Synopsis: 2005 Annual Meeting of the American Society of Andrology

The 30th Annual Meeting of the American Society of Andrology (ASA) was held in Seattle, Wash, April 2–4, 2005. The Annual Meeting was preceded by the Testis Workshop, held from March 31 to April 2. Dr Michael Palladino chaired the Andrology Laboratory Workshop. This full-day "Sperm Morphology Workshop," held on April 2, was a laboratory-based class with hands-on training in the microscopic assessment of sperm morphology according to classification methods currently used in clinical laboratories.

The program for the 30th Annual Meeting with the theme "Androgens and their Target Organs" featured 6 plenary lectures, 1 plenary debate, and 5 symposia. The meeting opened with the ASA Lecture "Genes, Gender, and Germ Cells," presented by David Page, PhD, from the Whitehead Institute, Massachusetts Institute of Technology, Boston. In an outstanding career spanning over 20 years, Dr Page’s studies have addressed and answered fundamental questions concerning the mechanisms that underpin gonadal sexual differentiation. His work has been instrumental in elucidating the contribution of genes on the Y chromosome to male development, while illustrating potential mechanisms by which this chromosome has evolved to its current form in the human. His ASA Keynote Lecture described recent work in his laboratory that addresses the absence of information about how an ovary develops in the absence of a stimulus to become a testis. He points out that although it is clear that the somatic cell lineage, specifically the pre-Sertoli cells in the fetal gonad, drive formation of the testis, little to no information is available about how an ovary forms.

As his tale of the 3 PhD students (Menke, Baltus, and Koubova) unfolded, we learned what happened when the team posed the hypothesis that the germ cells were the driver of somatic differentiation in the ovary. In an effort to identify genes involved in gender-specific gonad development, a subtractive hybridization experiment yielded predominantly somatic and male-specific genes, a disappointing blow to the original hypothesis. However, in reevaluation of the published gender-specific expression of the retinoic acid receptor-responsive gene, Stra8, Page’s team learned that this gene was both female- and germ cell-specific in the fetal gonad, being expressed in accordance with the onset of meiotic prophase and directly preceding expression of other meiotic markers. A similar pattern was seen in the postnatal male germ cells, and additional experiments demonstrated that fetal expression of Stra8 correlated with germinal male germ cell differentiation into the female lineage, regardless of somatic and germ cell genotype. Production of a Stra8<sup>-/-</sup> mouse resulted in the cessation of germ cell development in both males and females coincident with meiotic onset, demonstrating the critical role of Stra8 in this stage of gametogenesis. To investigate this phenomenon further, organ culture experiments have illustrated the reliance of Stra8 expression on retinoic acid, and this can be manipulated in both male and female gonads, again illustrating the independence of this phenomenon from genotype. With additional culture data, Dr Page illustrated the potential importance of the P450 enzymes, which degrade retinoic acid, to suppression of Stra8 in the developing male gonad. The site and nature of the relevant enzymes remain to be determined, as does the manner in which these events affect somatic cell differentiation. The ASA Lecturer presented an elegant and convincing case for the importance of retinoic acid and germ cells as mediators of male gonad differentiation, and these studies provide the first demonstration of how the onset of meiosis is regulated in mammals.

The International Lecture (ParentPlus Lecture) titled "Mutations in Male Infertility: Of Mice and Men" was given by Yoshitake Nishimune, MD, from Osaka University, Institute for Microbial Diseases, Osaka, Japan. ASA delegates were treated to an overview of the recent wide-ranging accomplishments of Dr Nishimune and his laboratory associates who are working to identify key genes involved in control of mammalian spermatogenesis. An estimated 25% of the mammalian genome is expressed specifically during the haploid phase of spermatogenic differentiation, and these are logical targets for fertility intervention and infertility investigations. Nishimune and colleagues set out to catalog these spermatid-specific genes in the mouse using a combination of cDNA expression library screening with spermatid-specific antibodies and cDNA subtractive hybridization studies. Dr Nishimune illustrated the identification and characterization of 80 genes, termed TISPs (transcripts increased in spermatogenesis). Many TISPs are characterized by the absence of introns (observed in 50%) and a TATA box, in addition to having an unusually high frequency of cyclic AMP response promoter elements (CREs) and being...
CpG rich. The derivation of knockout mice lines displaying male and female sterility has convincingly demonstrated their key roles in mammalian gametogenesis (DMC1, tektin-t, HANP1, TPAP, Prm1, Prm2). With the objective of understanding the contribution of gene mutations to the 60%–70% of cases in human infertility that are currently unexplained, Professor Nishimune and his colleagues began a search for single nucleotide polymorphisms (SNPs) that were associated with human male infertility in a set of 226 fertile and 270 infertile patients. SNPs in 4 haploid genes (protamine 2, TNP1, haspin, and SCOT-t) were identified within the infertile sample set, and the effect of the DNA modifications was described and discussed. In several cases, SNPs were identified that were not associated with male infertility (TNP2, Prm1). Dr Nishimune stressed the need to continue screening a larger set of patients to understand the contribution of haploid-specific contributions to human fertility.

On the same theme, Barbara Wakimoto, PhD, from the University of Washington presented “Toward a Comprehensive Genetic Analysis of Male Fertility Using Drosophila.” Drosophila melanogaster is a model organism that can be used to better understand the genetic basis of male infertility. Drosophila has several advantages, including well-characterized genetics and the availability of powerful tools, favorable cytology with the different developmental stages organized in chronological order in a single tube, and the presence of prototypical flagellated sperm. Dr Wakimoto presented an update on fertilization and paternal effect mutations that have resulted from a large-scale screen she and her colleagues have carried out in a search for male sterile mutations within the Zuker collection, a resource for the analysis of autosomal gene function in drosophila. Of over 2000 male sterile lines that were characterized, 81% failed during spermatogenesis, whereas 19% showed post- oratogenic abnormalities. Of the post- oratogenic sterile males, mutants were found that were defective in mating, sperm transfer, or storage and sperm-egg recognition. An interesting class of “paternal effect” mutants defective in sperm activation, pronuclear formation and competence, and paternal chromosome maintenance were of particular interest. Mutations in 5 different genes (eg, snky, a gene conserved in mouse and human) caused problems with plasma membrane breakdown. Characterization of the snky mutation suggested that the acrosome functions as a signaling vesicle rather than just a secretory vesicle in Drosophila, raising questions about conservation between species. Some mutants helped identify genetic pathways, such as those involved in acrosome biogenesis during spermiogenesis. Dr Wakimoto also described a reverse genetics technique, TILLING (targeting induced local lesions in genomes), she has been using more recently to uncover novel molecules involved in reproduction. In TILLING, one starts with a gene in which the mammalian homolog has a proposed or proven role in fertility and then looks for mutations in the gene in the Zuker collection. Dr Wakimoto expects this new high-throughput approach to facilitate the identification of multiple mutations within a given pathway. Given the conservation of cellular and molecular events between organisms, these efforts to identify factors that control male fertility in Drosophila should provide important information and insights for studies in humans.

The Woman in Andrology Lecture on “Novel Glycolytic Enzymes and Sperm Motility” was delivered by Deborah O’Brien, PhD, University of North Carolina. Dr O’Brien’s research has focused on this aspect of sperm biology. Previous research has shown that cytochrome C knockout mice are fertile, an unusual observation because this is the only cytochrome C source in sperm. Moreover, blocking oxidative phosphorylation does not impede fertility. Therefore, Dr O’Brien’s work addresses the hypothesis that the glycolytic pathway is required for male fertility. Previous in vitro studies indicated that glycolysis is required for sperm hyperactivation and protein tyrosine phosphorylation, 2 events required for fertilization. Moreover, substances that are metabolized to (S)-3-chloroalactaldehyde, a glyceraldehyde-3-phosphate analog, are able to block fertilization. Interestingly, sperm glycolytic enzymes are located in the principal piece, not in the mitochondria, leading to the hypothesis that ATP is synthesized locally to support axoneme function and motility. Therefore, they isolated the mouse sperm fibrous sheath and conducted proteomic analysis to reveal a number of tightly bound glycolytic enzymes. Two enzymes are of particular interest: glyceraldehyde 3-phosphate dehydrogenase-S (GAPDS) and phosphoglycerate kinase 2 (PGK2), because they are germ cell-specific and their inhibition blocks glycolytic ATP production. Sperm GAPDS (GAPD2 is the human ortholog), possesses a highly conserved, proline-rich N-terminus extension that is not present in somatic cell enzymes. The protein is expressed in late spermatids, although the mRNA is present much earlier during spermiogenesis. Proteolysis experiments indicate that this N-terminus is likely involved in GAPDS binding to the fibrous sheath and that this tight association is consistent with the hypothesis that GAPDS is important for sperm motility. GAPDS knockout mice were indeed infertile, although testis morphology, sperm production, and sperm structure appeared normal. Motility analysis, however, revealed that the sperm from the GAPDS knockouts was very poor (0.1% progressively motile vs 61% for controls). As well, sperm proteins from the knockout mice were unable to undergo tyrosine phosphorylation as in controls. ATP production in these sperm was about 10% of the levels in control sperm, strongly suggesting that GAPDS is required for sperm ATP syn-
thesis, which is required for motility and fertilization. Dr O’Brien’s team also generated PGK2 knockout mice, and very recent data indicate these animals show severe male subfertility, although fertilization occasionally occurs. These mice also demonstrate a major defect in sperm motility, but to a slightly lesser degree than the GAPDS knockouts. Again, the PGK2 knockouts demonstrate low sperm ATP production (approximately 10% of control levels).

Both of these germ cell–specific glycolytic enzymes are required for normal fertilization. The potential of these enzymes as contraceptive targets has also been broached with a structure-based design of inhibitors of sperm glycolysis. A recombinant form of GAPDS (without the proline-rich N-terminus region) was expressed, and homology models were generated. Inhibitor docking screening was then conducted to identify inhibitors that are predicted to fit into a functionally important pocket of GAPDS, according to the model. Taken together, this research provides great insight into the molecular mechanisms of sperm function and fertility and is pertinent to understanding certain causes of male infertility in addition to contraceptive development.

The clinical lectures included Wayne Hellstrom, MD, Tulane University, who spoke on “Gene Therapy for Erectile Dysfunction.” Dr Hellstrom gave an enlightened and informative plenary lecture that began with a brief history of erectile dysfunction (ED) treatments, historically managed by sex therapists until the 1960s, when penile prosthesis surgery garnered the interest of urologists. He mentioned that the prevalence of ED is expected to increase over the next 30 years from the current 152 million men worldwide to an estimated 322 million men on the basis of current population trends. In speaking about the causes of ED, Dr Hellstrom reminded the audience that “erectile function is a monitor of the vascular system in general,” as conditions that affect the endothelium throughout the body such as diabetes, hypertension, and dyslipidemias likely also affect penile erections. Dr Hellstrom defined the need for more research into gene therapy strategies for ED because 30%–40% of men do not respond to currently available phosphodiesterase-5 inhibitors (PDE-5i). Possible indications for gene therapy in ED include patients who are PDE-5i nonresponders, cannot tolerate PDE-5i side effects, are not candidates for PDE-5i therapy, or are fearful of taking more oral medication. The advantages of gene therapy for ED could include restoration of complete function, no need for “planning” for sex that is currently required for PDE-5i’s, reduction in use of concomitant oral therapies, and targeting therapy to specific abnormalities of erectile function.

Six different approaches to ED gene therapy were then reviewed. Endothelial nitric oxide synthase (eNOS) gene therapy has undergone the most research, especially in diabetic and aging rodent models. Data from a working rodent model using an adenovirus vector to deliver eNOS intracorporally showed impressive responses in treated vs placebo (citrate) controls with minimal intracavernosal fibrosis or reaction. A second approach involves naked DNA transfer of maxi-K+ channel sensitizers that might reduce the stimulus needed for an erectile response. This therapy has been studied in aging and diabetic rodents and is currently undergoing phase I clinical trials in humans (data not available). A third approach attempts to release free radical and superoxide anion presence, molecules that bind with intracellular NO and reduce its availability for intracellular signaling during the erection process. Dr Hellstrom reported his findings from experiments in which adenovirus-mediated superoxide dismutase was transfected into diabetic rats to reduce reactive oxygen species with impressive responses. A fourth method of gene therapy for ED involves replacing important vascular growth factors, including VEGF and BDNF, that might restore function in arteriogenic and venogenic impotence. Manipulation of the RhoA/Rho kinase cascade upstream of the NO cascade is another approach to ED therapy. When activated, this pathway stimulates vascular constriction and might be responsible for why “men don’t walk around with an erection.” If this cascade is inhibited, then smooth muscle relaxation and erections are possible. A double mutant model affecting this cascade was discussed. Last, Dr Hellstrom reviewed his recent work with mesenchymal stem cells (MSC) to treat ED. Injection of bone marrow–derived undifferentiated MSCs with and without adenovirus vectors and eNOS was discussed as potentially viable ways to restore normal endothelial function in corporal smooth muscle. Dr Hellstrom is very excited about the potential of these therapies and predicts that they will need a decade or so of further development before human treatments would become available.

The American Urology Association Lecturer for 2005 was Ian Thompson, MD, University of Texas Health Sciences Center, San Antonio. He spoke on “Androgens and the Prostate Cancer Prevention Trial.” Dr Thompson reviewed results of the Prostate Cancer Prevention Trial (PCPT), a randomized, double-blind, placebo-controlled study to determine whether the 5α-reductase type 2 inhibitor, finasteride, prevented prostate cancer in men at relatively low risk (with serum prostate-specific antigen [PSA] < 3 ng/mL). Follow up of men in the study was planned every 3 months for 7 years with an end-of-study prostate biopsy in all subjects. However, the PCPT was closed 15 months early because of a strong overall reduction in prostate cancer with finasteride treatment vs placebo, both in end-of-study biopsies and those done for cause (eg, elevated PSA). Further analysis revealed a reduction in low-grade (Gleason 5–6), but an increase in high-grade (Gleason 8–10), prostate cancer.
A major controversy regarding the results of the PCPT is whether finasteride induced a real or artifactual increase in high-grade prostate cancer. Dr Thompson presented several lines of evidence that supported an artifactual rather than a real increase in high-grade prostate cancer with finasteride treatment: 1) In finasteride-treated men, the incidence of high-grade prostate cancer did not increase with time, as might be expected if finasteride increased the biological aggressiveness (and grade) of prostate cancer. 2) Androgen deprivation induced by an alternative method to finasteride (ie, gonadotropin-releasing hormone [GnRH] agonist treatment) resulted in a higher grade appearance of prostate cancer. 3) The proportion of Gleason 8–9 grade prostate cancers that showed degenerative changes was similar in finasteride- and placebo-treated men, arguing against an increase in tumor aggressiveness and grade induced by finasteride. 4) Gleason 7–10 grade prostate cancer was found bilaterally and in a larger percentage of cores (both measures of the disease extent within the prostate) more frequently in men treated with placebo than finasteride, and the pathological stage and positive margin rate was similar in finasteride- and placebo-treated men. 5) The percentage of men with high-grade prostate cancer was higher in finasteride- compared with placebo-treated men only in the end-of-study prostate biopsies, but not in the biopsies done for cause (eg, elevated PSA).

Dr Thompson’s lecture was followed by the first symposium titled “Regulators of Prostate Cancer Growth: Potential Novel Therapies. Steven Balk, MD, Beth Israel Deaconess Medical Center spoke on “Androgen Receptors as a Therapeutic Target in Androgen Independent Prostate Cancer.” Relapse of prostate cancer after initial androgen deprivation therapy is associated with relative refractoriness to androgen deprivation and is referred to as androgen-independent prostate cancer. However, renewed expression of the androgen receptor (AR) and AR-regulated genes implies ongoing AR transcriptional activity despite androgen deprivation. The AR appears to become hypersensitive to low levels of androgens. Dr Balk presented several mechanisms for AR transcriptional activity that have been reported in androgen-independent prostate cancer and presented 2 examples of potential therapeutic strategies involving the AR for the treatment of androgen-independent prostate cancer. With the use of gene microarray analysis of primary, androgen-responsive prostate cancer vs metastatic, androgen-independent prostate cancer, he found severalfold (2- to 5-fold) increases in gene expression of AR and several enzymes involved in androgen biosynthesis and metabolism (including 17-β-hydroxysteroid dehydrogenase type 5 [aldo-keto-reductase (AKR)1C3], UDP-glucuronosyltransferase UGT 2B15, 5-α-reductase type 1, and AKR 1C2 and 1C3, enzymes that catabolize DHT to 3-α- and 3-β-androstanediol and androstenedione to testosterone). AKR 1C3 expression is slightly increased in primary prostate cancer, but markedly increased in recurrent and metastatic prostate cancer. Dr Balk reported that clinical trials were ongoing with 17-β-hydroxysteroid dehydrogenase type 5 inhibitors and ketoconazole (an inhibitor of androgen biosynthesis) alone or in combination with dutasteride (a 5-α-reductase type 1 and 2 inhibitor) for the treatment of androgen-independent prostate cancer.

Dr Balk also presented evidence that enhancement of AR corepressor recruitment might be a useful strategy to treat androgen-independent prostate cancer. Nuclear receptor corepressor, N-CoR, is a nuclear corepressor that inhibits DHT-stimulated AR transcripption. In vitro, AR antagonists (eg, bicalutamide) enhances N-CoR recruitment, but mifepristone (RU486) markedly enhances AR–N-CoR interaction. The latter is mediated by the C-terminal nuclear receptor interacting domains of N-CoR and N-terminal domain of the AR. Mammalian 2-hybrid systems are being used to screen for drugs that enhance AR–N-CoR binding as potential therapeutic agents for prostate cancer.

This was followed by “Stress Induced Antiapoptotic Genes and Clusterins as Targets for Prostate Cancer Therapy” presented by Martin Gleave, MD, University of British Columbia.

Dr Gleave presented 2 examples of stress-induced antiapoptotic genes as potential targets to delay the progression of prostate cancer to androgen independence. With the use of gene microarray analysis, the expression of several genes increased with progression of prostate cancer to androgen independence, including BC12, IGFBP-2 and -5, clusterin, and heat shock protein (HSP)27. The expression of clusterin, a stress-induced cell survival gene, is increased in high-grade prostate cancer, after androgen ablation therapy, in metastatic prostate cancer and with progression of prostate cancer to androgen independence. Antisense inhibitors of clusterin expression increase the sensitivity of prostate cancer to therapy both in vitro and in vivo. A potent 21-mer, antisense, phosphorothionate, 2′-O-(2 methoxy-)ethyl, oligonucleotide, OGX-011, has entered clinical trials. A phase I trial of OGX-011 was recently completed in men with localized prostate cancer undergoing radical prostatectomy and who were pretreated with an antiandrogen plus GnRH agonist. A single infusion of OGX-011 at doses from 40 to 640 mg increased tissue concentrations and decreased clusterin expression in a dose-dependent manner. A 640-mg dose of OGX-011 every week was determined as the optimal biological dosage for phase II trials. HSP27 is a chaperone protein that also increased with androgen ablation therapy in metastatic prostate cancer and with progression of prostate cancer to androgen independence. Antisense inhibitors of HSP27 increased ex-
pression of prosurvival and decreased expression of apoptotic genes and increased the sensitivity of prostate cancer to androgen ablation and chemotherapy. The combination of antisense HSP27 and OGX-011 enhanced prostate cancer cell loss. Dr Gleave suggested that a possible future initial therapeutic approach for prostate cancer is androgen ablation at the same time targeting of prosurvival and antiapoptotic genes, followed by tumor growth inhibitors.

The third speaker for this symposium was Peter Nelson, MD, Fred Hutchinson Cancer Research Center, University of Washington. He spoke on "Prostate Cancer Genomics: Identification of Potential Therapeutic Targets." Dr Nelson reported on the use of isotope-coded affinity tags and liquid chromatography followed by tandem mass spectrometry for quantitative proteomic analysis of androgen-regulated proteins in microsomal protein preparations from LNCaP prostate cancer cells in the presence and absence of androgens. A total of 1064 androgen-responsive proteins were identified, of which 19.8% increased ≥2-fold and 1.6% increased ≥4-fold in response to androgen exposure. With the use of this proteomic method, prostate-specific antigen (PSA), a known androgen-regulated protein, increased 4-fold with androgen stimulation. This finding was confirmed by Western analysis. Identified androgen-responsive proteins were assigned to several cell process categories. Of the 1064 proteins identified, ~19% were in the unknown process category, ~9% were associated with signal transduction, ~5% each were associated with energy generation and vesicular transport, and ~4% were associated with lipid metabolism. When considering proteins associated with energy regulation and lipid metabolism, androgen exposure of LNCaP cells stimulated proteins associated with the anabolic pathways of fatty acid and cholesterol synthesis (eg, fatty acid synthase) and glycogen metabolism and repressed catabolic pathways of fatty acid oxidation, tricarboxylic acid cycle, and glycolysis. The increase in proteins associated with energy generation (eg, oxidative phosphorylation) in androgen-deprived prostate cancer cells suggested that mitochondria in these cells were metabolically stressed. This was confirmed by increased cell death induced by agents that perturbed the respiratory chain (eg, rotenone, CCCP, antimycin A, and oligomycin) in androgen-deprived compared with -exposed prostate cancer cells. Understanding of the androgen regulation of proteins involved in the metabolism of prostate cancer cells could provide insights into future therapeutic strategies.

In general, androgen-regulated transcript and protein expression correlated, but there were some exceptions. For example, prostate/KLK4 is prostate-specific transcript that is increased with androgen exposure. However, KLK4 protein is not found to increase with androgen exposure, perhaps as a result of protein degradation. Some proteins that were increased with androgen exposure were also released by prostate cancer cells into the media (eg, HAI-1 and APLP2). The former is found in the blood of men with primary and metastatic prostatic cancer. Identification of secreted proteins from prostate cells might be useful in the diagnosis and monitoring of prostate cancer.

The second symposium, honoring C. Alvin Paulsen, MD, consisted of 3 speakers who were selected to address new developments in "Androgen Action in the Male Reproductive System." Dr Paulsen, the third president of ASA and winner of the Society's Distinguished Andrologist and Distinguished Service awards, was a recognized leader in andrology research focusing on androgens generally and their application to hormonal contraception in particular. At the start of the session, the chairs David DeKretser and Richard Sherins acknowledged Dr Paulsen's contributions. Dr Paulsen, who was in the audience, stood and was warmly accorded a standing ovation.

The first talk was delivered by Michaela Luconi, PhD, a recent graduate of the postdoctoral fellowship program at the University of Florence on the subject of "Nongenomic Steroid Receptors on Sperm—Signaling and Function." It has been established that several of the steroid hormone receptors have an additional mechanism of signaling that does not follow the classical genomic pathway. In the genomic pathway, the receptor is located in the cytoplasm, and subsequent to binding of the steroid, the ligand-receptor complex moves into the nucleus, where it acts as a transcription factor to modulate gene expression. Dr Luconi reviewed data that showed the existence of rapid, nongenomic signaling pathways in sperm that appear to involve membrane forms of the estrogen (ER) and progesterone (PR) receptors. A 29-kd variant of the ER and a 57-kd variant of the PR were shown to be present in the membrane of human sperm. Steroid derivatives conjugated to bovine serum albumin that act only at the cell surface were seen to modulate calcium flux in sperm.

The second talk, titled "Androgens and Epididymal Genomics," was delivered by Shayesta Seenundun, a postdoctoral student at McGill University. After a general introduction to the action of androgen, referring to both the classical genomic and nongenomic signaling pathways, she described the general procedure for studying the effects of androgen on responsive tissues. Historically, this has involved castration to study the effects of androgen withdrawal, followed by administration of exogenous testosterone to study androgen-dependent events. The epididymis is unusual in that testosterone enters the organ by 2 routes: via the seminiferous tubule fluid coming in from the efferent ducts from the testis and from the blood supply. Ms Shayesta described an experiment to profile the patterns of epididymal gene expression on days 1, 2,
5, and 7 postcastration. Programmed cell death (apoptosis) occurs in the epididymis after androgen withdrawal, and starts proximally with the initial segment, proceeding distally to the cauda. Clusterin, a protein that is known to be repressed by androgen, is among the gene products that initially have elevated expression on days 1 and 2. By day 7, however, expression levels are down. In the middle corpus segment of the epididymis, a surprising number of genes are increased in expression. The general trend is that androgen-regulated genes decline, that cell survival and apoptosis-related genes transiently increase and then decline, and that a subset of cell survival genes shows a late increase. Finally, Ms Shayesta reviewed the role of 5-α-reductase activity in epididymal function because this enzyme is abundantly expressed and converts testosterone to dihydrotestosterone, a more potent androgen. There are 2 isoforms: type 1 is in the initial segment, and the amount of type 2 is higher in the caput. The activity of 5-α-reductase enzyme can be suppressed with an inhibitor, PNU 1557706, and the consequences assessed through gene profiling.

The third speaker was Laurent Morel, PhD, of the University Blaise Pascal in Clermont, France. His talk, titled “Androgen Regulation of Vas Deferens Proteins,” explored the downstream events in vas epithelial cells subsequent to androgen stimulation. Interestingly and like the other speakers, Dr Morel included nongenomic pathways as among those that need to be considered when examining androgen action. The phosphoinositide-3 (PI3) kinase is a key mediator of androgen in the vas. Blocking PI3 kinase prevents the protective effect of androgen on apoptosis. Another key androgen mediator in the vas is the serine threonine kinase Akt. Dr Morel concluded that it is essential to study androgen effects in the normal cytokine environment of the tissue, which will include epithelial growth factor (EGF). Several facets of the androgen response are modulated by EGF, including cell cycle arrest. Interaction of EGF and the androgen pathways in vas epithelial cells was studied by blocking the mitogen-activated protein kinase. Overexpression of androgen receptor in vas epithelial cells could provide new avenues of investigating prostate cancer, another glandular epithelium that is sensitive to androgen.

The third symposium on “The Cell Biology of Fertilization” featured 3 speakers. Each one of these speakers presented some new insights into some long-standing mysteries in andrology about how the sperm interacts with the egg and other issues related to conception and infertility. John Herr, PhD, University of Virginia, spoke on “Sperm Acrosome Membranes, Matrix and Exocytosis” and focused on the identification of a new sperm protein, equatorial segment protein (ESP), which was identified recently in his lab as an antigen for antisera from men diagnosed as having anti-sperm antibodies. ESP is a complex, multidomain protein of 349 amino acids, including an osteoglycan-like domain, a cytolsin-like domain, and a type II membrane-binding domain. ESP is localized in the acrosomal matrix of acrosome-intact sperm and retained on acrosome-reacted sperm; anti-ESP antibodies reduce the binding to and fusion of human sperm with hamster eggs. Insights from these ongoing studies of ESP could provide some clues into sperm-egg membrane interactions and also about one potential cause of reduced fertility in men and women with anti-sperm antibodies because 28% of male and female patients with anti-sperm antibodies had antibodies to ESP. ESP was also proposed by Dr Herr to be a candidate gene involved in globozoospermia because of its localization during acrosome biogenesis.

Janice Evans, PhD, Johns Hopkins University, spoke about “Regulation of Sperm-Egg Interactions and Egg Membrane Dynamics” and discussed recent studies in her lab investigating how the fertilizing sperm induces a change in egg membrane function from receptive to sperm to nonreceptive to sperm. This change in egg membrane function constitutes a membrane block to polyspermy. The Evans lab has previously shown that sperm-induced calcium signaling is crucial for membrane block establishment in mouse eggs. On the other hand, studies in other labs and her own have shown that parthenogenetic activation of eggs did not induce establishment of a membrane block to polyspermy. Considering that parthenogenetic stimuli induce calcium signals that are very different from those induced by sperm, they hypothesized that spermlike calcium signals are required for membrane block establishment. However, eggs injected with an extract of soluble sperm proteins experience calcium signals virtually identical to those induced by normal fertilization and yet do not establish a membrane block. This led to the hypothesis that other factors from the sperm could be involved in triggering the egg to establish the membrane block. This hypothesis was tested with several variations on improved techniques for intracytoplasmic sperm injection (ICSI) with mouse eggs; however, none of these ICSI stimuli induced membrane block establishment. Taken together, these data indicate that other events of bona fide fertilization, such as a membrane fusion event or sperm-induced cortical remodeling in the egg, are part of the pathway, together with calcium signaling, that lead to establishment of the membrane block to polyspermy.

Tanya Hoodbhoy, PhD, National Institutes of Health, presented work from Jurrien Dean’s lab on “Sperm Binding and Humanized Zona Pellucida (ZP)” of the egg. The work of her and her colleagues has addressed the 3 major models for sperm-ZP interaction and the ZP block to polyspermy. She discussed the interesting questions about why human sperm did not bind to the ZP of eggs expressing human ZP2, ZP3, or both. Dr Hoodbhoy inves-
tigated whether ZP4 is important for human sperm binding. There are 4 syntenic loci for ZP genes in the human, mouse, and rat genomes. In the mouse, the hypothetical ZP4 gene contains multiple stop codons and thus appears to be nonfunctional; moreover, ZP4 is not detected in native mouse ZP by mass spectrometry. In contrast, the ZP4 genes in both the human and rat appear to be functional, and Dr Hoodbhoy reported that their mass spectrometry data of native rat ZP indicate that the rat ZP does contain ZP4. Sperm-ZP binding assays revealed that capacitated human sperm do not bind to rat eggs, indicating that the presence of ZP4 in the ZP matrix, along with ZP1, ZP2, and ZP3, is not sufficient to support human sperm binding.

“Male Contraception” was the fourth symposium of the ASA annual meeting. C. Yan Cheng, PhD, Population Council Center for Biomedical Research, spoke on “Agents Acting Directly on the Testis: Will They be Reversible?” Dr Cheng began by emphasizing the importance of Sertoli cell–germ cell contacts and interactions in the testis. He suggested that the Sertoli cell–germ cell junctions might be an interesting potential target for male contraception. Occludin is a tight junction integral membrane protein whose second extracellular loop is crucial for tight junction function. Dr Cheng described an approach to target and inactivate the outermost region of the second extracellular loop of occludin that used a mutated form of the follicle-stimulating hormone (FSH) as a delivery system; the mutated form of FSH was shown to have no biological activity of FSH in cell culture. He showed that intraperitoneal injection of a 22-amino acid occludin peptide conjugated to the mutant form of FSH could perturb the integrity of the blood testis barrier. Next, Dr Cheng described experiments that used the mutated FSH to target the germ cell toxin AF-2364 to the testis. When given systemically, AF-2364 is known to affect other tissues and cause liver toxicity. Dr Cheng’s goal was to find a way to target the agent directly to the testis and thus be able to use it in lower doses than those that cause systemic effects. He found that intraperitoneal injection of the mutated FSH conjugated to AF-2364 led to germ cell loss at doses that did not have effects on cellular junctions in other tissues, such as the small intestine and kidney, suggesting good germ cell selectivity. Current studies are aimed at understanding the mechanisms underlying the germ cell effects of the mutant FSH, occludin peptide, and drug conjugates.

Barry T. Hinton, PhD, University of Virginia followed with a talk on “Will There Ever Be an Epididymal Male Contraceptive?” The development of an epididymal contraceptive would have the important advantage of inducing a rapid onset of infertility and a corresponding rapid return of fertility. Several approaches to epididymal contraception are possible, including targeting transport, sperm within the lumen, or modifying the function of the epididymal epithelium. The initial segment has a key role to play, as demonstrated by infertility in c-Ros–deficient mice; these mice have an underdeveloped initial segment. The initial segment is affected by both circulating and testicular factors. Dr Hinton first described his studies of the signaling pathways involved in the effects of testicular factors on initial segment function. On the basis of efferent duct ligation and other experiments, Dr Hinton proposed a model whereby testicular factors regulate signaling pathways (through the fibroblast growth factor pathway), which in turn affect either the PEA3 family or other factors, which then affect downstream genes leading to effects on initial segment function. Dr Hinton went on to describe protection and survival mechanisms of initial segment cells. After efferent duct ligation and the subsequent loss of testicular factors, apoptosis is noted in the initial segment. To understand the signaling pathways involved, a screen for altered proteins was carried out; of 18 proteins affected, 11 were known to play a role in apoptosis. Gene expression studies were carried out to determine the specific downstream genes controlled by testicular luminal fluid factors. The gene expression studies indicated that loss of testicular factors led to changes in cell transduction pathways, which in turn caused a down-regulation of antioxidative genes, a subsequent loss of protection from reactive oxygen species, followed by up-regulation of alarm signals and mediators, leading to apoptosis. Together, although more research is needed, the studies presented have begun to identify important genes and proteins within the epididymis and support the postulation that promising potential epididymal contraceptive targets include altering the luminal environment or altering protection and survival mechanisms.

The third speaker, Amiya P. Sinha-Hakim, PhD, Harbor-UCLA Medical Center and Los Angeles BioMedical Research Institute discussed “Combination of Hormonal and Physical Agents—Are They More Efficacious and Why?” Dr Sinha-Hakim began by discussing the limitation of current approaches to male hormonal contraception, which are indirect and have a slow onset of action, and suggesting that a direct action on the testis might be advantageous. In the rat, different stages of spermatogenesis are sensitive to apoptosis as a result of hormone deprivation and heat treatment. He postulated that a combination of hormone and heat treatments might be more effective than either treatment alone. With the use of microarray analysis, more genes were affected (both up-regulated and down-regulated) after combined treatment than after heat or hormones alone. Initial studies in cynomolgus monkeys indicated that the combination of testosterone and heat resulted in a rapid suppression in sperm count. Similar effects were seen in man, with a more rapid and effective suppression of spermatogenesis in a group
of men treated with testosterone and scrotal hyperthermia than in those receiving either treatment alone. Together the studies suggested additive and synergistic effects of hormones and heat across species; however, the underlying mechanisms were unclear. Dr Sinha-Hakim went on to show that the mitochondria-dependent intrinsic signaling pathway plays a key role in germ cell apoptosis in subhuman primates as it does in rodents. Mechanistic studies to date, for the most part carried out in rodents, indicate that both hormones and heat appear to induce apoptosis through the same downstream pathways. However, the precise upstream events still need to be delineated to determine how stage- and cell-specific apoptosis are induced differentially by heat and hormones.

The last Symposium was “Hot Topics in Andrology.” The first speaker, Richard Berger, MD, University of Washington, discussed “Prostatitis: What Is New?” Dr Berger indicated that patients with chronic prostatitis (CP) or chronic pelvic pain syndrome (CPPS) might have symptoms because of infection, inflammation, or psychological reasons. Although men with CP routinely receive anti-inflammatory and antimicrobial therapy, several studies indicated that factors other than bacteria and leukocytes contribute to symptoms associated with CP/CPPS. The National Institutes of Health Chronic Prostatitis Cohort Study found no association of symptoms with infection and injection. Other studies showed that bacteria cultures from prostatic biopsies of men with CPPS did not differ from those of a healthy donor, suggesting that prostatic bacteria obtained by biopsy might not be etiologically related to the symptoms in the majority of men with CPPS. Thus, the etiology of CP/CPPS is unknown, and the symptoms of CP/CPPS might result from interplay between psychological factors and dysfunction in the immune, neurological, and endocrine systems. With regard to the methods for detection of inflammation, Dr Berger also suggested that evaluation of expressed prostatic secretion with stained smears to detect neutrophils and macrophages in men with CP/CPPS might yield more informative results. Dr Berger commented that studies have shown that reactive oxygen species (ROS) were found to be predictive of *Ureaplasma* infection. He commented that in patients with no white cells but inflammation, the relationship of ROS/TAC (total antioxidant capacity) was altered because of an increase of the ROS and a decrease of the TAC. According to the Comet assay, CPPS patients were found to have an increase in DNA damage. Cytokines (TNF-α, IL-1β, IL-6, and IL-8) have frequently been found and elevated in the expressed prostatic secretions from men with CPPS; seminal cytokine levels could provide an objective measure of disease and determine the therapeutic strategies in these patients. The presence of ROS, DNA damage, and cytokines in the sperm environment could have a deleterious effect on male fertility. Dr Berger considered CPPS as a pain syndrome, and treatment with quinolone antibiotics was generally not effective, but in some studies, alpha blockers and 5α-reductase inhibitors appeared to have some positive effects. Despite this, antibiotics are still the most common therapy for CPPS.

Chawshang Chang, PhD, from the University of Rochester described work from his laboratory on “Cell-Specific KnockOut of the Androgen Receptor in Testis—What Are the Physiological Implications?” Dr Chang began by indicating that tissue or cell-specific knockouts (KOs) of the AR (Exon 2: ARKO) in male and female animals have been developed. He commented that with these ARKO models in the prostate, epithelial AR is a proliferation suppressor, stromal AR is a proliferation activator, and AR is a suppressor on prostate tumor metastasis. He then mentioned that with such tissue-specific ARKOs, AR has been shown to be involved in female fertility, rheumatoid arthritis, antibacterial activity, wound healing, and insulin resistance. Dr Chang’s group has developed 4 testicular cell–specific KO models. With a cre-lox conditional knockout strategy, they reported the generation of ARKO mice. Phenotype analysis revealed that ARKO male mice had a femalelike appearance and body weight. Their testes are 80% smaller, serum testosterone concentrations are lower than in wild-type (wt) mice, and spermatogenesis is arrested at the pachytene spermatocyte stage. In addition, the number and size of adipocytes are also different between the wt and ARKO mice. The average number of pups per litter in homologous and heterozygous ARKO female mice is lower than in wt female mice, results that suggested potential defects in ovulation, female fertility, or both. Sertoli cell–specific ARKO mice (S-AR[−/y]) were infertile, with spermatogenic arrest predominately at the diplotene premeiotic. Sertoli cell–specific ARKO mice also had lower serum testosterone concentrations and higher serum leuteinizing hormone concentrations than wt animals. The authors also demonstrated that S-AR[−/y] mice had defects in the expression of anti-Mullerian hormone, androgen-binding protein, cyclin A1, and sperm-1, all of which play important roles in the control of spermatogenesis, steroidogenesis, or both. He then described that the Leydig cell–specific ARKO mice showed a variable phenotype, with some mice showing marked decrease in T levels with arrest of spermatogenesis. An increase in apoptosis and disruption of proliferation was observed. With regard to the animal’s fertility, Dr Chang described that in the Leydig cell ARKO animals, despite females showing normal plug formation after mating, decreases in sperm count and motility were found. Germ cell–specific ARKO mice had no specific phenotype. The AR in Sertoli, Leydig, and peritubular cells were unaffected. Finally, Dr Chang summarized some of the findings on the peritubular myoid...
cell ARKO. These animals had normal urogenital distance, as well as normal spermatogenesis, but had a reduced sperm count and reduced fertility.

Ina Dobrinski, PhD, from the University of Pennsylvania spoke on “Germ Cell Transplantation: Where Will This Technology Go?” Spermatogonial stem cells have the ability of self-renewal and production of differentiated daughter cells. They serve as a model to study the basis of spermatogenesis and provide their functional definition. Dr Dobrinski commented that the studies showed transplantation of germ cells from infertile Sl/Sld mutant male mice to infertile W/Wv or Wv/W54 mutant male mice restored fertility to the recipient mice. Thus, transplantation of spermatogonial stem cells from an infertile donor to a permissive testicular environment can restore fertility and result in progeny with the genetic makeup of the infertile donor male. Dr Dobrinski discussed what is currently known about xenogenic germ cell transplantation and spermatogenesis in the mouse with the use of cells from other species. Although the model has worked in rat and hamster, the studies done using rabbit, dog, pig, bull, horse, and primate stem cells showed colonization in the mouse but not differentiation, indicating that cells from these species do not enter into meiosis. When germ cell transplantation was done in goat, repopulation of the testis with the donor stem cell was achieved, indicating that the process could work in immunocompetent although phylogenetically distant animal species. Autologous germ cell transplantation could be used as a treatment to restore fertility in cancer patients after therapy; however, the procedure might restore the cancer in the patient if cancer cells are reintroduced during transplant. Dr Dobrinski then described her work on transplantation of pig and monkey testicular xenografts into mice, which resulted in spermatogenesis and generation of spermatozoa used in ICSI. Dr Dobrinski commented on studies done in the human, in which variable degrees of spermatogenesis were achieved with testicular biopsies from different infertile men grafted into mice; however, advanced germ cells did not survive. She concluded that the studies up to the present time would indicate the effectiveness of the procedure of cell and tissue transplantation in animal species, suggesting that when spermatogenesis should be preserved, cryopreservation of testis or cell suspension could be suitable for development of xenografts for sperm production followed by ICSI.

The safety of ICSI represents one of the most important areas for ongoing scrutiny by the Andrology Society. Dolores J. Lamb, PhD, Baylor College of Medicine, and Peter N. Schlegel, MD, Cornell–New York Presbyterian Hospital, debated whether or not “There Are Significant Increased Health Risks in Offspring Resulting from ICSI.” Speaking for the pro side, Dr Lamb noted that ICSI has revolutionized the treatment of male infertility and allowed severely infertile men to experience parenthood. ICSI is used to treat a variety of failures of spermatogenesis, including immotile sperm, poor sperm performance in the hamster egg penetration test, severe oligozoospermia, nonobstructive azoospermia, obstructive azoospermia, congenital absence of the vas, and DNA-damaged sperm. It is noteworthy that urologists performing testicular extraction in nonobstructive azoospermia can search for hours to find a single rare sperm for use in ICSI. Of major concern is that many candidates for ICSI might have genetic defects that cause their sterility, and the defect might be inherited by the offspring. By-passing natural fertilization and the barriers that could eliminate defective sperm, ICSI might increase the incidence of infertility genes in the gene pool. Important unresolved questions in the field include: Will men who are genetically programmed to produce abnormal sperm father children who will be infertile? Will the children have genetic problems or birth defects? Do the genes that cause poor fertility cause other systemic problems later in life? Data appear to be accumulating slowly in answer to these critical questions and there are few long-term assessments. The ICSI field was initiated in 1992, and the first male offspring of ICsIs are only now beginning to enter puberty. It is yet unknown whether the infertilities of ICSI fathers will be frequented upon their sons. If this occurs, ICSI could result in human lineages dependent on ICSI for their propagation. Of all births, the incidence of ICSI babies ranges currently from 1.7% to 4% in various European countries. A recent report in the United States indicates more than half of all in vitro fertilization (IVF) cycles involve ICSI. Potentially problematic is the use of ICSI to treat minor infertilities. This practice might impede the ability to assess the safety of ICSI in the treatment of severe male factor infertility.

Dr Lamb categorized potential problems with ICSI as increased incidences of poor obstetrical outcomes: birth defects, genetic defects, developmental defects, and systemic defects. There might also be risks related to the ICSI method itself, including introduction of viral DNA during the procedure. A controlled, prospective cohort study of 3372 ICSI children and fetuses and 8015 naturally conceived children and fetuses after the 16th week of gestation indicated an increased risk of major congenital malformation; infertility-linked risk is highly probable, and technique-related risk could not be ruled out. Dr Lamb concluded that the literature on birth defects is controversial, and data analyses in many cases are flawed. Emphasis is placed on statistical significance of incidences at birth rather than odds ratios. A recent meta-analysis presented odds ratio estimates that suggest more birth defects after Assisted Reproductive Technology (ART). Others compared congenital malformations in infants at 5 years of age after conception by ICSA, IVF, and natural...
conception. ICSI children had more major congenital malformations from, in part, increased urogenital abnormalities. ICSI and IVF children were also more likely to have had a significant childhood illness, surgical operation, or medical therapy with hospital admission. With ICSI being used to treat a range of infertilities of known genetic causes, such as Klinefelter syndrome, Y chromosome microdeletions, round-headed sperm, and immotile cilia and flagella, studies are now appearing that show transmission to ICSI offspring of genetic causes like Y chromosome microdeletions and imprinting errors. The latter observations are, however, relatively rare, making it difficult to estimate frequencies at present. Although the incidence of chromosomal abnormalities in the general population is 0.85, nearly 4% of infertile couples have one partner with a chromosomal change. These chromosomal anomalies occur with equal frequency in men and women and include 2.1% translocations, 1.1% chromosomal mosaics, 0.3% XXY, 0.24% marker chromosomes, 0.14% inversion, and 0.05% duplication. Nearly 5% of infertile couples had Y chromosome microdeletions. Patients with ICSI (76%) are more likely to have aneuploidy in their cytogenetic analysis of the products of conception than conventional IVF (41%). Dr Lamb concluded that ICSI children are at increased risk for congenital abnormalities; chromosomal abnormalities; Y chromosome microdeletions; structural and numerical abnormalities of chromosomes; imprinting errors; and childhood illness, surgical operation, or medical therapy with hospital admission.

Speaking for con, Dr Schlegel argued that ICSI is both an effective and a safe technique and has become the standard treatment for male factor infertility. ICSI treats all defects in sperm function, including patients with low sperm concentration, defects in motility, patients with anti-sperm antibodies, and defects in morphology. Pregnancy rates with ICSI are now up to 50% per attempt. These pregnancy rates are equivalent or better than that observed after IVF. Current data suggest that birth defect rates might be increased by ICSI, but the differences are minimal and can be explained by confounding factors, study design, detection bias, or a combination of factors. Both retrospective and prospective studies of ICSI have been published. These studies compare outcomes in ICSI, IVF, and natural conceptions. No differences in birth defects are identified when ICSI and IVF are compared. Pooled analysis of data from multiple studies evaluating birth defect rates after ART that included 2500 different studies and 22 comparisons of IVF vs ICSI outcomes have determined an odds ratio of 1.0, indicating ICSI is safe. A Swedish study of congenital malformations in 1139 infants born from ICSI when stratified by region, birth date, and maternal age, with detection of cases/malformations parallel and after stratification for singleton/
needs to be more information. An ICSI registry with patient follow-up would provide evidence from which infertility practitioners might proceed with greater certainty and provide accurate counseling to their patients. Both speakers noted that the underlying genetic causes of much male infertility are presently unknown and that such knowledge would significantly improve the ability to assess transmission to ICSI offspring. If most male infertility treated by ICSI has a genetic cause, then increases in carrier frequencies of infertility genes might be higher after ICSI than we now appreciate.

Submitted on behalf of the ASA 2005 Program Committee by Janice Bailey, PhD; Janice P. Evans, PhD; Matt Hardy, PhD; John C. Herr, PhD; Kate Loveland, PhD; Alvin Matsumoto, MD; Jacquetta Traster, MD, PhD; Paul J. Turek, MD; Monica Vasquez-Levin, PhD; and Christina Wang, MD (Chair).