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Review

The 25th Volume: President's Message: Andrology in the 20th Century: A Commentary on Our Progress During the Past 25 Years

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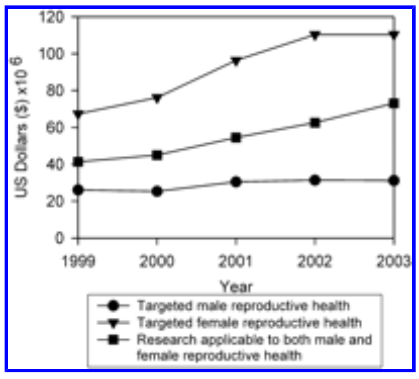
In a heartbeat, we are there. Twenty-five years ago, Dr Nancy Alexander, President of the American Society of Andrology (ASA), delivered a Presidential Address at the 1980 ASA Annual Meeting in Chicago where she shared with us her perceptions for the future of andrology by the year 2000. This "state-of-the-art" address, titled "Andrology in the Year 2000," was published in its entirety in the first volume of the *Journal of Andrology* (*J Androl.* 1980;1:149–157). It's a wonderful set of predictions, and we encourage our readers to go back to this manuscript and read (or reread) her insightful comments made at a time when the field of andrology was relatively new. The focus of Dr Alexander's comments and predictions for the year 2000 was not the entire field of andrology, but rather, 2 facets that are her area of expertise: 1) advances in male contraception, and 2) basic and clinical studies on development and maintenance of male fertility. In celebration of the Silver Anniversary of the *Journal of Andrology,* the 2004 ASA Presidents now reflect on Dr Alexander's comments and describe how the subsequent events during the past 25 years have confirmed or changed her predictions for andrology in the year 2000.

Funding for Male Reproductive Research

In 1978, National Institutes of Health (NIH) funding for population research had grown considerably from the previous 15 years and reached a total of $112 million set aside for the year. The ratio of male-female reproductive system funding was approximately 1:2, which was a vast improvement over the 1:4 ratio in 1972 (Alexander, 1980). The future for male reproductive research was promising. To address the current status of NIH funding for reproductive research, we asked the National Institute of Child Health and Human Development (NICHD) of the NIH to provide data on their funding levels during the past 5 years (1999–2003) in the areas of male and female reproductive research. It is important to stress that these numbers reflect NICHD funding only and do not include male reproductive system research by other institutes such as the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), the National Institute on Aging (NIA), and the National

Institute of Environmental Health Sciences (NIEHS), all of which have research programs that include the male reproductive tract. Nonetheless, the NICHD is considered the primary institute for reproductive research and can be used as an indicator of fiscal commitments to reproductive research. Three categories were defined for analysis and are shown in the Figure: 1) targeted male reproductive health (research that applies only to male reproductive health [eg, endocrine regulation of germ cell apoptosis in the male, examinations of male fertility, Sertoli cell development]), 2) targeted female reproductive health (research that applies only to female reproductive health [eg, gonadotropin secretion during lactation, progestin regulation of uterine hemostasis and angiogenesis, prevalence and etiologic predictors of vulvodynia]), and 3) research applicable to both male and female reproductive health and not included in categories 1 or 2 (eg, sperm–egg interactions, human immunodeficiency virus [HIV] prevention methods, efficacy of infertility treatments). Data collected for these 3 categories between 1992 and 1998 indicated a steady funding level at a 1:2 ratio for male-female targeted research, as it was in 1978. With the concerted effort to double the NIH budget between 1998 and 2002, there was a marked increase in research funding in all 3 categories (Figure). That's the good (great) news. The bad news is that funding increases in male reproductive health research lagged behind those for female (category 2) and male and female (category 3) reproductive health research, bringing the simplistic male-female research ratio close to 1:4 again, the value in 1972. Explanations for the disparate funding increases are multifactorial and include the initiation of the Women's Reproductive Health Research career development programs and the NICHD's collaboration with the Office of Women's Health to administer the "Building Interdisciplinary Research in Women's Health." Nonetheless, it is disheartening to see a return to greater disparity between male and female reproductive health emphasis at the NICHD rather than the reduced disparity predicted by Dr Alexander 25 years ago. Several key factors also contributed to the increased level of funding for research that is applicable to both male and female reproductive health during the past 5 years. For instance, the NICHD is now supporting the development and operation of a Biological Testing Facility and a Peptide Synthesis Facility. These facilities help researchers develop and assess the potential clinical uses of new compounds and formulations. Another contributing factor is that the NICHD has taken the opportunity to fund research under Center Core Grants that the National Institute of Allergy and Infectious Diseases manages. All of these activities include research applicable to male reproductive health, so there is optimism in these numbers as well. We hope that the expected downturn in NIH funding levels during the next several years will not too negatively affect the field of andrology.



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Trending data for funding levels (in millions US dollars) at the National Institute of Child Health and Human Development earmarked for male and female reproductive health research or both between 1999 and 2003 (see text for description). Data supplied by the Office of Science Policy, Analysis, and Communication, NICHD/NIH/DHHS.

Male Contraception

Dr Alexander mentioned several aspects of the hormonal control of human spermatogenesis and the initial approaches to contraception using endocrine (steroid) administration. She predicted that by 2000, we would not have a male pill but that potential products would be undergoing testing. Both of these predictions have proven correct. However, she predicted that the approaches being tested in the early years of the new millennium would not be steroids. This prediction was not correct; all the major trials in recent years have involved steroids, particularly regimens combining an androgen (various forms of testosterone) and progestins (such as desogestrel, levonorgestrel, depot medroxyprogesterone acetate, norethisterone enanthate). Such regimens are now quite effective; the pharmaceutical industry (at least in Europe) has taken notice, and a multicenter trial, sponsored by Organon and Schering, is under way using testosterone undecanoate and 3-keto desogestrel. Unfortunately, the planned participation by 2 US centers in this study was prevented by the Food and Drug Administration, which is requiring additional animal data. Dr Alexander suggested that superactive analogs of gonadotropin-releasing hormone (GnRH) combined with testosterone would provide adequate spermatogenic suppression. This approach has been disappointing, but the possible utility of antagonist analogs of GnRH, particularly in induction regimens, is under active investigation in combination regimens with various testosterone compounds and formulations.

The prediction of Dr Alexander that, by 2000, we would be closer to an immunologic method of preventing male fertility has not proven to be correct. These approaches have foundered on difficulties with reliable induction of fertility suppression, unpredictable return to fertility, and adverse side effects. No such technique is in clinical trials, nor are animal studies particularly promising. Substantially greater understanding of the basic control mechanisms of immunology will be required prior to readdressing the clinical application of immunologic approaches.

Finally, Dr Alexander's own findings of increased atherosclerotic disease following vasectomy in animals have not been confirmed in extensive studies of men. Therefore, her prediction that this putative complication would decrease the numbers of vasectomies has not been borne out by subsequent clinical experience. Vasectomy remains a very effective, safe, and widely used method for permanent fertility control in men.

Epididymal Function

Extensive research on the epididymis has been conducted during the past quarter century, and several of the issues addressed by Dr Alexander with regard to epididymal function, protein secretions, and histology are beginning to be unraveled. For example, one set of experiments has shown the importance of the initial segment, at least in mice. Knockouts of the orphan tyrosine kinase receptor c-Ros show an undeveloped initial segment and male infertility (Sonnenberg-Riethmacher et al, 1996). The infertility defect appears to be due to a defect in tail angulation, and thus, the sperm fail to reach the egg. Tantalizing evidence suggests that the infertility phenotype is due to the failure of sperm to regulate their cell volume, which may be due to an altered epididymal luminal fluid microenvironment (Yeung et al, 2000). In 1980, although a few components of epididymal secretions had been identified, we had no idea about their role in sperm maturation, their maintenance during storage, or their activity after ejaculation. Scientists now have some evidence-based ideas. One current hypothesis is that organic solutes secreted into the epididymal lumen are osmolytes that regulate water movement into and out of both sperm and epididymal epithelial cells, similar to their role in the kidney. That solutes such as inositol, L-carnitine, glycerophosphorylcholine, and glutamate are found in the 50–60 mmol/L range (Hinton and Palladino, 1995) lends support for this role. Furthermore, osmolytes may protect sperm cells from rapid changes

in osmolarity, which is important, since epididymal luminal fluid is hyperosmotic.

In 1980, Dr Alexander stated that "Only initial studies have been done on the various protein components... of the epididymal secretions" and predicted that their identification would eventually lead to new treatment methods for infertility. Following the revolution in molecular biology, this is currently the most studied aspect of epididymal biology, and many proteins have been recently discovered. While some proteins are unique to the epididymis, others are ubiquitous. The challenge to uncover their role(s) remains, since only a few secreted proteins have been assigned some kind of function. For example, the epididymis secretes defensins and defensin-like molecules, presumably for the protection of sperm and the epididymis itself (Von Horsten et al, 2002; Rao et al, 2003). CRISP-1 is a secreted protein that may be involved in either capacitation or sperm–egg binding (Cohen et al, 2000; Roberts et al, 2003). Other proteins have been identified with a putative function (eg, proteases, protease inhibitors, other enzymes), but again, their role in sperm maturation (if indeed there is one) is unknown. One recent leap forward is the identification of transporters in both sperm and epididymal epithelial cells, which has helped researchers understand how the epididymis forms the specialized luminal fluid environment. For example, several water, ion, and organic solute transporters have been identified and include the aquaporins; hydrogen plus adenosine triphosphatase for hydrogen ion transport; NHE-RF, a transporter involved in sodium/bicarbonate transport; and OCTN2, which transports L-carnitine (Breton et al, 1998; Bagnis et al, 2001; Rodriguez et al, 2002; Cheung et al, 2003). It is likely that no single secretory component is responsible for sperm maturation, but rather, that this process involves a complex series or cascade of events involving multiple cell–cell interactions.

A call was made for the development of research tools for the localization of cellular components. Considerable advances have been made in this field, and more and more proteins have been localized in different epididymal cell types. With laser capture technology, it is now possible to capture individual epithelial cells and perform reverse transcriptase-polymerase chain reaction (Kirby et al, 2003), generate complementary DNA libraries, and perform gene arrays. Hence, it is predicted that, in a few years, we will have a more thorough idea of the function of each epididymal cell type. Nonetheless, we still lack an understanding of the fundamental cell biology of epididymal function—protein synthesis, trafficking, secretion, and endocytosis, for instance, and this remains an area of future need. With advances in imaging, we can now perform in situ hybridization with immunohistochemistry, observe calcium movements in real time, and track epididymal development and fluid movement with time-lapse microscopy; thus, further advances are on the horizon. With more gene promoters being analyzed, it will not be too long before it will be possible to target gene silencing agents to specific epididymal cell types, as is already done to some regions of the epididymis. Hence, these approaches may also provide valuable information on the function of some genes/proteins in a cell type in a particular epididymal region in the very near future.

Semen Standards

Major advances were made during the past quarter century to standardize semen analysis within the andrology community. Andrology laboratories in the United States now are included under the Clinical Laboratory Improvement Act (CLIA), which considers semen analysis a high-complexity test. Among other regulations, this designation requires adherence to strict standards, including daily quality control, laboratory certification or accreditation with attendant inspections, and oversight of a board-certified, doctoral-level laboratory director. Consequently, proficiency testing is now available from many providers for sperm concentration, viability, morphology, antisperm antibody assessment, and, most recently, motility. This semiannual assessment demonstrates a laboratory's ability to accurately analyze these male reproductive measures. Changes to CLIA now include periodic

technologist competency testing, which is commercially available.

The World Health Organization (WHO) *Laboratory Manual for the Examination of Human Semen* is now published in its 4th edition and, since 1980, has set performance and evaluation recommendations that are used worldwide (WHO, 1999). Although semen analysis standards were not published by the ASA as Dr Alexander thought appropriate, many current and past ASA members have been intimately involved in writing these and other guidelines with the worldwide community. That we are now working from the 4th edition speaks for the fact that semen analysis, like all laboratory testing, requires continuous updating and reevaluation. This is most apparent in the area of sperm morphology, where evaluation systems have changed markedly over the years. In fact, this past year at the 2004 ASA Annual Meeting, a full-day Sperm Morphology Workshop was held that focused on laboratory-based training of the 2 most popular sperm morphology classification systems used by fertility specialists today: the WHO 3rd edition and the WHO 4th edition, also known as Strict Criteria. Unfortunately, clear standards for these systems are lacking, making the reproducibility of analysis difficult. We continue to need consistency and training for the clinical laboratory as well as for toxicology and industrial studies, which are now mandated in the United States.

During the past 20 years, we witnessed the introduction and use of computer-assisted semen analysis (CASA) systems for sperm concentration and motility analysis in the clinical and research andrology laboratory. While broadening and quantifying our information on motility parameters, these CASA systems, when used correctly, can reduce subjective variability in semen analysis across technicians in a single laboratory and even between laboratories. However, useful clinical correlates for all of the new motility information are still lacking and remain an area of future research. The application of CASA to morphometric analysis of sperm is relatively new, and its use is hampered by a lack of clear morphology standards. The cost and complexity of most CASA instruments remain significant barriers to their widespread adoption.

Andrology Laboratory Tests

The past 25 years have brought enormous advances and uses for andrology testing and application above and beyond what was anticipated by Dr Alexander or anyone else, for that matter. The Hamster Ova–Sperm Penetration Assay or SPA, introduced by Rogers (1985), became a standard tool for the comprehensive andrology laboratory as predicted. Throughout the 1980s and 1990s, the SPA was widely used for evaluating the fertilizing potential of human spermatozoa with a discriminating power greater than that of the semen analysis alone (Rogers, 1985). As predicted, this technology was marketed by several commercial ventures. Reference laboratories developed systems for overnight transportation of sperm samples, which allowed centralized laboratories to perform the SPA for clinicians across the country and made this test widely available. Additionally, several companies sold frozen hamster ova, which made offering this assay a possibility in andrology laboratories that did not have prior access to an animal facility. As in vitro fertilization (IVF) became common practice for achieving pregnancy in the infertile couple, the SPA proved to be predictive of fertilization success in vitro. However, with the advent of intracytoplasmic sperm injection (ICSI), the use of the SPA has waned considerably. Although this test was shown to be predicative of the need for ICSI (Gvakharia et al, 2000), the success of ICSI and its subsequent widespread use and application in almost every IVF clinic have rendered the labor-intensive and costly SPA obsolete.

It was predicted that cervical mucus penetration assays would become a routine andrology laboratory test and, indeed, this came to pass. This aided the physician in making choices for the use of artificial insemination or even sperm donor insemination when "hostile" mucus was encountered for the sperm from a female's partner. Antisperm antibody testing not only became standard in the andrology work-up but also highly specific with the introduction of the Immunobead Test, which

identified the immunoglobulin subtype present on sperm or within the female genital tract (Carson et al, 1988). However, once again, with the commonplace use of IVF-ICSI for establishing pregnancies in the infertile couple, the use of both of these assays has waned considerably from their peak usage in the 1990s.

Newer andrology laboratory tests have been introduced during the past decade and, although not commonplace in the standard andrology laboratory, their availability in centralized reference laboratories has allowed their implementation when clinically necessary. The Sperm Chromatin Structure Assay assesses sperm DNA fragmentation and has been found to correlate with fertility potential (Evenson and Jost, 2000). In addition to its routine prognostic value, this cytometry assay is useful for evaluating men at increased risk for DNA damage that can follow occupational exposures, that can occur with aging, or that can follow freeze-thaw procedures. Discoveries in the field of genetics have led to Y-chromosome deletion testing for infertile men, and commercial kits are available for this purpose. Full deletions or microdeletions in the long arm of the Y chromosome (azoospermic factor or AZF regions) have been shown to cause azoospermia, oligozoospermia, and related male infertility problems, and the ability to screen for these in the infertile patient has vastly improved diagnostic capabilities (Kent-First et al, 1996; Reijo et al, 1996). The use of testicular sperm extraction (TESE) from azoospermic men, combined with IVF-ICSI to achieve fertilization, permits the transmission of Y-related infertility to the male offspring, making this testing modality imperative for informed decision making by the patients who undergo these procedures.

The past 25 years have seen the rise, as well as the subsequent decline, of intensive andrology testing in the work-up of the infertile couple. The ease and availability of assisted reproductive technologies (ART) have led many clinicians to implement these techniques quickly and to forgo a full diagnostic male evaluation. Although this approach may lead to a pregnancy in the short term, it carries the risk that the cause of infertility will go undiagnosed. Since infertility can be a marker of serious medical problems or toxicant exposures, denying the male partner a full work-up has important health consequences. Furthermore, several studies have shown that the cost-effectiveness (cost per live delivery) of treating more common male factor problems such as varicocele or vasal and/or epididymal obstruction is much greater than the initial use of IVF-ICSI (Kolettis and Thomas, 1997; Schlegel, 1997). Thus, the continued full evaluation and treatment of the male partner remains an important part of fertility treatment for the couple.

Testicular Biopsy—TESE and ICSI

IVF and its variations have revolutionized the field of reproductive medicine during the past 25 years in ways that could not have been foreseen. This is particularly true for the treatment of the infertile male. Prior to the advent of IVF, severe male factor infertility had very limited and largely unsuccessful treatment options, and infertile couples either used donor sperm or adopted. In its initial years, IVF permitted fertilization attempts by severe oligo-, terato-, and/or asthenozoospermic males, although their success in fertilizing the ovum in vitro was markedly lower than that of the normospermic male. However, the introduction of ICSI in the 1990s allowed successful fertilization by a single isolated, immotile, and even, in some cases, dead sperm (Van Steirteghem et al, 1996). The combination of ICSI with TESE created treatment options for men whose ejaculate was azoospermic due to obstructive or nonobstructive causes, including those patients in whom only isolated pockets of spermatogenesis existed within the testes (Silber et al, 1995). Subsequent advances in this treatment modality include the cryopreservation of TESE sperm, which permits the advanced removal and storage of sperm prior to initiating an IVF cycle (Prins et al, 1999; Habermann et al, 2000). In 1980, Dr Alexander predicted that the use of testicular biopsy

would decline, since it did not provide useful treatment options. It is noteworthy, however, that Dr Alexander predicted a renewed interest in testicular biopsies if biochemical and metabolic studies led to effective treatments for pathologic conditions that were previously unrecognized or untreatable. This prediction has been partially realized— however, it was through TESE-ICSI rather than biochemical methods. In fact, testicular biopsy can be combined with TESE–sperm cryopreservation both to diagnose and treat infertility in a single procedure (Schoor et al, 2002), and this approach is now used in many centers worldwide.

Artificial Insemination—Sperm Cryopreservation

When the first IVF baby was born 25 years ago, the common practice for treating the infertile couple was artificial insemination using either the partner's sperm (AI-partner) or donor sperm (TDI). AI-partner then became the first line of therapy prior to the more expensive ART approaches but, in recent years, has been used less as practitioners realized the greater effectiveness of ART in treating male factor infertility. The epidemic of HIV that began in the 1980s radically changed the practice of sperm donor screening and use. Today, all TDI procedures use sperm that was frozen and stored in liquid nitrogen "quarantine" while the donor was extensively screened for genetic abnormalities and infectious agents. This practice led to needed improvements in the cryopreservation approaches for human sperm that Dr Alexander had requested in 1980. The improvements in freeze-thaw outcomes are due, in part, to the development of complex semen extenders and buffer systems that are now commercially available (Weidel and Prins, 1987). However, we still lack and need a testing modality that will predict the freezing success and subsequent fertilizing potential of frozen-thawed human sperm prior to the commencement of sperm freezing.

Men's Health

Dr Alexander called for an increased focus on the interaction of the male reproductive system with other body systems—"the organism as a whole." What insight she had. In the past few years, the field of Men's Health has emerged as a new health emphasis area. In fact, this topic was the theme of the 2004 ASA postgraduate course titled "Men's Health: On the Horizons of Andrology." Renowned experts presented lectures on androgen physiology in men, cardiovascular repercussions, sexual and psychosocial health, osteoporosis in men, use and abuse of anabolic steroids in sports, and trans-sexualism to provide a framework and vision to facilitate future contributions by andrologists to these important areas. The ASA shares its commitment to this endeavor with many organizations, including the NIH, in recognizing Men's Health as an important issue for the 20th century. Dr Alexander's vision and hope for a holistic approach to andrology is finally being realized.

Summary

From time to time, it is useful for a profession to review its history and take stock of its progress and obstacles. We are fortunate that our past President Dr Alexander provided the ASA with a blueprint for the future that now becomes a lens to focus on our past accomplishments and failures. We challenge one or several of our members to replicate her audacious insights with predictions for the next quarter century.

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