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Male Contraception: Views to the 21st Century, Bethesda, MD, USA, 9–10 September 1999

Louis V. DePaolo, Barry T. Hinton and Robert E. Braun

For men who still wish to father children, the contraceptive options currently available are withdrawal and the condom. Although significant progress has been made on hormonal and vaccine-related approaches to male contraception, a marketed product is, at best, several years away. Therefore, the National Institute of Child Health and Human Development convened a workshop to discuss novel strategies for development of male contraceptives that focused on the testis and epididymis. Participants recognized that exploration of these new approaches will necessitate considerable investment of funds and research efforts.

L.V. DePaolo is at the Reproductive Sciences Branch, Center for Population Research, National Institute of Child Health and Human Development, NIH 6100 Executive Boulevard, Room 8B01 Bethesda, MD 20892-7510, USA. B.T. Hinton is in the Department of Cell Biology, University of Virginia Health Sciences Center, Charlottesville, VA 22908, USA. R.E. Braun is in the Department of Genetics, University of Washington, Seattle, WA 98195, USA.

As the world population continues to grow and critical resources become limited, there is a call, once again, for more and improved contraceptive options. This is particularly true for males who currently have four options: abstinence from heterosexual intercourse, withdrawal prior to ejaculation, condoms and vasectomy. Unfortunately, for those men who wish to father children at a later time, withdrawal and condoms are the only options because vasectomy is essentially irreversible. Despite convincing evidence that hormonal approaches to male contraception based on inhibition of pituitary gonadotropins can render men azoospermic or severely oligospermic and therefore do result in effective contraception[1], research on hormonal, as well as vaccine-related approaches to male contraception have yet to yield a marketed product.

The long-standing belief that men would not accept the responsibility for contraception has been one of the many hindrances to gaining momentum for worldwide efforts in developing male contraceptives. Interestingly, however, it is estimated that one-quarter to one-third of all contraception in the United States is, in fact, accomplished with male methods[2]. Furthermore, a recent poll has found that 70% of men are willing to use a contraceptive[3]. Thus, the question is posed; 'Is the time ripe for research and development efforts in new approaches to male contraception?' Based on the apparent willingness of men to bear the burden of

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contraception, and the possibilities for novel approaches that grow out of the seemingly boundless advances in biomedical research and technology taking place today, the answer is a resounding yes!

Given that the climate appears to be opportune for male contraceptive research and development, the National Institute of Child Health and Human Development (NICHD) convened this workshop to identify novel strategies for developing a male contraceptive in the 21st century. Focus was given to strategies that involved testicular and epididymal targets. Basic and clinical scientists working in the field of male reproduction, andrology and retrovirology, as well as representatives from the World Health Organization and industry were invited to participate. In all, the agenda included 17 presentations and a breakout session in which the participants were divided into three working groups that addressed three questions: (1) What are the unique processes in the male reproductive tract that could be targets for new contraceptives?; (2) what are the barriers to developing a male contraceptive?; and (3) what reagents/expertise are needed to further our understanding of testis/epididymal biology?

• Testis as a Target

For the testis, not surprisingly, one approach considered was the direct interference with various aspects of spermatogenesis. These strategies included interference with meiosis and particularly the cell-cycle checkpoints, disruption of RNA–protein interactions (post-transcriptional control) and disruption of junctional complexes between Sertoli cells and germ cells. For a strategy that involves meiosis inhibition, an ideal target would be a protein that is both germ cell- and meiosis- (not involved with mitosis) specific. Two such proteins, Dmc1 and MutS homolog 5 (MSH5), appear to be reasonable candidates. Indeed, chromosomes fail to pair in either Dmc1 or MSH5 null-mutant mice[4,5]. Alternatively, a number of checkpoints in the meiotic process are being identified where meiotic arrest can occur, such as the metaphase I transition checkpoint. Consequently, it was suggested that development of compounds that activate checkpoint arrests may prove to be effective in blocking meiosis, and thus, the production of haploid spermatozoa.

Another strategy involving the disruption of spermatogenesis centered on the post-transcriptional control of gametogenesis. In this regard, it is now known that ≈13% of human azoospermia can be attributed to *de novo* deletions of the Azoospermia Factor (AZF) region of the Y chromosome, which encodes several RNA binding proteins[6]. Thus, one is led to speculate that synthetic molecules that disrupt RNA–protein interactions may mimic mutations in genes encoding essential RNA binding proteins, and, therefore, result in infertility.

A third strategy that is well developed in terms of contraceptive applicability targets the tight junctions between Sertoli cells and germ cells. The disassembly and reassembly of these junctions is critical in promoting sperm differentiation, and, thus, any disruption of this highly regulated process can be expected to impact fertility. Indeed, data were presented at the workshop to show that administration to rats of two novel inidazole carboxylic acid derivatives that disrupt Sertoli cell–germ cell junctional complexes resulted in infertility which was restored upon cessation of treatment. Although these compounds are known to exert toxicity that could hamper their use as contraceptives, no histological changes were observed in the liver, kidney or epididymis at the doses administered that caused anti-fertility effects (C.Y. Cheng, pers. commun.).

• Epididymis as a Target

The other target discussed at this workshop was the epididymis. The epididymis has traditionally received much attention as a potential contraceptive target because it is well known that the process of sperm maturation and storage occurs in the various segments of the epididymis. It is thought that post-testicular contraception would have the advantages of being immediate in onset and readily reversible, avoiding endocrine impairment of libido and presenting minimal risk for mutagenic damage. Fortunately for the sperm, and unfortunately for the scientists attempting to develop a post-testicular contraceptive aimed at the epididymis, this organ has developed an elaborate system to protect sperm via the presence of a blood–epididymal barrier. In addition to a physical barrier, a 'knowledge' barrier exists in that there is a relative lack of understanding of how sperm come to mature in the epididymis and how mature sperm, capable of motility, remain quiescent in the cauda epididymidis waiting to be released upon ejaculation.

Nevertheless, significant progress is being made in our understanding of epididymal biology. For example, it is now appreciated that changes in surface charge, surface proteins, lectin-binding properties, nuclear disulfide bonds, lipid and carbohydrate composition and membrane fluidity occur in spermatozoa during their transit through the epididymis, suggesting that prevention of one or more of these changes could interfere with sperm maturation. Moreover, several genes within the initial segment are regulated by luminal factors of testicular origin, and various factors secreted in one segment of the epididymis are likely to affect function in either another segment or within the same segment through lumicrine mechanisms.

Recently, gene knockout strategies have been employed to elucidate functions of epididymal-derived factors. In particular, mice deficient in the production of the epididymal-specific protein, c-ros, an orphan tyrosine kinase receptor, lack a fully developed initial segment and are infertile[7]. Interestingly, sperm taken from these mice are capable of fertilization *in vitro*, thereby pointing to a motility defect as a result of c-ros deficiency.

The tissues of the male reproductive tract, including the epididymis (and even the testis), are target tissues for androgens. However, very little is known about the mechanism of action of androgens on these tissues. Androgens are found in high concentration in the epididymal lumen, and the

importance of androgens, particularly dihydrotestosterone in sperm maturation is well known. Indeed, androgen withdrawal results in apoptotic cell death along the entire epididymis[8]. Thus, it becomes critical to identify the genes that are controlled by androgens. In this regard, a murine epididymal retinoic acid-binding protein, belonging to the lipocalin family of proteins, was recently characterized and found to be androgen regulated and expressed in the caput epididymidis[9]. As dihydrotestosterone levels are high in the caput region, it is reasonable to propose that blockage of 5α-reductase activity with a compound such as finasteride would provide an effective means to block the expression of androgen-regulated genes that are important for sperm maturation at least in the caput region.

• Barriers to Developing Testis/ Epididymal Male Contraceptives

The need to minimize unwanted side effects is as important as having an effective and reversible contraceptive option. Consequently, contraceptives that can be delivered to the site of action are highly desirable. However, the blood–testis and blood–epididymal barriers pose a major obstacle for delivering contraceptive agents to their site of action. Since the blood–brain barrier presents a similar obstacle for development of neuropharmaceuticals, a presentation was made in which progress on this front was discussed. The delivery system that is currently being pursued takes advantage of receptors localized on capillary endothelial cells. Accordingly, the therapeutic entity is conjugated to the ligand for the receptor or a receptor antibody that can cross endothelial cells. Likewise, work was presented on current attempts to target retroviral vectors to specific cell types. Such work is important for successful gene therapy strategies. Again taking advantage of cell membrane receptors, it was reported that successful infection of cells expressing epidermal growth factor receptors (EGFR) with the subgroup A avian leukosis virus (ALV-A) was accomplished *in vitro* by the construction of a soluble bridge protein comprised of Tva, the cellular receptor for ALV-A, fused to EGF (Ref. 10). These exciting discoveries hold great promise for targeted delivery strategies for contraception because a number of candidate receptors exist on male germ cells, somatic cells and reproductive tract tissues, and the list will undoubtedly expand. One prominent example discussed at this workshop was the receptor for follicle-stimulating hormone, which could be used for delivery of compounds/genes to the Sertoli cell.

Aside from physical barriers, other barriers to the development of contraceptives were identified and included acceptance of methods by diverse cultural, religious and political groups, the possibilities (and likely probabilities) for litigation and trust of the female partner.

• Reagents and Expertise Needed

Enhancing the knowledge base on testis and epididymal biology notwithstanding, a variety of reagents and expertise were identified as necessary for development of male contraceptives targeted to the testis and epididymis. The list included the establishment of immortalized cell lines, identification of testicular stem cell markers, development of expansive databases for male reproductive genomics and proteomics, more efficient utilization of combinatorial chemistry, high throughput technologies and computer modeling, and further development of novel delivery systems to introduce contraceptive agents to their site of action. Finally, it was recognized that success in developing new contraceptive options requires collaborative efforts not only from the people doing the science, but also from government agencies, private foundations and industrial entities that provide support for the research.

• Final Thoughts

The two-day workshop was considered a success on two fronts. First, it provided a platform for excellent scientific presentations, congenial exchanges and serious but enjoyable brainstorming. Second, it provided a launching point for possible future research collaborations and funding initiatives that address the development of male contraceptives. Development of novel contraceptive approaches will require support for basic, applied and clinical research, as well as technology development and information management. In conjunction with other federal agencies, private foundations and industry, the Center for Population Research at the NICHD is challenged with designing and implementing appropriate funding strategies to support the spectrum of efforts needed to develop novel male contraceptives. Clearly, this is no small task. However, success on this front is obligatory for any serious efforts in providing the male public with safe, effective, reversible and acceptable options to control their fertility.

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Sex is All in the Brain: Report of a Novartis Foundation Symposium on the Neuronal and Cognitive Effects of Oestrogens, London, UK, 7–9 September 1999

Lisa Melton

Suspicions of the impact of oestrogen beyond its traditional role in sex maturation were confirmed with the discovery of oestrogen receptors in a great variety of tissues other than just in sex organs. Recently, research findings have indicated the central nervous system as one of the prime target organs – not only does oestrogen modulate the production and actions of serotonin, acetylcholine, dopamine and norepinephrine, but it also encourages the growth of new synapses and enhances neuronal survival. Whether the many effects of oestrogen operate through classical oestrogen receptors or alternative channels remains under intense investigation. During this three-day symposium, researchers and clinicians discussed a wide-range of oestrogen-related subjects from novel mechanisms of action to the influence of oestrogen on cognition and behaviour, and results from clinical studies that suggest oestrogen therapy after menopause may protect from Alzheimer's disease.

Until 1996, scientists struggled to explain how ovarian steroids could influence functions other than those relating to reproduction. Only the oestrogen receptor α (ERα) was known and, although abundant in the uterus and the hypothalamus, it was sparsely scattered in other non-reproductive organs and regions of the brain. Moreover, a single receptor could not explain the contrasting effects of oestrogen in different tissues. When Jan-Åke Gustafsson *et al.* (Huddinge, Sweden) discovered the second receptor, ERβ (Ref. 1), it prompted researchers to re-evaluate their ideas about the cellular mechanisms of oestrogen. The mRNA for ERβ is more widespread than for ERα, being found in the cerebral cortex, hippocampus, and the cerebellum, as well as in the cardiovascular and immune systems and other tissues. Moreover, ERβ can exert opposite effects to ERα, and each appears to have its own signalling pathway. The Swedish team now believes that stimulating the ERβ pathway may dampen ERα activity – a possibility they are currently exploring in mice in which the gene encoding for ERβ has been deleted. Gustafsson has also observed that, although ERα and ERβ bind oestradiol-17β equally well, they differ in their ability to bind other oestrogenic compounds, such as phytoestrogens.

However, whether oestrogen only operates through nuclear receptors is still debatable. The classical genomic route presents ERα and ERβ as transcription factors, binding oestrogen in the nucleus, where it regulates gene expression. However, the more rapid effects of oestrogen cannot be explained through this genomic mechanism. Increasing evidence supports the existence of a fast, non-genomic pathway initiated by a receptor located in parts of the cell other than the nucleus, and possibly associated with membranes. Ellis Levin (Long Beach, CA, USA) proposed an *in vitro* model, where nuclear and membrane receptors work in unison to accomplish the biological functions of oestrogen[2]. However, the membrane receptor has yet to be isolated.

Dominique Toran-Allerand (New York, NY, USA) suggested that the receptor has not been detected yet because it is sequestered within small vesicular invaginations of the plasma membrane called caveolae. These 'crowded little caves' are highly enriched in caveolin – a scaffold-forming protein onto which the oestrogen membrane receptor and other signalling molecules dock. By forcing a huge variety of molecules into close proximity, calveolae can thus expedite the activation by oestrogen of the Ras–Raf– MAPK (mitogen-activated protein kinase) cascade[3].

• Sexual Behaviour, Motor Coordination and Mood

Despite the puzzling absence of detectable ERs in many areas of the brain, scientists have continued to explore the effects of oestrogen on the central nervous system. Jill Becker (Ann Arbor, MI, USA) has found that oestrogen modulates neurochemical activity in the striatum, affecting sensorimotor function as well as sexual behaviour[4]. Becker measured the pacing strategy employed by female rats to ensure that copulation results in impregnation. Oestrogen facilitates this reproductive behaviour, and enhances task performance by boosting dopamine release in striatal nerve endings.

L. Melton is at the Novartis Foundation, 41 Portland Place, London, UK W1N 4BN.

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