical College, New York, NY

A decision by healthcare providers to give testosterone replacement therapy to an older male patient should rely on careful consideration of the potential benefits and risks of such therapy. Each patient, however, offers has at least a subtly different clinical presentation, so weighing the relative benefits and risks for a specific patient is not always straightforward. The clinical evidence to support testosterone replacement for older men are relatively limited. During this symposium, several brief clinical cases will be presented to highlight some typical clinical situations. Each case will be followed by a review of the current literature as it pertains to the treatment issues being considered. Cases scenarios will include management of a hypogonadal man with cardiovascular disease, who has both fatigue and decline in physical function. We will also discuss treatment considerations for a hypogonadal man with erectile dysfunction who also has a history of radical prostatectomy for prostate cancer.

MONDAY, APRIL 7, 2014
1:45 p.m. – 3:15 p.m.

SYMPOSIUM III – Spermatogenesis, Post-Testicular Sperm Maturation and Male Fertility

QUANITATIVE AND QUALTITATIVE ASPECTS OF THE HORMONAL CONTROL OF SPERMATOGENESIS REVISITED

Ilpo Huhtaniemi, MD, PhD, FMedSci, Olayiwola Oduwole, PhD and Hellevi Peltoketo, PhD
Institute of Reproductive and Developmental Biology, Hammersmith Campus, Imperial College London, London W12 0NN, UK

The manipulation of gonadotropin action in genetically modified mice has provided us with novel information about qualitative and quantitative aspects of the hormonal control of spermatogenesis. We tested in the hypogonadal luteinizing hormone receptor knockout (LuRKO) mouse the concept of the hormonal male contraception, i.e. that a single dose of testosterone (T) supplementation can suppress gonadotropins and testicular T production while simultaneously maintaining extragonadal sexual and anabolic androgen actions. It was found that the dose-responses of all extragonadal and intragonadal actions of T were practically identical. Hence, a single dose of T that would produce suppression of gonadotropin and testicular T production without simultaneously turning on spermatogenesis could not be defined. This explains why the hormonal male contraception with T has insufficient efficacy. In another study we crossed the LuRKO mice with a transgenic mouse expressing a constitutively activated mutant of follicle-stimulating hormone receptor (FSHR-CAM). While the LuRKO mice are azoospermic, the FSHR-CAM mutant males have no apparent phenotype. Interestingly, the LuRKO/FSHR-CAM double mutants had normal spermatogenesis. This was initially interpreted to be due to stimulation of Leydig cell T production by Sertoli cell-derived paracrine factors stimulated by enhanced FSHR function. However, spermatogenesis persisted in the double mutant mice when they were treated with antiandrogen (flutamide). This indicated that missing androgen stimulation of spermatogenesis can be compensated for by enhanced FSH action. Hence, it appears that T and FSH have additive and complementary effects on spermatogenesis. It was shown earlier that FSH/FSHR knockout male mice have largely normal spermatogenesis. Here we demonstrate that enhanced FSH stimulation can compensate for the absence of androgens in the maintenance of spermatogenesis.

MONDAY, APRIL 7, 2014
1:45 p.m. – 3:15 p.m.

SYMPOSIUM III – Spermatogenesis, Post-Testicular Sperm Maturation and Male Fertility

CA2+ AND CAMP SIGNALING CROSSTALK DURING SPERM CAPACITATION

Pablo Visconti, PhD
Department of Veterinary and Animal Sciences. University of Massachusetts, Amherst.

Mammalian sperm become fertilization competent in the female tract in a process known as capacitation. This process is correlated with functional changes in sperm parameters such as the activation of sperm motility known as hyperactivation and the preparation to undergo a physiologically induced acrosome reaction. Taking into consideration the highly differentiated and compartmentalized nature of sperm, it can be postulated that the molecular basis of capacitation should account for independent changes occurring in different sperm compartments such as the flagellum (e.g. hyperactivation) and the head (e.g. preparation for the acrosome reaction). At the molecular level, capacitation is associated with the activation of a PKA-dependent phosphorylation cascade and with hyperpolarization of their membrane potential. It has been shown in multiple species that activation of PKA is needed for hyperactivation and to prepare the sperm for the acrosome reaction. Capacitation is also associated with the increase in intracellular Ca^{2+} concentrations. Work from our laboratory indicates that there is a crosstalk between the cAMP and the Ca^{2+} pathway. On one hand Ca^{2+} regulates cAMP synthesis and also its degradation. On the other hand, cAMP and PKA are upstream of the increase in Ca^{2+} needed for hyperactivation and for the sperm to acquire fertilizing capacity.

MONDAY, APRIL 7, 2014
1:45 p.m. – 3:15 p.m.

SYMPOSIUM III – Spermatogenesis, Post-Testicular Sperm Maturation and Male Fertility

AGING AFFECTS GERM CELLS FROM GENES TO FERTILITY

Bernard Robaire, PhD
Departments of Pharmacology & Therapeutics and of Obstetrics and Gynecology, McGill University, Montreal, Canada

The age of paternity is increasing and there is growing societal concern regarding the potential consequences of this increase to progeny. Several epidemiological studies have established clear links between paternal age and an increased incidence of conditions such as autism, diabetes, cardiovascular anomalies, and schizophrenia in the next generation. Using animal studies, we have found that increasing paternal age affects progeny outcome, sperm quality, and the response to oxidative stress. We found significantly altered expression of genes involved in DNA damage/repair, the response to oxidative stress, and cell adhesion in isolated pachytene spermatocytes, but not in round spermatids, from young and aged rats. Further analysis of pachytene spermatocytes demonstrated that genes involved in the base excision repair (BER) and nucleotide excision repair (NER) pathways were specifically altered during aging. These studies established that aging is associated with differential reg-

ulation of DNA repair pathways. Furthermore, in aged males there was an increase in 8-oxo-2´-deoxyguanosine (8-oxodG) immunoreactivity in the testes and in the number of spermatozoa positive for 8-oxodG; thus, downregulation of the BER pathway led to oxidative-stress related deficient repair of 8-oxo-dG lesions in germ cells. We also found changes in the expression of over 70 transcripts involved in cell adhesion; of these, at least 20 are specifically involved in junction dynamics within the seminiferous epithelium. The mRNAs and proteins of many cell adhesion/junction markers were decreased by at least 50% in aged spermatocytes. We saw a gradual collapse of the blood-testis barrier between 18 and 24 months. The damage to spermatogenic cells from aged rats led us to hypothesize that spermatogonial stem cells may be affected. Using CD9+ enriched GFP-marked spermatogonial cells from young and aged rats and transplanting them into the testes of busulfan-treated nude mice, we found that both colony numbers and size were affected by age. The transcriptomes of FACS-isolated spermatogonial cells were analyzed to evaluate molecular changes occurring in these cells with age. In the aged CD9+ enriched cells, an altered gene expression was found for transcripts involved in mitosis and in DNA damage response. These molecular alterations in the spermatogonial enriched population of cells from the testes of aged rats imply that stem/progenitor spermatogonia are contributors to the germ cell origin of reproductive aging.

These studies were funded by the Canadian Institutes for Health Research.

MONDAY, APRIL 7, 2014
3:30 p.m. – 4:15 p.m.

LECTURE III

THE STRESS HORMONE CORTICOTROPIN-RELEASING FACTOR ACTS IN THE BRAIN AND THE TESTES TO REGULATE TESTOSTERONE SECRETION

Catherine Rivier, PhD
The Salk Institute for Biological Studies, La Jolla, CA

Objectives: Testosterone (T) secretion is usually considered hormonally regulated by hypothalamic gonadotropin-releasing hormone (GnRH), the ensuing secretion of LH and the feedback provided by testicular steroids. However, dissociated LH and T release is observed under a variety of stressors. This led us to propose the existence of a multisynaptic neural pathway between the brain and the testes, independent of the pituitary that inhibits T secretion. Evidence for this pathway was further indicated by the ability of intracerebroventricularly (icv) administered corticotropin-releasing factor (CRF) or monoamines, to block the T response to hCG.
Methods: We injected the retrograde tracer pseudorabies virus (PRV) into the testes, lesioned specific sites of the proposed circuit and identified the brain regions of the proposed pathway by double labeling with PRV, CRF and/or and tyrosine hydroxylase (TH).
Results: PRV staining was found in the spinal cord, the locus coeruleus (LC) and the paraventricular nucleus (PVN) of the hypothalamus. Co-labelling of CRF and PRV was found in the PVN, and co-labelling of PRV and TH in the PVN, the LC and the ventral norepinephrine pathway of the brain stem. Spinal cord transection at T7-T8 prevented brain staining, and restored hCG-induced T release in rats injected with CRF or monoamines icv. The inhibition of these icv treatments is not due to sympathetically-mediated vasoconstriction of, or decreased blood flow to the testis, and is mimicked by their microinfusion into the PVN. CRF, isoproterenol or alcohol also decreased testicular levels of the steroidogenic acute regulatory protein and the peripheral-type benzodiazepine receptor.
Conclusions: We propose that in the male rat, Leydig cell function depends on both a fast, pituitary-independent neural pathway, as well as a slower hormonal pathway represented by the classical hypothalamic GnRH/pituitary LH connection. CRF and catecholamines may act as neurotransmitters in the brain-testicular circuit. Alcohol and other stressors may inhibit male reproductive functions not only through their known effects on hypothalamic GnRH and/or pituitary LH, but also through the proposed neural circuit.

Funding provided by by NIH grant AA 12810.

MONDAY, APRIL 7, 2014
4:15 p.m. – 5:00 p.m.

LECTURE IV

PHARMACOLOGICAL REGULATION OF STEROID BIOSYNTHESIS: FROM TESTIS TO BRAIN

Vassilios Papadopoulos, PhD
The Research Institute of the McGill University Health Centre and the Department of Medicine, McGill University, Montreal, Quebec, Canada

Gonadal and adrenal steroidogenesis are increased by pituitary hormones which accelerate the delivery of the substrate cholesterol from intracellular stores to mitochondrial CYP11A1. Placenta and brain make steroids in a hormone-independent manner, in the case of placenta to satisfy fetal-maternal requirements, and in the case of brain to form small amounts locally needed to control neuronal function. Considering the role of steroids as mediators of development, reproduction, body homeostasis, adaptation and behavior, it is obvious that changes in the rate of steroid formation could result in pathological states. In the testis, reduced serum testosterone (T) is common among subfertile and infertile young men. Reduced T is also common in aging men and is often associated with mood changes, fatigue, depression, decreased lean body mass, metabolic syndrome, and reduced sexual function. Although T-replacement therapy has been the treatment of choice in both young and aging men, the undesired side-effects associated with flooding the body with large amounts of T drove the search for the development of repair therapies designed to restore the ability of the testis itself to make T. In contrast, in the case of excessive steroid production associated with Leydig cell tumors, inhibitors of steroid formation might be used to control the rate of excessive steroid synthesis. In the brain, steroids have both long-term and rapid effects, acting as local regulators of neural development and excitability. Changes in neurosteroid levels are linked to the development of neuropsychiatric and neurological disorders such as depression, anxiety and neurodegeneration. Local administration of neurosteroids is unfeasible, and treatment of patients with large amounts of neuroactive steroids is unsafe. Thus, there is a clear need for developing repair therapies that restore the brain's ability to make neurosteroids. Progress in the development of compounds that target proteins involved in cholesterol transport into mitochondria in the testis and brain, and in this way help to control steroid biosynthesis in these organs, will be discussed.

SPEAKER ABSTRACTS

TUESDAY, APRIL 8, 2014
8:00 a.m. – 9:15 a.m.

SYMPOSIUM IV – PSA and Prostate Cancer Debate: Is PSA Screening A Rational Approach?

PSA AND PROSTATE CANCER SCREENING DEBATE

William J. Catalona, MD
Northwestern University Feinberg School of Medicine, Chicago, Illinois, USA

The US Preventive Services Task Force (USPSTF) and American Urological Association (AUA) guidelines take steps in the wrong direction for patient-centered care and, if implemented, would deprive many men of the opportunity to pursue shared decision-making about life-saving PSA testing. A more forward-looking approach is needed.

These guidelines are based on incomplete data and inaccurate estimates of the benefits and harms of PSA testing. Guidelines panels rely on evidence from randomized clinical trials (RCTs) and statistical modeling studies, but the available RCTs provide little reliable evidence, and some are profoundly flawed. Many medical organizations have reviewed the same body of evidence and formulated vastly divergent guidelines, ranging from the USPSTF recommending *against* PSA testing for any man, to the European Association of Urology recommending a baseline PSA test beginning at age 40-45, with follow-up testing for all men with a life expectancy of ≥10 years, always with shared decision making between the man and his doctor.

The RCTs were conducted over a limited time period and do not reveal true information about absolute benefits of screening over a lifetime. The use of RCT data to estimate benefits and harms of PSA testing underestimates benefits and exaggerates harms. In assessing benefits, the USPSTF and AUA panels focused solely on prostate cancer death without considering avoiding suffering from metastases that might not have resulted in a cancer death. An analogy would be a study of the benefits of wearing seatbelts in cars. Is the benefit only the deaths prevented, or should it also include the catastrophic injuries prevented that did not result in death? Avoiding metastases significantly shifts the balance of harms and benefits, as men diagnosed with metastases ultimately require more treatments and have more side effects.

In assessing the harms of testing, the panels cast a net over a variety of side effects of PSA testing, biopsy, and treatment that range from minor to serious. The possible harms of a simple blood test should not be linked with those of biopsy and treatment, and few of these side effects reach the extreme of a prostate cancer death.

The AUA guidelines do not recommend screening men <55 years old with an average risk of prostate cancer. The primary objective of baseline testing in men in their 40s is to assess the risk for subsequent life-threatening prostate cancer. Men in their 40s in the top 10% of PSA levels for their age group account for almost half of all prostate cancer deaths up to 30 years later, and those with levels above 1 ng/mL warrant more careful monitoring. A high baseline PSA in a man in his 40s is a stronger risk factor than African heritage or a positive family history. It is impossible to fully assess whether a man is at high risk without measuring a baseline PSA in early middle age.

The AUA did not recommend testing men <55 years is that the RCTs have not adequately tested PSA screening in this age group. The available evidence suggests it is beneficial. Starting testing at age 55 is too late. There is no reason to believe that if PSA testing works in men 55 to 69 years old, it would not also work in men 45-55 years old. Although the AUA guidance document explains that the panel does not recommend *against* PSA testing for men 40- 55 years old, the actual guidelines statement uses the language, "we do not recommend." Rather, it should read, "there is insufficient evidence to recommend *for or against* early detection in men younger than 55."

The AUA panel's suggestion for longer testing intervals needs to be reconciled with the realization that less frequent testing limits the ability to detect aggressive cancers that have the shortest preclinical phases and that, with less frequent testing, there remains the undesirable effect of detecting all of the low-risk cancers (length-time bias), possibly doing more harm than good.

The AUA also does not recommend routine testing in men >69 years old, despite the fact that 50% of prostate cancer deaths occur in men diagnosed after age 75. Age 70 is too young to stop testing in healthy men who have a 10-15 year life expectancy. Therefore, testing in men over 70 should be performed on an individual basis with shared decision-making. In the absence shared decision making, men are more than twice as likely *not* to undergo testing.

There has been a 75% reduction in metastatic disease at the time of prostate cancer diagnosis and more than a 45% decrease in the age-adjusted prostate cancer mortality rate in the U.S. during the PSA era, largely attributable to PSA testing. Similar trends have been observed in other countries where PSA testing is widely practiced. Restricting PSA testing too much would significantly compromise these benefits.

TUESDAY, APRIL 8, 2014
8:00 a.m. – 9:15 a.m.

SYMPOSIUM IV – PSA and Prostate Cancer Debate: Is PSA Screening A Rational Approach?

CHOOSING WISELY ABOUT PSA TESTING: WHY SAYING "NO" IS A GOOD HEALTH-CARE CHOICE

Timothy Wilt, MD, MPH

Questions remain whether PSA screening and subsequent early treatment for screen detected prostate cancer provides lifetime benefits that exceed harms. Yet, PSA screening for prostate cancer is common. However, current data indicate that this balance is not favorable, especially as currently practiced in the U.S. through at least 15 years and results in large health care costs. Implementation of high value prostate cancer care requires a change in practice through science-based educational and policy initiatives. A review of the goals of cancer screening strategies, best evidence regarding the main benefits and harms of prostate cancer screening, current prostate cancer screening recommendations as well as the principals and ethics of high-value care will be presented. I will provide suggestions on guiding clinicians in implementation of high-value prostate cancer care and helping their patients to choose wisely about PSA testing.

SPEAKER ABSTRACTS

TUESDAY, APRIL 8, 2014
8:00 a.m. – 9:15 a.m.

SYMPOSIUM IV – PSA and Prostate Cancer Debate: Is PSA Screening A Rational Approach?

TARGETED APPROACH TO PROSTATE SPECIFIC ANTIGEN (PSA) BASED PROSTATE CANCER DETECTION: THE RATIONAL CHOICE

H. Ballentine Carter, MD
Johns Hopkins School of Medicine

Objectives: Review the rationale for a targeted approach to prostate cancer screening using prostate specific antigen (PSA) to assess risk.
Methods: A systematic literature review was commissioned by the American Urological Association (AUA) to inform the practice of prostate cancer detection. A methodology team reviewed over 300 studies that evaluated outcomes important to patients (prostate cancer, incidence/mortality, quality of life, diagnostic accuracy and harms of testing). A multidisciplinary panel (general internal medicine, cancer epidemiology, health policy, and medical, radiological and urological oncology) interpreted the evidence and formulated statements to assist the clinician and the *asymptomatic average risk* man in decision-making regarding prostate cancer detection.
Results: There was no evidence to address the outcomes of interest to patients other than with PSA based prostate cancer screening. PSA based screening in the US was estimated to have contributed approximately 50 percent of the overall 40 percent reduction in prostate cancer mortality that occurred over the last two decades. This would be consistent with the decline in prostate cancer mortality reported in randomized prostate cancer screening trials in which there was minimal contamination of controls and low prescreening rates. However, an approach to screening that assumes that benefits will be shared equally among all ages and risk groups (non targeted), and results in treatment of most individuals after diagnosis regardless of cancer aggressiveness, resulted in over treatment rates that are estimated to be 30 percent or more. Thus, a more targeted screening approach is necessary to reduce over treatment of prostate cancer and is supported by the AUA. The strongest evidence that benefits may outweigh harms was in men age 55-69 years undergoing PSA based screening. This led the panel to recommend shared decision making for these men at average risk, but recommend against *routine* screening for other age groups at average risk. Further, to reduce the harms associated with screening (false positive tests, over diagnosis, over treatment), the panel recommended against annual screening for those who choose to be screened.
Conclusions: A panel under the auspices of the AUA recommended a targeted approach to PSA based screening that involves shared-decision making for the average risk asymptomatic man between ages 55-69 years.

TUESDAY, APRIL 8, 2014
9:15 a.m. – 10:15 a.m.

INTERNATIONAL LECTURE

PHARMACOGENETICS OF FSH

Manuela Simoni, MD, PhD
Unit and Chair of Endocrinology, Dept of Biomedicine, Metabolic and Neural Sciences, University of Modena & Reggio Emilia, Modena, Italy.

Objectives: FSH acts through its receptor, the FSHR on Seroli cells. Recently, several single nucleotide polymorphisms (SNP) were found to be associated both with biological parameters of FSH action and with the pharmacological response to FSH. Here, I will assess the potential pharmacogenetic use of FSH for infertility treatment.
Methods: Critical review of the literature and genomic databases. In vitro experiments, using human granulosa-lutein cells and transiently transfected COS7 cells. Study of the activated signal transduction pathways by Western Blotting. SNP assessed: rs6166 (c.2039A>G, p.N680S), rs6165 (c.919A>G, p.T307A), rs1394205 (c.-29G>A) in *FSHR* and rs10835638 (c.-211G>T) in *FSHB*. Literature search via PubMed. Blast analysis of genomic information available in the NCBI nucleotide database. Prospective, randomized clinical trial assessing FSH effects on sperm DNA fragmentation index in idiopathic infertile men with oligo-asteno-theratozoospermia selected according to their *FSHR* genotype.
Results: All SNPs appear first in *Homo*, result in reduced FSH action and are present with variable frequencies and combinations worldwide. Stringent clinical studies demonstrate that the *FSHR* genotype influences serum FSH levels and gonadal response. Serum FSH levels depend on the -211G>T SNP, influencing transcriptional activity of the *FSHB* promoter. Genotypes reducing FSH action are overrepresented in infertile subjects. The response to FSH in infertile men depends on the *FSHR* genotype
Conclusions: Considering the combination of *FSHR* and *FSHB* genotypes has the potential for a much stronger clinical impact than either one alone. About 20% of people are carrier of the alleles associated with lower serum FSH levels/reduced *FSHR* expression or activity, possibly less favorable for reproduction. Prospective studies need to investigate whether stratification of infertile patients according to their *FSHR-FSHB* genotypes improves clinical efficacy of FSH treatment compared to the current, naïve approach. A relative enrichment of less favorable *FSHR-FSHB* genotypes may be related to changes in human reproductive strategies and be a marker of some health-related advantage at the costs of reduced fertility.

SPEAKER ABSTRACTS

TUESDAY, APRIL 8, 2014
10:30 a.m. – 12:00 p.m.

SYMPOSIUM V – Innovations in Male Environmental Health Protection

REVOLUTION IN TOXICITY TESTING AND RISK PREDICTION FOR CHEMICALS IN THE ENVIRONMENT

Thomas Knudsen, PhD
US EPA/ORD/NCCT, Research Triangle Park, NC

Addressing safety aspects of drugs and environmental chemicals relies extensively on animal testing; however, the quantity of chemicals needing assessment and challenges of species extrapolation require alternative approaches to traditional animal studies. Newer in vitro and in silico approaches focus on predictive modeling of adverse outcome pathways (AOPs) using computational and high-throughput screening (HTS) data for thousands of chemicals and hundreds of HTS assays in EPA's ToxCast inventory. Virtual Tissue Models (VTMs) built for developmental processes simulate multiscale disruptions in the system and provide a quantitative spatio-temporal prediction of how chemicals might impact embryo-fetal development. Virtual embryo models integrate empirical data with embryological information to simulate dynamic biological tissue architectures relevant to specific AOPs. This approach is being used to evaluate chemical effects on development, such as disruption of blood vessel formation (angiodysplasia), palatal fusion (cleft palate), limb outgrowth (ectrodactyly) and urethral fusion (hypospadias) among other systems. Simulations of endocrine and vascular pathways can be parameterized in this way, using in vitro data for chemical prioritization and early lifestage exposure considerations.
This work was funded by the US EPA under its Chemical Safety for Sustainability Research Program but does not reflect US EPA policy.

TUESDAY, APRIL 8, 2014
10:30 a.m. – 12:00 p.m.

SYMPOSIUM V – Innovations in Male Environmental Health Protection

RESPONSE OF HUMAN FETAL TESIS XENOTRANSPLANTS TO ENVIRONMENTAL TOXICANTS: IMPLICATIONS FOR RISK ASSESSMENT

Kim Boekelheide, MD, PhD, and Daniel J Spade, PhD
Brown University

Objectives: Male rats exposed in utero during critical periods of reproductive development to an active phthalate, such as di-n-butyl phthalate (DBP), have alterations in the developing testis, including effects on the seminiferous cords and suppressed Leydig cell steroidogenesis. Interestingly, however, male mice similarly exposed in utero are resistant to the anti-androgenic effects of phthalates. This study used human fetal testis xenotransplants to determine the response of human fetal testis to phthalates.
Methods: Adult male athymic nude mice were castrated, and human fetal testis fragments (gestational week 16-22) were xenografted into the renal subcapsular space. Hosts were treated with human chorionic gonadotropin for 4 weeks to stimulate testosterone production. During weeks 3 and 4, hosts were exposed to DBP (500 mg/kg/d po) or abiraterone acetate (75 mg/kg/d po), a potent irreversible CYP17A1 inhibitor.
Results: Abiraterone acetate significantly reduced host testosterone and the weights of androgen-sensitive host organs, while DBP had no effect on androgenic endpoints. DBP produced a near-significant increase in multinucleated germ cells in the xenografts, an indication of an effect upon seminiferous cords.
Conclusions: We have developed an assay, similar to the Hershberger assay, that evaluates human fetal testis for anti-androgenic effects of environmental toxicant exposure. Abiraterone acetate dramatically reduced steroidogenesis in human fetal testis xenografts. Similar to the mouse, but unlike the rat, 500 mg/kg/d DBP had no effect on human fetal testis testosterone production. These results provide novel, human-relevant mechanistic insight into the effects of phthalates on the developing male reproductive tract.
Funding: Supported by grants from the National Institute of Environmental Health Sciences of the National Institutes of Health (R01 ES017272 to KB, T32 ES007272 to DJS).

TUESDAY, APRIL 8, 2014
10:30 a.m. – 12:00 p.m.

SYMPOSIUM V – Innovations in Male Environmental Health Protection

TRANSLATION OF THE SCIENCE IN MALE REPRODUCTIVE AND ENVIRONMENTAL HEALTH FOR EVIDENCE-BASED DECISIONS BY CLINICIANS, REGULATORS AND THE PUBLIC

Paula I. Johnson, Patrice Sutton and Tracey J. Woodruff
Program on Reproductive Health and the Environment, University of California - San Francisco

Patient exposure to toxic environmental chemicals is ubiquitous, and preconception and prenatal exposures can have a profound and lasting impact on reproductive health across the life course. Organizations such as the American Congress of Obstetricians and Gynecologists and the Endocrine Society have called for timely action to prevent harm. In the clinical sphere systematic reviews are used to inform risk/benefit decisions for patient care. However, due to differences in the evidence stream and decision context, there is no established corollary to making recommendations about environmental exposures. Beginning in 2009, a collaboration of 22 clinicians and scientists developed the Navigation Guide systematic review methodology, modeled after best practices in evidence-based medicine and environmental health science. As part of proof of concept we have applied the Navigation Guide methodology to the question: What is the impact of exposure to the antimicrobial triclosan on male reproductive health? We adapted established clinical medicine and healthcare quality and risk of bias tools to assess individual studies and to rate the quality and strength of an entire body of evidence for toxicity. The adoption of an efficient systematic and transparent method will speed the incorporation of research into clinical and policy decision-making to protect patient and public health.
The development of the Navigation Guide methodology and proof-of-concept was funded by grants from New York Community Trust, California Environmental Protection Agency, Clarence Heller Foundation, Passport Foundation, Heinz Endowments, Fred Gellert Foundation, Rose Foundation, Kaiser Permanente, UC San Francisco Institute for Health Policy Studies, Planned Parenthood Federation of America, National Institute for Environmental Health Sciences (ES018135), US Environmental Protection Agency EPA STAR (RD83467801) and USEPA through a contract with Abt Associates (GAIA-0-6-UCSF 17288), and appointments to the Internship/Research Participation Program at the National Center for Environmental Economics, USEPA, administered by the Oak Ridge Institute for Science and Education through an interagency agreement between the US Department of Energy and EPA.

