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Introductory Overview of "The Epididymis: Present Progress, Future Directions" (Proceedings of the Fifth International Conference on The Epididymis)

Editorial

This Special Issue of the *Journal of Andrology* consists of the Proceedings of the "Fifth International Conference on the Epididymis (Epididymis V)," which took place October 25–28, 2010, in Águas de São Pedro, São Paulo, Brazil. Based upon the central theme "Epididymis: Present Progress, Future Directions," peer-reviewed papers from 18 of the invited speakers are gathered herein. They provide a unique opportunity for reproductive biologists to become updated on the many recent discoveries and advances in epididymis structure and function. Here, we present meeting highlights and an introductory overview of the papers, organized by their main themes.

History and Goals of The Fifth International Conference on The Epididymis (Epididymis V)

The central theme of the Fifth International Conference on the Epididymis (Epididymis V), held in Águas de São Pedro, Brazil, October 25–28, 2010 was "The Epididymis: Present Progress, Future Directions" (see Supplemental Figure 1, available online at www.androlgyjournal.org). The first quadrennial meeting to take place in Latin American, Epididymis V carried on the tradition of previous meetings focused specifically on the epididymis: Epididymis I, organized by P. Wong (China, 1992); Epididymis II, organized by R. Jones and M. Holland (Australia, 1998); Epididymis III, organized by T. Turner and B. Hinton (United States, 2002); and Epididymis IV, organized by J. Drevet and T. Cooper (France, 2006).

Why hold an entire meeting devoted to the epididymis? Historically, the epididymis has received the least attention with respect to basic and clinical research on male reproductive function and health, especially when compared with the testis and prostate. The original organizers of this series of international conferences, enthusiastic about the unique roles and new discoveries in this tissue and their relevance to male fertility, created the successful meeting format that has been used since Epididymis I in 1992. Researchers from countries around the world have found in these meetings a stimulating intellectual environment, based on well-organized scientific programs, with opportunities to meet international colleagues and interact with old friends. Inclusion of senior researchers, new trainees, and young investigators provides an ideal format for exploring the current status and perspectives of research in the area and opening avenues for ongoing and future collaborations that accelerate research advances in epididymal biology.

Epididymis V was small in size (94 participants from 10 countries) and international in scope, maximizing participant interactions and strengthening scientific

The meeting would not have been possible without the important financial and scientific support received from the Institutions/Agencies/Scientific Societies in Brazil (Universidade Federal de São Paulo, UNIFESP; UNIFESP Research Foundation, FAP-UNIFESP; São Paulo Research Foundation, FAPESP; The National Counsel of Technological and Scientific Development, CNPq; Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, CAPES; Universidade de Campinas, UNICAMP; Universidade Estadual de São Paulo–UNESP-Botucatu, Colégio Brasileiro de Reprodução Animal; and the Brazilian Society of Cell Biology), United States (International Research and Training Grant in Reproductive Biology USA/Brazil, Fogarty International Center/National Institute of Child Health and Human Development/National Institutes of Health; American Society of Andrology, ASA), and France (ANDROLOGIE: the periodic of the French Andrology Society, SALF). A special recognition should also go to the funds received from The International Society of Andrology (Germany) and The Lalor Foundation (USA) that made possible the distribution of awards to trainees/young investigators who were speakers in the "Oral Communication Sessions" and to 22 trainees/young investigators who presented posters. The Society for the Study of Reproduction (SSR, USA) made available complementary registration for 2 trainees, from a country other than United States or Canada and selected for "Best Poster Presentation," to attend the 44th SSR Annual Meeting. Furthermore, we also thank donations made by the local companies Life Technologies/Invitrogen do Brasil, Applied Biosystems do Brasil, WR Research Products, Interprise, Spectrum Bio Engenharia Médico Hospitalar Ltda, and Banco do Brasil S/A.

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Figure. Invited speakers of the Fifth International Conference on The Epididymis. Clockwise from top left: Conference Speakers Sylvie Breton (United States), Bernard Robaire (Canada), and Terry Turner (United States); Symposia Speakers Michelle Welsh (United Kingdom), Thomas Carroll (United States), Barry Hinton (United States), Yong-Lian Zhang (China), Jean-Luc Gatti (France), Ilpo Huhtaniemi (United Kingdom), Barry Shur (United States), Louis Hermo (Canada), Rex Hess (United States), Maria Christina W. Avellar (Brazil), Mark P. Hedger (Australia), Joel R. Drevet (France), Matti Poutanen (Finland), Brett Nixon (Australia), Debora Cohen (Argentina), Gail Cornwall (United States), Wilma DeGrava Kempinas (Brazil), and Michael O'Rand (United States).

relations between numerous researchers. The meeting featured 22 speakers representing countries from nearly every continent (the Figure). The broad scope of the scientific program included topics on basic and clinical aspects of epididymal biology from cellular, molecular, and hormonal regulation, to aspects of organ development, to the central role of the epididymis in sperm maturation and fertility, which contributes to new approaches for treating male infertility and developing male contraceptives. During the activities, views and questions on the state of the art in epididymal research were presented, highlighting unanswered research questions and visions for the future, as well as identifying challenges that investigators will have to overcome to keep this research area growing. Epididymis V also provided the opportunity to recognize and honor Marie Claire Orgebin-Crist (United States) for her pioneering and longstanding contributions to the field of "epididymology" over the past 50 years.

In addition to the main program, two sessions were devoted to oral "short communications" presented by trainees and young investigators competitively selected from abstracts submitted to the meeting (see Supplemental Figure 2). A total of 54 abstracts were presented in poster format, most with trainees as first authors. A special 1-hour activity for trainees and young investigators called "Young Reproductive Biologists: Let's Talk," consisted of working groups of 5–6 people that discussed opinions, expectations, and thoughts about the future of epididymis research and developed strategies to increase trainee interactions and professional networking.

This Special Issue of the *Journal of Andrology* includes 18 peer-reviewed papers submitted by invited speakers who participated in Epididymis V. Each of the papers represents a unique opportunity for reproductive biologists to be updated on the many recent discoveries over the last few years and to be made aware of ongoing developments in various areas of epididymal research from laboratories around the world.

Overview of the Proceedings of the Fifth International Conference on The Epididymis

In contrast to sperm from invertebrates, birds, reptiles, and other lower vertebrates, mammalian sperm leaving the testis do not have the ability to recognize and fertilize the egg, requiring a transit through the epididymis to acquire their fertilizing ability. The epididymis is a several-meter-long unique and convoluted tubule lined by a tight layer of epithelial cells, forming an organ that is classically subdivided into at least 3 major anatomical regions (caput, corpus, and

cauda). Spermatozoa, transported through the duct, may be stored in the cauda epididymis, vas deferens, or both. During their transit along the epididymis, sperm mature, but it is only when they reach the cauda region that almost all spermatozoa will have acquired their natural fertilizing ability. This posttesticular maturation process is unique to mammals and involves the interaction of sperm with the complex luminal fluid provided by the successive epididymal regions. During this process, the sperm plasma membrane undergoes constant remodeling, with attachment and shedding of molecules that depend highly on a well-synchronized series of events, whereby gene expression occurs in a segment- and cell-specific pattern along the epididymis. For this reason, the epididymis is considered an ideal research target organ for the understanding of the molecular mechanism(s) of sperm maturation, providing new thoughts on pathophysiological mechanisms underlying male infertility, novel diagnostic markers, and therapeutic interventions for the control of male infertility related to posttesticular malfunction, as well as drug design for male contraception.

Without a fully developed and functional epididymis, male infertility results. Very little is known, however, about the process of epididymis development or the nature and causes of congenital epididymal defects that can lead to male infertility. In the first paper of this Special Issue, the reader is invited by Hinton et al to reflect on these topics and the intriguing question of how 6 m of epididymis can fit inside a human scrotum. The authors review fundamental biological aspects and dynamic mechanisms involved in the formation of this tube during organogenesis and provide interesting ideas and hypotheses about how the developing epididymis (Wollfian duct) elongates, coils, and then forms its characteristic shape and size. The authors discuss facets of the differential regulation of these morphogenic events during embryonic and postnatal development by androgens, mesenchymal secretions, and lumicrine factors.

In spite of significant progress in the last years toward identification and characterization of the role of the different epididymal cell types involved in the establishment of the optimal lumen environment for sperm maturation and storage, the different players in and mechanisms by which tight cell regionalization and regulation of gene expression and protein secretion are attained along the epididymis remain a subject of investigation. The next 2 papers present new and relevant insights into cell-cell cross-talk and both intra- and intercellular mechanisms involved in water transport and luminal acidification, two essential determinants of normal epididymal function. Hermo and Smith review aspects of water content regulation within the lumen in the male reproductive tract and its importance for sperm differentiation, maturation, and transport. In this scenario, the authors focus on the diverse and varied cell types and region-specific patterns of the expression of different aquaporins along the testis, efferent ductules, and epididymis. They illustrate the unique association of these proteins with specific membrane domains within the efferent ductules and epididymis epithelium, as well as the dynamics of their complex expression pattern. The interesting ''thirsty business'' that emerges from the authors' data suggests that the epididymis has built-in safeguards to ensure maximum efficiency, redundancy, and selectivity in the ability of its constituent epithelial cells to transport water and small uncharged solute molecules at critical sites, thereby serving to support and maintain health and viable spermatozoa in the lumen.

The second paper, by Breton's group (Shum et al), reviews both the importance of a low luminal bicarbonate ion concentration and low pH to maintain sperm quiescence during their maturation and storage in the epididymis and the mechanisms by which luminal acidification is achieved and regulated. In this scenario, they provide more fascinating and novel perspectives on how epididymal epithelial cells work in a concerted manner, together with spermatozoa, to establish and maintain this acidic luminal environment. The authors focus on the physiological aspects of the epididymal epithelium, describing selected aspects of the proton-pumping ATPase (V-ATPase) and its regulation by luminal and paracrine factors, and illustrate the elaborate communication network present between principal and clear cells and between basal and clear cells that controls luminal acidification. The reader also learns about the remarkable property of the basal cells to extend long and slender cytoplasmic projections that cross the tight junction barrier to monitor the luminal environment.

The next paper brings us up to date on new ideas about the functional regulation of the epididymis. Zhang's group describes the use of microarray and small-RNA library screening to unravel the identification of spatially and temporally regulated known and novel small regulatory RNA molecules (microRNAs). The authors also discuss their exciting progress on the use of the RNA interference (RNAi) approach as a transient conditional gene knockdown tool to investigate some newly discovered epididymis-specific genes and their roles in vivo. They emphasize how these methodologies are helping to decipher the function of several sperm motility- and capacitation-related proteins in the rat epididymis, highlighting results from their work of several years on different beta-defensin genes that are differentially expressed along the rodent

epididymis. An interesting model is proposed at the end of the reading, suggesting how multiple genes may work in a coordinated sequence to affect sperm motility development as it takes place along the rat epididymis.

Although it is well established that epididymal structure and functions are maintained by a complex interplay of endocrine, lumicrine, and neuronal systems, among which androgens are the most important component, several aspects underlying the hormonal regulation of epididymal functions and gene expression by androgens and other steroid hormones, such as estrogens and glucocorticoids, are still poorly understood.

The next 2 papers in this Special Issue review the well-established mechanism of androgen action in the epididymis, whereby the androgen receptor (AR) functions as a transcription factor to regulate gene expression that affects epididymal function. Robaire and Hamzeh further synthesize their recent data on the initial and subsequent roles of androgens in altering cellular architecture and function in an androgen-deprived condition. By using Affymetrix rat genome microarray chips and pathway assist software, they highlight the existence of novel sequences of gene activation or suppression that occur in the androgen-deprived tissue on the readministration of dihidrotestosterone and estradiol, in this latter case revealing new insights into the role of this steroid hormone in the epididymis. Based on the use of a PC1 mouse–derived epididymal cell line, their studies also reveal the existence of an intracellular signaling pathway in which insulin-like growth factor-1 and epidermal growth factor receptors are found to be important mediators of AR-mediated rapid responses through activation of the MAPK/ERK pathways, opening new avenues into how androgens regulate the function of epididymal cells.

Although the coexistence of genomic and nongenomic mechanisms to mediate androgen action under normal circumstances in the epididymis still need to be confirmed, the different usage of AR coregulators has been cited to explain the existence of differential gene expression induced by androgens in different epididymal regions. The original paper presented by Huhtaniemi's groups (Sipilä et al) expands on this concept by using global expression profiling of microarray analysis to reveal the presence of numerous AR coregulators in the epididymis, with potential involvement in the androgen-dependent regulation of epididymal genes. Because some of these coregulators were found to be expressed differentially along the different epididymal regions, the authors hypothesize that they might have roles in modeling androgen-dependent gene expression in an epididymal region-specific manner.

The potential function of estrogens in male reproduction and fertility is then discussed by Hess and collaborators from 2 other laboratories. The authors present an informative overview of the structure, localization, and signaling pathways of the estrogen receptors ERS1 and ERS2, as well as the expression of the G-protein–coupled estrogen receptor 1 (GPER) along the efferent ductules and epididymides in various species. They raise considerations of the role of estrogens in the function of these tissues on the basis of cumulative data obtained over the last several years by the different laboratories. The paper also provides discussion about the interplay between the relative importance of estrogen receptor subtypes in relation to AR and other nuclear steroid receptors, as well as their combined activities in cells that coexpress nuclear receptor cofactors in the epididymis.

In the next paper, from Avellar's group (Silva et al), glucocorticoids emerge as another potential regulatory factor of epididymal function. They synthesize the expression, cell- and region-specific localization, hormonal regulation, and function of the glucocorticoid receptor (GR) and review the role of glucocorticoids in the male reproductive tract and epididymis, highlighting the potential significance of these steroid hormones and GR and their interplay with androgens as a counter-regulatory mechanism during the course of responses related to stress, including infection. The authors also illustrate the cellular and biochemical machinery by which the epididymis mounts an effective host response via Toll-like receptor 4 against gram-negative bacteria infection, highlighting the potential interplay between glucocorticoids and androgens in the modulation of epididymal function during both normal and infection/inflammation conditions.

Along related lines, Hedger reviews, in the next paper, the immunophysiology and the pathology of inflammation, emphasizing our current understanding of the innate and adaptive immune systems as they relate to the testis and epididymis. The author provides the reader with an update on the effects of systemic inflammation and illness on the testis and epididymis, blood-epithelial barrier and compartmentalization, distribution of immune cells, immune privilege, immunoregulation, and innate immunity in these 2 tissues. The paper ends with the recognition that although relatively little is known about the innate and adaptive immune systems as they relate to the testis, even less is understood about the interactions of these 2 immune systems in the epididymis, with instructive considerations about future research needs in this area.

The next paper from Drevet's group (Noblanc et al) reviews the protective mechanisms by which the fine control of reactive oxygen species (ROS) actions is regulated in the luminal compartment of the epididy-

mis. The authors explore the interesting and important role of the glutathione peroxidase (GPX) gene family that encodes bifunctional enzymes. They show how such enzymes can work either as classical ROS scavengers or as thiol-peroxidases, introducing disulfide bridges in thiol-containing proteins which, in turn, are a facet of sperm maturation along the epididymis. They nicely summarize ''lessons learned'' in the last several years from different mouse GPX knockout models, revealing the roles played by the sperm-associated GPx4 and GPx5 in both structural maturation of sperm cells and protection of sperm against ROS-induced damage during their journey across the mammalian epididymis.

Considering that mammalian spermatozoa have little transcriptional capacity and are unlikely to generate new proteins, the secretory activity of the epididymal epithelia is acknowledged as playing a key role in promoting mammalian sperm maturation. The relevance of epididymal fluid components for mammalian sperm maturation and fertilization, as well as for the development of male contraceptives, is reviewed in the next group of papers.

The first, from Gatti's group (Guyonnet et al), gives the reader an excellent overview of how recent transcriptomic, proteomic, and secretomic (protein synthesis and the related secretion activity of the epididymal epithelium) studies are contributing new information on the functions and importance of the regionalization of the epididymis to the understanding of epididymal fluid composition. After considering how control mechanisms for protein synthesis, secretion, and reabsorption/degradation operate in several different species, the authors highlight recent methodological advances that allow the analysis of specific sperm surface protein changes along the epididymal duct. They go on to illustrate the dynamics of the sperm and luminal fluid proteomes in modulating sperm membranes during epididymal maturation and show how the sperm acquire their physiological properties in response to the sequential changes in the surrounding luminal fluid.

The next paper, by Nixon's group, adds an evolutionary perspective to this Special Issue. The authors describe how monotremes provide a useful model for studying the evolutionary forces that have driven the need for sperm maturation in higher mammals. More specifically, the authors show that epididymal maturation in Australian monotremes involves the formation of remarkable sperm bundles that enhances their motility, likely through specific proteins secreted by the epididymis. They suggest that this intriguing cooperative strategy might represent an early form of epididymal maturation, far less complex than that operating in eutherian mammals, which has possibly evolved to provide sperm with a presumed advantage in ascending the female reproductive tract.

Although the role of epididymal proteins in monotreme sperm maturation still needs to be confirmed, existing evidence indicates that epididymal maturation in eutherian mammals involves an extensive remodeling of the sperm plasma membrane, mainly as a consequence of the acquisition of new proteins of epididymal origin. This point is well exemplified by Cohen et al, from Cuasnicú's group, showing that one of the main epididymal secretory proteins, cysteine-rich secretory protein 1 (CRISP1), associates with the sperm surface during maturation and plays different roles in fertilization through its varied associations with, and localizations on, spermatozoa. Using genetically modified mice, the authors show that CRISP1 is a multifunctional protein involved in capacitation-dependent protein tyrosine phosphorylation, sperm–zona pellucida binding, and gamete fusion. These findings contribute to a better understanding of the molecular mechanism involved not only in sperm maturation but also in sperm-egg interaction.

Besides their direct roles in sperm maturation, luminal proteins also contribute to creating the permissive environment for the occurrence of this process. One such enabling protein is the cystatin-related epididymal spermatogenic (CRES) protein, present within the epididymal lumen as a soluble monomeric form and also as insoluble high–molecular mass aggregates, possibly amyloid. In a clear and informative review, Cornwall et al discuss the biological significance of protein aggregation in the epididymis and other tissues and describe studies carried out in the mouse to determine both if amyloid is present in the epididymal lumen and if CRES is associated with these structures. As indicated by the authors, the presence of CRES amyloid protein in the epididymal lumen, in the absence of pathology, opens the interesting possibility that CRES is a new example of a functional amyloid protein with roles in epididymal function.

Contrasting with all other eutherian mammals in which fluid protein composition shows major differences from one segment to the other, protein composition of the different epididymal segments is quite similar in humans, suggesting that human spermatozoa might not undergo the biochemical modifications underlying sperm maturation in other mammalian species. Using microarray analysis of the epididymal transcriptome, Sullivan et al provide added insight into this question by showing that gene expression in the human epididymis is also highly regulated and segment specific. The authors also present evidence that vasectomy significantly affects the transcriptome along

the epididymis and that some sequelae of vasectomy on the epididymis are not reversible after vasovasostomy, thus permanently affecting some aspects of sperm maturation and functions. A better understanding of the potential consequences of these alterations for spermatozoa will provide important information on the complex processes specific to human sperm maturation.

Because of their relevance for sperm function and fertility, epididymal proteins have always been considered excellent targets for male contraception. One potential candidate is EPPIN (official symbol SPINLW1), a sperm surface epididymal protease inhibitor studied by O'Rand's laboratory for the past 15 years and reviewed in this Special Issue. EPPIN binding to the seminal vesicle protein semenogelin inhibits sperm motility; as prostatic specific antigen (PSA) hydrolyzes semenogelin in the ejaculate coagulum, spermatozoa gain progressive motility. Interestingly, male monkeys immunized with EPPIN exhibited complete and reversible infertility, strongly supporting epididymal proteins as target candidates for male contraceptive development. O'Rand et al also describe the mechanism underlying EPPIN function, as well as the recent development of a screening method to look for compounds that inhibit EPPIN-semenogelin interaction that could be important for development of nonhormonal male contraceptives.

Neuronal and nonneuronal factors are known to be important physiological determinants of the mechanisms underlying the contractility of the smooth muscle surrounding the epididymal tubules. These mechanisms, in turn, regulate sperm transport through the epididymis. Kempinas' group (Bellentani et al) presents an original paper revealing the potential of drugs that interfere with autonomic activity to affect sperm epididymal transit. The authors treated adult male rats with sibutramine, a drug with sympathetic action currently used for the treatment of obesity, and report consequent acceleration of sperm transit through the epididymis, which decreases sperm reserves in the cauda region. The authors close by highlighting the importance of understanding how drugs that interfere with the autonomic activity of the epididymis smooth muscle could have downstream effects on male fertility.

Finally, with the simple yet provocative title "Why This, Why Now," Turner invites the reader to look to the future of epididymal research in the last paper of this Special Issue. The author guides the reader through the current status of epididymal research in the scientific literature, highlights challenges of this area and suggests ways in which this area can be kept alive and growing in the coming years. The paper represents a summary of the take-home messages presented by Turner in the "Concluding Remarks" session of the meeting that can be readily appreciated by all readers, both inside and outside the field. His reference to a quote from Winston Churchill—"... a puzzle inside a riddle wrapped in an enigma ..."—aptly describes how the epididymis is understood and represented in male reproductive biology. In Turner's words "to a scientist, that is an appealing quality for a subject to have, and it is the reason most of those interested in the epididymis find it fascinating in the first place." In fact, we hope that all the papers contained in this Special Issue will convince readers that important mysteries remain and will motivate them to join the field and become epididymologists like us!

Overall, our hope as organizers of the Epididymis V meeting is that this Special Issue becomes an essential update for the reader, as well as an important tool of dissemination of the current thinking in epididymis biology. We also hope the ideas and discussions contained in this Special Issue will be inspirational to more people, so they join us for Epididymis VI, and we may pave the future of andrology... together.

Following the idea to move this series of Conferences to cities around the world, the sixth edition of this Conference will take place in Shanghai, China, in 2014, organized by Dr Yong-Lian Zhang from the Chinese Academy of Sciences in Shanghai (see Supplemental Figure 3). As we look to China, where our meetings began in 1992, it is important to note how far we have come in these series of international meetings. Indeed, just as "a journey of a thousand miles begins with a single step" our ongoing journey through epididymal biology has progressed far from its origins, but we still need to be mindful of making progress by walking together—basic scientists, applied scientists, and clinical scientists—a step at a time.

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