Report on the 31st American Society of Andrology Annual Meeting

The 31st American Society of Andrology Annual Meeting was held in Chicago on April 8-11, 2006. This year’s theme was “Contemporary Dimensions of Male Reproductive Physiology and Health”. By all accounts the program was a great success, made possible by the combination of excellent speakers, interesting topics and an engaged, conversant, inquisitive audience. President Sally Perreault-Darney, incoming President Christina Wang, the entire Program Committee and I would like to thank all of the participants – speakers, moderators, and presenters alike for their efforts. Below is a summary of the lectures and symposia to give a flavor for what was presented, learned, and discussed. This program was specifically designed and developed to be of interest to a wide variety of ASA members, spanning both basic science and clinical medicine, while bridging benchwork and patient care. Contributions to the authorship of the summary included Janice Bailey, Kirk Lo, Janice Evans, Erwin Goldberg, Gail Prins, Sally Perreault Darney, Shane Russell, Robert Viger, Patricia Cuasnicu, Terry Turner, Mark Sigman, Kim Boekelheide, Robert Brannigan, and Robert Oates.

We were favored by an eloquent first lecture from our ASA Keynote speaker John Robert Aitken. Dr. Aitken directs the ARC Center of Excellence in Biotechnology and Development and is Professor of Biological Sciences at the University of Newcastle in NSW, Australia. In his talk entitled “Molecular Mechanisms Regulating Sperm Function: From Proteomics to Peroxidation,” John deftly synthesized a large body of work showing that oxidative stress, including that associated (and accumulating) with age, is detrimental to sperm function, impairing both motility (and therefore fertilizing ability), via lipid peroxidation of sperm membranes, and DNA integrity (and therefore the ability of the sperm to support embryonic development), via the formation of DNA and protein oxidative adducts. He then explained that despite the dangers of oxygen free radicals, low levels of oxidative stress are beneficial, and in fact necessary to trigger sperm capacitation. The challenge to Andrologists and others working to improve in vitro fertilization in humans, wildlife and agriculturally important species, is to balance pro- and anti-oxidative forces to insure successful fertilization without damaging the sperm DNA. Using a proteomics approach John reported the identity of widely sought after specific tyrosine kinases and their regulators that are involved in the calcium-initiated, cAMP-dependent molecular signaling cascade that effects sperm capacitation. An understanding of this cascade obviously contributes to our understanding of the regulation of sperm function at the molecular level, including those genes and their products that may be altered in infertile or subfertile men.

Our own Gail Prins then delivered an insightful and elegant presentation on the Regulation of Prostate Development and Morphogenesis: Relationship to Prostate Carcinoma. Gail was selected to give our AUA Lectureship this year and a better choice could not have been made. Of interest to clinicians and basic scientists, she began by discussing that prostate gland morphogenesis occurs during fetal development in humans and postnatally in rodents. Thus postnatal development of the rat prostate serves as an animal model for human prostate development which allows for easy hormonal
manipulation and analysis. The gland initiates when buds of urogenital sinus (UGS) epithelium grow into the UGS mesenchyme and elongate and branch in a complex pattern. Androgens, most notably DHT, are essential and sufficient for prostate morphogenesis and differentiation. In addition, other steroids affect the developmental process, most notably estrogens via stromal ERalpha and ERbeta and retinoids via RAR/RXRalpha, beta and gamma which are found in a cell-specific manner. Several developmental genes were characterized in the developing rat prostate and some were shown to be downstream targets of androgen and estrogen action. The secreted morphoregulatory factors, Fgf10 and Shh were demonstrated to have a specific role in stimulating ductal outgrowth and distal tip branching. Inhibitory factors Bmp4 and Wnt5a where shown to be produced by the mesenchyme and function to tightly regulate branching at specific sites. Homeobox genes Hoxb-13 and Nkx3.1 are expressed in the developing prostate epithelium and were shown to have an important role in driving epithelial differentiation once the ducts have formed. Both were shown to be up-regulated by androgens and suppressed by estrogens. Gail then elaborated upon an emerging hypothesis for prostate carcinogenesis as development gone awry. Evidence was shown for increasing canonical Wnt gene expression and activation of beta-catenin during cancer progression in a rat model. The developmental estrogenization model was introduced as another example of aberrant development which predisposes to prostate cancer. In this model, early exposures to high doses of estradiol permanently change the developmental program of the prostate resulting in prostatic intraepithelial (PIN) lesions and tumors with aging. Evidence was presented to show aberrant expression of several developmental genes as a result of the early estrogen imprint. In total, the data indicate that alterations of the developmental program early in life have the capacity to sensitize the prostate gland to a neoplastic state with aging. Members of the ASA who work with the prostate gland were left with a tremendous amount to think about after Gail’s comprehensive lecture.

**Symposium I** was entitled Sex Chromosomes and Spermatogenesis. Co-chairs were Robert D. Oates, MD and Kirk C. Lo, MD. The presenters included Julian Lange (PhD Candidate), Whitehead Institute for Biomedical Research (The Cost of Palindromes in the Human Y Chromosome); Manyuan Long, PhD, University of Chicago (Male Genes Out of X: Phenomena and Mechanisms); Ronald Swerdloff, MD, Harbor-UCLA Medical Center (Why and How is the Extra X in 47, XXY Males so Harmful). Y chromosome microdeletions can lead to male infertility phenotypes. The Y chromosome is structurally quite interesting in that there are long stretches of palindromic sequences, a necessary anatomy to allow self-chromosomal repair but with the downside that it also allows for microdeletion and the formation of isodicentric derivatives. Julian Lange, a graduate student from Dr. David Page’s laboratory at the Whitehead Institute for Biomedical Research, MIT, presented data that the point of “breakage” (the point at which the two arms of an isodicentric chromosome are fused) of the isodicentric Y chromosomes studied occurred in the palindromes and was caused by illegitimate homologous recombination – the same mechanism that leads to the microdeletions that we are all becoming familiar with such as AZFc. Certainly an interesting extension of the concept originally developed and demonstrated by the Page lab to explain Y chromosomal microdeletions. Mammalian sex chromosomes have undergone profound changes since
evolving from ancestral autosomes. Dr. Manyuan Long, professor of Ecology and Evolution at University of Chicago, presented his laboratory findings by examining retroposed genes in the human and mouse genomes, and demonstrated that the mammalian X chromosome has generated and recruited a disproportionately high number of functional retroposed genes, whereas the autosomes experienced lower gene turnover. Dr. Long gave an evolutionary example where the insertion of the sphinx gene 1-2 millions years ago may have changed the male to male courting behavior to the present male to female pattern. In addition, he also discussed the extensive gene traffic involving the human X-chromosome. Finally, Dr. Ronald Swerdloff presented one of the potential mechanisms that leads to infertility in males with Klinefelter (47, XXY) syndrome. Using a mouse XXY model, Dr. Swerdloff described a decrease in germ cell and pre-spermatogonial cell number by day 7 after birth. In addition, there is a loss of androgen receptor (AR) expression in the Sertoli cells of XXY testes by day 20, while there is no change in the Leydig and myoid cells. To demonstrate the functionality or the lack of ability of XXY testes to support germ cell development, Dr. Swerdloff’s laboratory transplanted green fluorescent protein (GFP) labeled normal testicular cells into XXY testes. They found Leydig cells and spermatogonial cell colonization, but no spermatogenesis. These three excellent presentations explored various aspects of the basic structure of the sex chromosomes all the while relating them back to the pathology we see clinically when things go awry.

The oral presentations were selected as the most highly rated 12 from the entire cohort of 128 abstracts submitted and each invited speaker gave an interesting and thought-provoking talk. **Oral Session 1:** Basic Biology of Sperm Function was chaired by Terry T. Turner and Prabhakara Reddi. This interesting session included presentations on sperm-associated proteins acquired during spermatogenesis or during sperm maturation in the epididymis. Dr. Steven Chang from Stanford University presented his and his colleagues’ work on histone modifications during spermatogenesis comparing the process between flies (Drosophila) and mice. Interestingly, the timing of histone modifications differed between the two widely-separated species and the investigators will continue to look at underlying regulatory mechanisms. Dr. Polina Danshina of the University of North Carolina presented work on the impact of phosphoglycerate kinase-2 (PGK2) on sperm function. *Pgk2* is expressed only during spermatogenesis, and working with a *pgk2* knock-out model, the investigators determined that male fertility, generally, and sperm motility, specifically, were significantly reduced in the absence of PGK2. PGK2 is a glycolytic enzyme, thus suggesting the enzyme might be an appropriate target for contraceptive research. Dr. Huili Zheng of the University of Nevada described BCLT, a novel, testis-specific member of the Bcl-2 family. *Bclt*-null mice have reduced sperm counts and fertility. The authors hypothesize that BCLT functions as a prosurvival factor during spermatogenesis. Dr. Debora Cohen from the Institute of Biology and Experimental Medicine in Buenos Aires presented data on the epididymal protein known as DE or Crisp-1. Using recombinant fragments of the protein, Dr. Cohen and colleagues determined which areas of the protein’s sequence were most important for binding to the egg surface, thus adding to our understanding of the mechanism of mammalian gamete binding and fusion. Dr. Genevieve Griffiths of the University of Delaware described her work on SPAM1, another protein secreted in the epididymis and known to be important
in fertilization. Dr. Griffiths and colleagues discovered that SPAM1 is transferred to the sperm surface by two mechanisms, one vesicle-mediated, and one unknown but likely to involve fluid-phase oligomeric aggregates or monomers. Finally, Dr. Allison Gardner of the Johns Hopkins University presented her work on the role of Ca\textsuperscript{++} signaling in the block to polyspermy. Dr. Gardner determined that activity of Ca\textsuperscript{++}/Calmodulin kinase II is a key effector molecule in Ca\textsuperscript{++} signaling in that it aids in producing a graded Ca\textsuperscript{++} response in the egg membrane which blocks further sperm entry into the egg after initial fertilization. These presentations on testis- and epididymis-derived proteins were helpful in further defining the mechanisms of spermatogenesis as well as fertilization success.

Held concurrently, Oral Session II, enigmatically titled “A Potpourri of Intriguing Topics” was moderated by Janice Bailey and Alan Diekman. Dr. Youngbing Pu (and collaborators from the University of Illinois at Chicago) presented data showing that testosterone regulates the development of the rat prostate gland in a lobe-specific manner by stimulating the expression of multiple morphoregulatory genes. Dr YH Lue (and colleagues from Harbor-UCLA) captivated the attendees by showing that bone marrow stem cells harvested from adult transgenic mice expressing the green fluorescent protein marker transdifferentiated into germ cells, Sertoli cells and Leydig-like cells when injected into the testes of recipient wild-type mice. Dr. Matthew Anway (and colleagues from Washington State University) presented evidence that embryonic exposure to vinclozolin, an anti-androgenic endocrine disruptor, induces transgenerational phenotype modifications of the rat prostate and transcriptome. Dr. Peter Chan (and collaborators from McGill University and University of Montreal) spoke about their prospective study on the andrological profiles of young adult men undergoing chemotherapy for testis cancer, Hodgkin’s or non-Hodgkin’s lymphoma, and concluded that cancer treatment negatively impacts the fertility and sexual function of such men. Dr. Carla Morrow (and colleagues from the University of Illinois at Urbana-Champaign) described abnormalities to the ventral prostate in Repressor of Estrogen Receptor Activity (REA) heterozygous knockout mice. Finally, Dr. Soumya Venuganti (and collaborators from the University of North Carolina) described the expression of two testis-specific isoforms of the glycolytic enzyme aldolase A during the later stages of spermatogenesis in the mouse, which are present in sperm and tightly bound to the fibrous sheath. In sum, it was a dynamic and well-attended session that was appreciated by all participants.

John Mulhall from Cornell and New York Presbyterian Hospital gave an invigorated lecture entitled: The Scientific Foundation for PDE5 Inhibitor use in the Preservation of Erectile Function for the Prostate Cancer Patient. From his first slide, it was obvious this would be a very interesting overview of the rapidly evolving field of erectile function preservation and rehabilitation following treatment for prostate cancer, including the role of PDE-5 inhibitors in this process. He started with a list of the spectrum of problems which can accompany prostate cancer treatment, including erectile dysfunction (ED), anejaculation, anorgasmia, painful ejaculation, urine leakage associated with intercourse of orgasm, penile length changes, and penile curvature/Peyronies. Dr. Mulhall made the point that very similar pathophysiologic processes leading to ED seem to be at work in patients following various forms of prostate cancer treatment, including radical retropubic prostatectomy (RRP), external beam radiation therapy (XRT), and androgen
ablation. In patients who have undergone RRP, predictors of recovery of erectile function include whether the procedure was nerve sparing, the age of patient, pre-operative erectile function, and post-operative penile hemodynamics (venous leak and arterial insufficiency). Factors that do not seem to predict post-op erectile return include such factors as tumor volume and pre-RRP PSA. Radiation, in the forms of XRT and brachytherapy, is known to cause dose-dependent detrimental effects on the health of penile tissue, nerves, and blood vessels. Radiation damage can adversely affect both endothelial function (at dosages of only 0.1-1Gy) as well as blood vessels (>20Gy needed for large vessel damage), both of which can lead to fibrosis of the penis. Nerve function can also be impaired with radiation dosages of 10Gy. Dr. Mulhall notes that standard prostate radiation treatments usually involve prostate dosages of 80-90Gy, with the proximal penis typically receiving about 7000cGy of exposure. Androgen ablation can also have a negative impact on the recovery of spontaneous erections following treatment for prostate cancer. Low androgen states can lead to structural changes in penile architecture in as little as 30 days from onset. Preservation of erectile function following radical prostatectomy is a current topic of much discussion and debate and the data for early “penile rehabilitation” was detailed. Dr. Mulhall reviewed his own post-RRP protocol, which includes starting men on maximum dose PDE-5 inhibitors several weeks after RRP. Those that respond stay on this treatment (at maximum dose 3 times a week and low dose the other days) and are followed every 4 months. PDE-5 inhibitor non-responders are started on intracavernosal injections 3 times a week and rechallenged with PDE-5 inhibitors every few months (when they respond they are switched to this medication and scheduled injections are stopped). The primary goal is to increase oxygenation of the penis and prevent potentially permanent damage through hypoxia-induced collagen deposition. In conclusion, Dr. Mulhall gave an excellent overview of the current state of research and clinical practice being utilized to maintain and enhance erectile function in men who must undergo treatment for prostate cancer. Dr. Mark Hughes, M.D., Ph.D. from the Genesis Genetics Institute gave a beautiful, state-of-the-art presentation on Preimplantation Genetics: The Technology, the Medicine and the Bioethics. True to the title, Dr. Hughes took the audience through the amazing technology used in PGD highlighting the many ways employed to achieve the goal being sought – single gene mutation identification all the way to simple distinction of whether the embryo is male or female. What are the disorders that he is presently asked about and his lab helping with? The list is expanding every day, assisting more and more couples in their efforts to conceive a healthy child. Prevention of disease is an often neglected part of medical practice in this day and age as just trying to cure or cope with illness a patient already has is a daunting task. Here, however, we can prevent at the earliest stages of a life – at the genetic stage. As eloquent as any speaker on the program, Dr. Hughes then discussed the ethical aspects of PGD and the audience was left with a sense that we are not headed down a slippery slope as long as we keep our focus on the basic tenets of medicine and to use PGD in the most honorable and honest way possible.

The ASA was delighted to have Mary Ann Handel of the Jackson Laboratory give the Women in Andrology Lecture on Genes and Infertility: Unbiased Discovery Strategies. Dr. Handel introduced the strategies that investigators can undertake to identify genes that are involved in reproductive processes. She highlighted the current approach being
used by her and her colleagues John Schimenti and John Eppig, which she calls "phenotype-driven mutagenesis" to contrast this approach to either making knockouts in candidate genes or discovering a spontaneous mutation that has an infertility phenotype. This strategy being used by the Reproductive Genomics project, supported by the NIH, is an unbiased one, using whole genome ENU mutagenesis and screening for the failure to produce offspring. The project's website, reprogenomics.jax.org, provides an excellent overview of the mutagenesis and mating strategy and of the assessments of possible causes of infertility in both males and females. Male mice are treated with ENU, and then mated in a three-generation breeding scheme to produce mice potentially homozygous for any mutant allele. Both females and males in the G3 generation are mated with wild type mice to test for their ability to produce offspring, and any infertile individual is subjected to a clinical work-up that Dr. Handel noted was analogous to human infertility clinic diagnostics. Dr. Handel reported that more the 12,000 mice had been screened, resulting in 35 that have been mapped and defined as separate distinct mutant lines. Of these 35, interestingly, a notable majority (71%, 26/35) shows infertility in males only. Of the remaining nine lines, two show female infertility, and seven show infertility in both males and females. The reason for this skewed ratio is not known, although in the discussion period Dr. Michael Holland of Monash University remarked that their own ENU mutagenesis project has resulted in the same bias for male infertility over female infertility in two genetic backgrounds. The ReproGenomics project at the Jackson Laboratory has successfully identified genes that have been previously characterized to have a role in reproduction. The repro11 mutant, which had male infertility due to spermatogenesis arrest, was an example of this. This mutant was mapped to chromosome 15, and ultimately identified to have a point mutation in the Smc1b gene (encoding a meiotic cohesin) that caused premature termination. Indeed, the phenotype of the repro11 mutant is very similar to the Smc1b knockout. The project has also identified several novel genes; Dr. Handel noted two, repro4 which produces spermatogenic arrest during meiosis and ferf1 which causes abnormalities in sperm function. Although the responsible genes have not yet been cloned, the phenotypes have interesting implications for infertility treatment and contraceptive development. Dr. Handel noted in closing that the ReproGenomics project leaders are willing to collaborate with those in the field who are interested in characterizing a mutant in more detail; for example, the program could conduct the genetic fine mapping that leads to gene identification.

Moving away from trying to enhance fertility and discovering reasons for infertility, Symposium II concentrated upon an area of equal interest to many ASA members: Contraception - Beyond Abstinence, moderated by Erwin Goldberg, PhD and Patricia L. Morris, PhD. Mike O’Rand opened the session with his presentation focused on the sperm protein Eppin. Eppin (SPINLW1; serine protease inhibitor-like, with Kunitz and WAP domains 1) is a member of the whey acidic protein (WAP)-type four-disulfide core (WFDC) gene family. Two isoforms of the Eppin protein are expressed; one is secreted and one lacks a signal sequence. On the surface of ejaculated human spermatozoa Eppin is found in a tri-molecular complex with semenogelin and PSA. Eppin inhibits PSA and anti-Eppin antibodies inhibit semenogelin from binding to Eppin. Eppin is a reasonable target for the development of a male contraceptive. Erv Goldberg reviewed three
categories of testis specific gene products as targets for contraceptive development; glycolytic enzymes, ion channels, and signaling molecules. Potential contraception is suggested by targeted disruption of the genes encoding GAPD-S and PGK-2 from the O’Brien and Eddy and O’Brien, Eddy and McCarrey laboratories, respectively. Mutant sperm had significantly lower ATP content, could not be hyperactivated and were unable to fertilize the egg. Modeling the 3 dimensional structure of these proteins is underway to map critical binding sites and identify lead molecules that perturb enzymatic activity. Goldberg is collaborating with Mitch Eddy to knock out the ldhc gene and determine its potential as a contraceptive target from the phenotype. Sperm specific ion channels include CatSper (described by the Clapham and Garbers labs) and a sodium hydrogen exchanger (sNHe) reported by the Garbers group. A spectacular technical achievement, patch-clamping sperm, will allow study of ion channel and their druggable properties for contraception. The soluble adenylyl cyclase (sAC) plays an important role in signaling events for sperm capacitation and hyperactivation. Conti showed that these processes are absent from mutant sperm after targeted disruption of the gene. SAC appears to be sufficiently different from the membrane bound cyclase to allow development of specific inhibitors that would have contraceptive properties. The final presentation by Don Johnston covered the basic steps in the drug discovery pathway and the use of transcriptional profiling, proteomics and bioinformatics to identify novel targets for the development of non-hormonally acting male contraceptives. In particular, detailed transcriptional profiling of the rat testis, rat epididymis and mouse epididymis was described as well as a cataloging of the human sperm proteome. Examples were provided on how these data could be compared to transcriptional profiling data of multiple non-reproductive tissue to identify reproductive-tract specific qualifiers for further characterization and target selection.

Symposium III was co-chaired by Paul Cooke and Jon Pryor and was entitled Endocrine Issues in Male Reproductive Failure. Stephanie Seminara from Massachusetts General Hospital led off with a fascinating talk on Idiopathic Hypogonadotropic Hypogonadism: An Update on Genetic Etiologies as the past 3 years have been witness to remarkable developments in the understanding of the genetics of idiopathic hypogonadotropic hypogonadism (IHH), including the discovery of the role of the metastin-GPR54 pathway. Mutations in the G protein-coupled receptor, GPR54, have been identified in patients with IHH, supporting the important role for this receptor, and by extension, its ligand, in sexual maturation in the human. Since these genetic discoveries, several investigators have demonstrated that when administered as a single bolus, metastin is a potent stimulus for GnRH-induced LH secretion in vivo. In contrast, continuous administration of metastin appears to desensitize the GPR54 receptor and lower LH levels. Strategies for Stimulating Androgen Production in Aging Men and Men with Secondary Hypogonadism was discussed by Alvin M. Matsumoto, M.D. (2007 Annual Meeting Program Chair). Testosterone (T) replacement remains the major therapeutic approach used to treat men with both primary and secondary hypogonadism. In men with complete or partial secondary hypogonadism (gonadotropin deficiency due to hypothalamic or pituitary disease, e.g. Kallmann syndrome), a feed-forward strategy utilizing human chorionic gonadotropin [hCG] or pulsatile gonadotropin-releasing hormone [GnRH] administration may be used to
stimulate endogenous T production by the testes. In men with partial combined secondary and primary hypogonadism (partial defects in both gonadotropin secretion by the pituitary and T production by the testis, e.g. aging men), an alterative strategy is to interrupt steroid negative feedback regulation using an estrogen receptor modulator, e.g. clomiphene or raloxifene, or aromatase inhibitor, e.g. anastrazole or letrozole, to increase endogenous gonadotropin secretion which in turn, stimulates endogenous T production. GnRH or gonadotropin therapy is usually utilized to stimulate spermatogenesis in addition to T production in men with secondary hypogonadism who desire fertility. Although the response to therapy and cost is comparable to gonadotropin therapy, the availability and practicability of pulsatile delivery of GnRH limit its use in clinical practice. The dosage of hCG administered twice weekly to every other day is titrated to achieve normal serum T levels, and is much lower than used previously. In men with post-pubertal acquired or partial prepubertal gonadotropin deficiency, treatment with hCG alone will stimulate T production but may also induce spermatogenesis. Men with more complete prepubertal gonadotropin deficiency (as evidenced by very small testes and low serum gonadotropin concentrations) usually require FSH (human menopausal gonadotropin [hMG] or recombinant human FSH) administration in addition to hCG, the cost of which may be prohibitive. Prior T therapy does not affect the spermatogenic or androgen response to gonadotropin therapy. However, the response to gonadotropin therapy is clearly reduced in men with a history of cryptorchidism or other primary testicular disease. At present, the evidence base for the use of estrogen receptor modulators and aromatase is limited. Studies have involved small numbers of men, been short-term and examined only hormone responses and surrogate outcomes. Longer-term clinical outcomes studies have not been performed. So, the long-term consequences of estrogen deficiency or estrogen receptor antagonism induced by these strategies on brain function (sexual function, mood and cognitive function), bone mineral density, cardiovascular risk and the prostate gland, and of chronic stimulation of the pituitary and testes are unclear. Therefore, the clinical utility of these feedback strategies to increase androgen production, especially in aging men, await further longer-term efficacy and safety studies examining their potential benefits and risks. V. S. Wilson from the US Environmental Protection Agency spoke about Environmental Anti-Androgens: Altered Development and Function in the Male Reproductive Tract. Dr. Wilson began with a brief review of the background on the issues surrounding the EDC (endocrine disrupting compounds) and the controversial human health issues (such as increasing rates of testicular cancer, increased incidence of hypospadias, declining sperm counts) that may be related to EDC exposure. This was followed by an overview of the work on environmental compounds that act as anti-androgens in laboratory studies. The bulk of the presentation and the major research focus were the effects of in utero phthalate exposure on the male rat offspring. Research presented included details on the suite of malformations seen in adult male offspring after in utero exposure to some phthalate esters including DEHP (diethylhexyl phthalate), DBP (dibutyl phthalate) and BBP (benzylbutyl phthalate). Exposures of the dam during gestation resulted in a wide range of reproductive effects on androgen dependent tissues in adult male offspring including hypospadias, undescended testes, epididymal agenesis, histological lesions in the testis and epididymis in adults and on androgen dependent processes resulting in reduced anogenital distance in males at post-natal day 2. The critical window of exposure to
produce these effects in the rat is during the period of sexual differentiation of the fetal reproductive tract. Current studies are examining the changes that occur in the fetal testis during this window of exposure. Ex vivo culture of gestational day (GD) 18 testes showed reduced testosterone production after phthalate exposure. Also, on GD18, insl3 (a gene responsible for gubernacular cord development and the first stage of testes descent) gene expression was significantly reduced. Expression of several other genes in the testis was also affected by phthalate exposure. For example, many of the genes involved in steroidogenesis were significantly reduced. Studies with binary mixtures of phthalates indicate that in utero exposure produces cumulative effects on many of these endpoints. Also, mixtures of anti-androgens and an anti-androgen with a phthalate which impact the same pathway through different mechanisms of action also produce additive effects. The action of phthalate esters on male reproductive development has primarily been evaluated in rats and there is no direct human data relating exposure of phthalates with adverse outcomes in humans. There are striking parallels, however, between this “phthalate syndrome” of effects seen in male rat offspring and the reported human testicular dysgenesis syndrome. It was also noted that the pathways/genes affected are highly conserved between rats and humans, the active metabolites responsible for the reproductive toxicity, the monoesters, are produced by both rat and humans and that these metabolites are commonly found in both the urine and amniotic fluid of humans. Also, a key event, the reduction of fetal testosterone synthesis, is believed to have a parallel mechanism of action in both species with respect to the resultant effects on androgen dependent processes. These parallels warrant further study. Dr. Niels Geijsen (Harvard Medical School and Center for Regenerative Medicine and Technology, Massachusetts General Hospital) gave an elegant and interesting presentation in the breakthrough area of germ cell derivation from embryonic stem cells (ES). Dr Geijsen’s novel research is centered on elucidating the mechanisms by which germ cells (the only truly totipotent cells of our bodies) maintain their pristine state, and in particular the reprogramming of epigenetic information that occurs during germ cell development. Since epigenetic control of gene expression limits the ability of somatic cells to transdifferentiate into cells of a different cell type, nuclear reprogramming holds the promise for the development of novel therapeutic treatments of diseases. Since germ cells normally undergo nuclear reprogramming, the germ cell is the model system of choice for studying this process. In the first half of his presentation, Dr. Geijsen described the system that he and others have developed to derive primordial germ cells (PGC) from mouse ES cells in vitro. Dr. Geijsen utilizes embryoid bodies (EB) to differentiate mouse ES cells into PGCs. EBs form when ES cells are cultured without pluripotency-maintaining factors. EB formation is an essential step for commitment to the germ cell lineage. EB-derived PGCs express multiple germ cell markers including the pluripotency markers SSEA1 and OCT4. These cells mimic in vivo PGCs in their ability to respond to BMP4, to erase parental imprints and interestingly, to colonize the mouse testis and produce viable sperm. Dr. Geijsen’s group has recently identified a rare subpopulation of EB-derived PGCs that can undergo meiosis to produce round spermatids (identified by classical morphology and markers of meiotic male germ cells) that have the capability to produce embryos when injected into mouse oocytes. This system has therefore the potential to advance the field of andrology by greatly facilitating the study of male germ cell development. Dr. Geijsen is currently refining this system through the characterization of alternatively cultured murine ES
cells, otherwise known as ACME cells. Dr. Geijsen ended his talk with a description of these cells and how they compare to traditionally cultured ES cells for the generation of PGCs. Dr. Geijsen’s talk was followed by an outstanding presentation given by Dr. Keith Parker (University of Texas Southwestern Medical Center at Dallas), an internationally recognized expert on the role of the nuclear receptor steroidogenic factor-1 (SF-1/NR5A1) in the hypothalamus-pituitary-gonadal axis. Dr. Parker began with a description of a novel strategy to identify genes that specify ovarian development. Unlike the testis, where many critical male-determining genes are known, the ovary remains a black box when it comes to genes that dictate female gonad development. He then moved on to a description of SF-1 function in the reproductive axis. Dr. Parker’s group was the first to demonstrate, via mouse knockout studies, that SF-1 is essential for early gonadal morphogenesis in both sexes. Mice lacking the SF-1 gene have a complex phenotype characterized by gonadal and adrenal agenesis and defects at the level of the hypothalamus and pituitary. To probe the role of SF-1 in its different target tissues, he also described the results of more recent tissue-specific SF-1 inactivation experiments. In the testis, inactivation of SF-1 in the steroidogenic Leydig cell lineage results in cryptorchidism presumably through a failed activation of the insulin like factor 3 (INSL3) gene that encodes a critical hormone required for normal testicular descent during fetal development. Dr. Parker is also currently employing this tissue-specific targeting strategy to examine the brain-specific role of SF-1. Dr. Parker ended by providing some insights into the role of SF-1 in human disease. Due to its critical role in maintaining proper adrenal function, human SF-1 gene mutations that severely affect its function are very rare. Dr. Parker’s strategy has been to identify milder mutations where gonadal, but not adrenal function, is compromised. His approach appears to be a good one since an increasing cohort of novel human SF-1 mutations are now being identified.

Symposium IV was chaired by Mark Sigman and Ajay Nangia: New Developments in Sex Determination and Testis Differentiation. Geert De Vries began with an enticing topic: Sex chromosomes and Sex Hormones At Odds in Creating Neural Sex Differences. Sexual differences in vasopressin neural projections were discussed. The male brain has more vasopressin neural projections than female brains. Neonatal male castration causes decreased vasopressin projections while neonatal testosterone exposure induces increased numbers of vasopressin projections. Cell death during embryogenesis is involved in cell distribution but not in the initial cell differentiation. Interestingly, during sexual differentiation, males express more progesterone receptors than females. It was hypothesized that neural differences between the sexes could induce differences in behavior such as aggression or sexual behavior. Alternatively, these differences could compensate for differences in physiology between the sexes. This might result in behavioral similarities such as social memory, parental behavior, or aggressive behavior. Homeobox Genes and Male Reproduction was presented by Jim Maclean. The homeobox domain, which binds DNA, is present in over 200 genes including Hox and Rhox. Rhox is expressed in reproductive tissues. The role of Rhox genes in the post-natal period and adult is unclear although in the adult they are expressed primarily in reproductive tissues, especially epididymis and prostate. The Rhox gene cluster is on the mouse X chromosome and consists of a cluster of 12 Rhox genes which are likely transcription factors. These may be regulated by androgens, especially in Sertoli cells. The rhox 5
knockout demonstrates decreased numbers of sperm and increased germ cell apoptosis. Interestingly Sertoli cell expression remains normal. Rhox somehow regulates some Leydig cell genes but is not expressed in these cells. Other possible regulatory roles include insulin II expression in Sertoli cells. The Role of Vascular Endothelial Growth factor (VEGF) in Testis Morphogenesis was presented by Andrea Cupp. Sertoli cells produce VEGF which induces mesonephric cell migration to produce sex cord development. The VEGF mRNA has alternative splicing sites yielding isoforms which have different functions on vascular growth. VEGF 164 is the most potent. The receptors for VEGF include KDR and FLT1. Inhibitors of VEGF result in arrest of both vascular development and seminiferous cord development.

The ASA was honored to have Dr Trevor Cooper present the International Lecture: Sperm Volume Regulation; A New Role for the Epididymis? The title hints at a new function for the epididymis in controlling sperm volume regulation. Starting from observations on infertile, transgenic, male mice, whose spermatozoa were swollen and unable to reach the oviducts, a hypothesis was presented that sperm osmolytes were provided by the epididymis to counter the osmotic challenges (up to100 mmol/kg) they experienced in the female tract of many species. Some evidence for this was presented in boars where the luminal fluid concentration and the sperm content of glutamate increased as they passed through the organ. Recent observations confirmed that similar osmotic challenges face human spermatozoa at ejaculation. Incubation of epididymal and ejaculated spermatozoa from mice and men in physiologically relevant hypo-osmotic media in the presence of inhibitors of channels involved in volume regulation of somatic cells identified K⁺ channels and Cl⁻/organic osmolyte channels to be involved in volume regulation in both species but the K⁺-Cl⁻ co-transporter only in murine spermatozoa. The location of the channels was the cytoplasmic droplet in both species and a discussion ensued on the different use of the term cytoplasmic droplet by basic scientists and clinicians. The presence of droplets on the majority of human spermatozoa demonstrated in live sperm preparations was not evident from routine air-dried smears which subjects spermatozoa to abnormally large osmotic shocks. The importance of sperm volume regulation was highlighted by reference to (1) naturally infertile domestic species, and possibly man (spermatozoa from fathers regulate volume better than those from patients), (2) sperm handling in the lab, and (3) the large osmotic shocks suffered by spermatozoa transferred to cryoprotectants. The final part of the talk returned to the concept of epididymal maturation. By comparing the different osmotic challenges given to mature and immature spermatozoa during the insemination procedure, and their differing ability to cope with the stress, it was concluded that a major reason for the poor fertilising capacity of immature spermatozoa was their underdeveloped volume regulation - not a new idea at all, but one that had been around since the 1930s!

In Symposium V: Value of Basic Science for Advancing Clinical Practice and Public Health, two talks were presented, including “Sertoli Cell Toxicants: Theory and Practice” by Kim Boekelheide, and “Tracking Distal Control Elements of FSHR by Comparative Genomics and Transgenic Analysis” by Leslie Lynn Heckert. “Sertoli Cell Toxicants: Theory and Practice” reviewed the important role of Sertoli cells in modulating germ cell production through the elaboration of both survival and killing factors. Sertoli cell
toxicants were defined as those toxicants that produced the first evidence of testicular damage in Sertoli cells, typically vacuolation seen by light microscopy or upregulation of a killing signal, such as FasL. The effects of Sertoli cell injury are dependent upon life stage and include phthalate-induced multinucleated gonocytes, associated with perinatal exposures, and irreversibly testicular atrophy, associated with high dose exposures in adults. Because of the potential for additive or synergistic adverse effects, exposures to mixtures of Sertoli cell toxicants or mixtures of toxicants that target different interacting cell types within the testis are important areas for future research.

“Tracking Distal Control Elements of FSHR by Comparative Genomics and Transgenic Analysis” reviewed the extensive research effort directed at elucidating the molecular control of expression of the FSH receptor (FSHR). The major focus of this work has been to identify the regulatory control elements in the promoter regions of FSHR responsible for tissue specific expression. This has been a daunting task because the FSHR gene is found in a region of DNA with widely separated genes, and therefore large stretches of repetitive sequence. Using traditional approaches to study the activity of the proximal promoter, it was rapidly discovered that the proximal promoter was insufficient to designate tissue specificity. Current approaches involve comparative genomics using widely divergent species—comparing chicken, mouse, and dog to human—with the expectation that highly conserved regions are likely important to regulation of expression. In addition, transgenic approaches with Yeast Artificial Chromosomes (YACs) have been used to express the rat FSHR in mice with large amounts of adjacent non-coding DNA. To date, a YAC with ~200 kb of upstream DNA was insufficient to restrict expression of FSHR to Sertoli cells, although these approaches hold promise to eventually identify the regulatory sequences.

All in all, the program displayed the talents of those who work in the field of Andrology. The ASA is greatly appreciative of the efforts put in by all of the presenters and invited speakers as well as the moderators and reviewers.

Respectfully submitted on behalf of the Program Committee: Janice Bailey, Janice Evans, Gail Prins, Sally Perreault Darney, Robert Viger, Patricia Cuasnicu (International Speaker Chair), Shalender Bhasin, Terry Turner, Gabor Huszar, Christina Wang, Mark Sigman, Kim Boekelheide, Wayne Hellstrom (Postgraduate Course Chair), Gary Klinefelter, Dorrie Lamb, Robert Brannigan, and Robert Oates (Program Chair).

July 18, 2006