# Editorial

**Advances in Andrology and Male Reproductive Health**

Andrology, the branch of medicine concerned with male reproductive health, emerged as a specialty at the end of the nineteenth century. However, it was not until the latter half of the past century that andrology had been recognized as one of the most intriguing sub-specialties of human reproduction. This is due to rapidly rising volume of scientific evidence that document the critical role of spermatozoa in the fertilization process. Due to the true nature of research involving the classical disciplines of physiology, biochemistry and molecular biology, scientific knowledge and application of andrology evolve continuously. Invariably, it is leading to a growth in andrological information and better understanding of the field of male reproductive biology.

The scope of modern andrology now covers a wide spectrum from genetic studies to pubertal changes in the male and from infertility and assisted reproduction techniques to disorders of the prostate, sexual function and contraception. In this Special Issue of *The Open Reproductive Science Journal*, we examine some of these technical developments by highlighting the most up-to-date advances and contentious issues in male reproduction in a collection of high quality reviews written by distinguished experts in their fields. These topics were chosen to demonstrate the exciting breadth of andrology and the opportunity it holds for both understanding and improvement of male reproductive health.

This Special Issue commences with a provocative commentary that presents a critical appraisal of the new 5$^{th}$ edition World Health Organization (WHO) reference values for laboratory semen evaluation. Reference values were obtained from data involving approximately two thousand men who were able to impregnate their wives in a period of twelve months or less. Apart from total sperm number per ejaculate, the cutoff limits of these distributions are lower than the 'normal' or 'reference' values of previous WHO manual editions. Despite the notable advance of using evidence-based data to obtain the new limits, a systematic review of the literature was not performed to identify all data on semen quality in various populations. Conversely, the generation of the new WHO reference limits was biased by pooling very few studies coming mainly from Northern European cities. From these data, it seems unsound to assume, as proposed by the 5$^{th}$ edition WHO manual, that the reference values represent global semen characteristics of fertile men. The authors go on by discussing other flaws of the newly proposed reference limits for the semen analysis parameters and how the strict adoption of such limits may have an impact on patient referral, diagnosis, treatment of recognized conditions such as varicocele, and on the indications of assisted reproductive modalities [1]. The authors conclude that more debate is needed before the universal adoption of the proposed WHO reference values and suggest that a better approach would be the presentation of reference values by percentiles rather than solely the lower cut off limits for those who consider adopting them. This commentary is very appropriate for two main reasons. First, it

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highlights the shortcomings of the routine semen analysis to discriminate fertile and infertile male populations. Second, it indicates that the time has come for technological developments in the field of andrology to bring robust and cost-effective clinically useful sperm function tests to fix the shortcomings of the routine semen analysis.

The second article is a comprehensive review of how basic andrological research can be translated into male infertility clinical practice [2]. The authors discuss several novel concepts that emerged in the recent years and their implications for diagnosis and treatment of male infertility. For instance, the clinical usefulness of two important tests recently added to the andrologic armamentarium, i.e., sperm DNA integrity and measurements of oxidative stress, and how they can be integrated to the conventional semen analysis to provide a more comprehensive evaluation of a man's fertility status. Molecular biology genetic testing involving the Y-chromosome can now correctly identify oligozoospermic and azoospermic men misdiagnosed as having idiopathic infertility. Moreover, Y-chromosome testing is of prognostic value for sperm retrieval in non-obstructive azoospermia. In the field of treatment, medication and surgical options for infertile males are expanding. Evidence now suggests that antioxidant therapy along with life-style modifications may improve the male reproductive health. The refinements of microsurgery are improving treatment success for infertile males. Microsurgical treatment of clinical varicoceles may optimize the reproductive outcome of couples undergoing intracytoplasmic sperm injection or microsurgical testicular sperm extraction. Established concepts have now been challenged by new ones. Men with non-obstructive azoospermia, elevated follicle-stimulating hormone levels and small testes, for example, are no longer considered sterile because modern retrieval techniques can be used to collect testicular sperm and produce a healthy biological offspring via assisted conception.

Alaa Hamada and colleagues follow this theme by presenting an insightful review on how modern andrology may unravel the mysteries of unexplained male infertility [3]. After ruling out female infertility factors, erectile problems and coital factors, modern andrology may help to analyze the unexplained male fertility problem on the basis of cellular and subcellular mechanisms. Furthermore, this analysis may lead to the selection of proper treatment options fitting the needs of patients with unexplained infertility. In this chapter, the authors highlight the fact that the understanding of sperm physiology and fertilization is far from complete. However, contemporary andrology has novel techniques and methods for the diagnosis of hidden sperm functional problems. Laboratory seminology is clearly moving from the assessment of conventional semen profile into the assessment of sperm function. Such strategy is likely to aid in the understanding of the underlying pathophysiology of male infertility and in suggesting options for treatment and prevention.

In terms of genetic contributors to male reproductive health, this Special Issue contains three insightful, comprehensive reviews that bring us up to date with the current status of this field. Rima Dada and her group [4] provide a comprehensive data summary that has been generated on the genetics aspects of male infertility and its implication on diagnosis and treatment. It begins by establishing the genetic causes of male infertility and expands by helping clinicians, scientists and infertile couples considering ART to better understand the ways to maximize the chances of having a healthy child. Genetic male infertility may be presented with physical phenotypical abnormalities or with sperm abnormalities of unknown origin. Genetic screening includes cytogenetic analysis to detect numerical and structural chromosomal rearrangement, single gene mutations and

assessment of nonspecific DNA damage. The authors present practical recommendations for testing and the possible implications of using spermatozoa from men with genetic abnormalities for assisted conception. Additionally, a timely review of DNA damage is included. It provides the key elements for understanding oxidative stress involvement in the etiology of defective sperm function and DNA damage. Sperm nuclei peripheral region has genes critical for early embryonic development. The DNA of these genes is bound to histones and therefore more prone to oxidative damage. Oxidative DNA damage may result in methylation errors in sperm that may increase the incidence of imprinting defects in ART conceived children. In such circumstances, early diagnosis and prompt antioxidant treatment may result in significant decline in free radical levels and oxidative DNA damage. Singh Rajender [5] reviews the role of epigenome and its impact on spermatogenesis and embryo formation. Robust paternal epigenetic contribution to embryogenesis requires that DNA, and chromatin structure as a whole, contain layers of regulatory elements that are sufficient to drive genes towards activation or silencing upon delivery to the egg. Changes in epigenome are now well known to affect gene expression, and several genes participating in spermatogenesis have been demonstrated to be epigenetically regulated. Differential methylation in promoter regions of certain genes contributes to male infertility, while epigenetic changes in others do not. The authors present information on epigenetic modifications not only affecting spermatogenesis but also disease susceptibility later in life. Endocrine disruptors known to adversely affect spermatogenesis could operate by affecting epigenetic modifications. This field is rather young and more investigation to explore epigenetic modifications in genes regulating spermatogenesis is required. Aspinder Singh [6] follows this theme by expertly reviewing the role of sperm chromatin integrity and DNA damage in male infertility. The chromatin structure is clearly a key aspect of sperm cell biology. Sperm DNA maturation and appropriate packaging are vital steps in the proper development of spermatozoa. Any defects in these processes can result in substantial sperm DNA damage which may then be transmitted to the embryo. Sperm DNA damage may come in the form of chemical modifications, abortive apoptosis or reactive oxygen species attack. The authors beautifully review how damage must be analyzed and understood, by means of various laboratory tests, and which steps can be taken to not only minimize but also to prevent DNA damage.

Understanding the mechanisms by which the acrosome reaction (AR) is regulated is central to models of fertilization. The acrosome reaction has been recognized as a biological phenomenon for several decades but only now we are beginning to understand its molecular mechanisms. The zona pellucida (ZP) matrix surrounding the oocyte is responsible for the binding of the spermatozoa to the oocyte and induction of the acrosome reaction (AR) in the ZP-bound spermatozoon. The AR is crucial for the penetration of the ZP matrix by spermatozoa. In humans, it has been recently demonstrated that N-linked glycans of ZP1 and ZP4 (in addition to ZP3), are critical for AR induction. In this chapter [7], the authors present a summary of their long-term research experience with *in vitro* acrosome reaction testing. Tests for detecting acrosome reaction (AR) and their clinical utility are presented in a concise manner. Due to the fact that acrosomal loss can be a result of sperm death, AR testing should be used in conjunction with an assay to monitor sperm viability. The authors' proposed method of using the hyposmotic swelling test in conjunction with fluorescence probes to monitor sperm viability seems to be a step towards simplification. Different stimulants, such as phosphodiesterase inhibitors, drugs and toxins have been investigated in their ability to affect the sperm ability to undergo *in vitro* acrosome reaction. Sperm acrosomes are also sensitive to the freezing-thawing process and strategies have

been described to minimize cryodamage. The assessment of the acrosome has been shown to be a stable parameter of sperm function and a valid tool to predict the fertilizing potential of human spermatozoa. The acrosome reaction following ionophore challenge (ARIC) has good predictability of sperm's fertilizing potential for assisted conception techniques including intrauterine insemination and conventional *in vitro* fertilization.

Mathur and colleagues [8] address the role of apoptotic cell death within the testes as a vital facet of survival and propagation of healthy, viable sperm. Nuclear factor-kappa B (NF-κB) is a family of transcription factors implicated in numerous stress responses including apoptosis within male testicular cells. NF-κB has proven to be of great importance when it comes to programmed cell death because of its ability to act as a double-edged sword, being proapoptotic in one instance and anti-apoptotic in another. The authors' hypothesis is that NF-κB has a key role in executing reactive oxygen species (ROS)-mediated apoptosis inside the testis following exposure to environmental toxicants. During severe stress NF-κB activation protects the Sertoli cells and simultaneously induces the activation of Sertoli cell gene(s) that exerts proapoptotic effects on germ cells. Dysregulation in NF-κB expression may be secondary to toxicant exposure and it is suggested that this mechanism have a negative impact on testicular function.

Major advances in biomolecular techniques along with the development of mass spectrometers of greater accuracy and sensitivity are leading to an unprecedented growth in the knowledge of spermatozoa function. The spermatozoon is an excellent target for proteomics because the functional transformation of this cell during its journey from the seminiferous tubules to the surface of the oocyte, takes place in the complete absence of contemporaneous gene transcription. Du Plessis *et al.* [9] review the current knowledge concerning the proteomic profiles of human spermatozoa and the potential applications of such knowledge on the understanding of sperm dysfunction.

Of course, not all aspects of sperm function are driven by changes in the proteome. A section of this Special Issue is fully dedicated to the influence of endocrine and environmental factors on male infertility. The role of thyroid hormones on male reproductive health is a novel concept. Thyroid hormone receptors have been recently identified in Sertoli cells and shown to be important for their development. Singh and colleagues [10] expertly summarized the data that has been generated on the role of thyroid hormone receptors on testicular cells and the implications of thyroid disease for male fertility. The possible link between exercise and male fertility/subfertility is presented by du Plessis and colleagues [11]. Their chapter highlights the fact that although physically active individuals have better semen parameters and hormone levels than sedentary men, growing evidence suggests that exercise (especially when excessively practiced) may lead to adverse effects on the reproductive system and fertility. In another article, a comprehensive summary of apoptotic regulators of male fertility is presented, including cytokines and B-cell lymphoma family of genes, as well as conditions that may lead to increased apoptosis such as heat, radiation, drugs, alcohol and smoking [12]. The authors expanded the theme of apoptosis by pinpointing how environmental toxicants affect apoptosis and male reproductive health. Numerous studies have identified the pathways through which specific toxicants trigger oxidative stress-induced testicular apoptosis. Several toxins have been examined such as alkylphenolpolyethoxylates, polycyclic aromatic hydrocarbons, bisphenol A, polychlorinated biphenyls, lindane, methoxychlor,

toluene, *tert*-Butyl hydroperoxide, phthalates, 2,5-hexanedione, 1,3-dinitrobenzene, nitrobenzene, and ethanol. This information is relevant for the prevention of environmental and occupational hazards because potentially harmful compounds, in particular, continue to proliferate in households and workplaces. The Cleveland Clinic reproductive research team pioneered on suggesting a possible link between electromagnetic wave field energy emitted by cellular phones and male infertility. Cell phone manufacturers are competing for providing the highest quality communication services. However, it is still unknown the impact of these advances on human health. Their authoritative review [13] depicts the physiopathologic mechanisms involved in this process and summarizes the current concepts and the emerging evidence suggesting that electromagnetic energy is a real threat to male reproductive health.

Currently, assisted reproductive technology (ART) is the only option for most men with severe male factor infertility to have their biological offspring. Success has been achieved with intracytoplasmic sperm injection (ICSI) in several male infertility conditions, including immunological infertility, severe sperm deficiencies and azoospermia. In this chapter [14], the authors prepared a comprehensive summary of their decade experience in the management of infertile males using assisted conception. Laboratory management of male infertility cases requires special attention because spermatozoa collected from men with severely impaired spermatogenesis are often compromised and fragile. The authors provide tips and pitfalls of handling such gametes inside the laboratory. Adherence to state of the art laboratory techniques and quality control are recommended to avoid jeopardizing sperm fertilizing potential and the chances of achieving a live birth. Several strategies are proposed for optimizing the chances of conception for men enrolled in ART. For instance, efforts should be made to improve the male health status prior to embarking on ART because current evidence suggests that improvements in male reproductive health may improve treatment outcome. Air quality control in the embryology laboratory and critical areas dealing with gametes is another issue and it may positively impact embryo development and pregnancy results. Sperm retrieval techniques using microsurgery offer the possibility of collecting testicular spermatozoa even in the most difficult cases of nonobstructive azoospermia.

Of great interest is the data suggesting a role of oxidative stress (OS) in the pathogenesis of diseases such as hyper- and hypothyroidism [10] and HIV/AIDS [15]. Oxidative stress has been identified as one of the main factors affecting fertility status, and OS has been extensively studied in recent years. Elevated levels of seminal ROS are found in men with thyroid diseases [10]. Moreover, increased levels of ROS are present at the onset of human immunodeficiency virus (HIV) infection [15] and have been implicated as a co-factor in the progression of acquired immunodeficiency syndrome (AIDS). Delayed response by the immune system upon HIV infection may be due to an initial depletion of antioxidants, which play a critical role in scavenging excess ROS to maintain normal physiological conditions. In this chapter, the authors discuss antioxidant treatment as a promising and cost-effective therapeutic approach in treating HIV-infected individuals on a global scale.

Overall, this Special Issue of "*The Open Reproductive Science Journal* gives an overview of the scope of modern andrology and its role in the male reproductive health. We recommend the contents to students and researches in the biological, veterinary and medical sciences or clinicians interested in following the exponential growth in our knowledge of the

mechanisms regulating the pathology and physiology of male reproductive system. Due to the multidisciplinary nature of andrology, unsolved problems present themselves and the opportunities for advancement continue to expand. We hope you appreciate this Special Issue of *The Open Reproductive Science Journal* and share our excitement in the study of andrology and male reproductive health.

**Ashok Agarwal and Sandro C. Esteves**
(*Guest Editors*)

Center for Reproductive Medicine
Cleveland Clinic
Cleveland
Ohio 44195, USA
Tel: (216) 444-9485
Fax: (216) 445-6049
E-mail: agarwaa@ccf.org

ANDROFERT
Andrology and Human Reproduction Clinic
Campinas, São Paulo 13075-460
Brazil
Tel: +55 19 3295-6992
Fax: +55 19 3294-6992
E-mail: s.esteves@androfert.com.br

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**CONFLICT OF INTEREST**

None Declared.

**REFERENCES**

[1] Esteves SC, Agarwal A. Impact of the new WHO guidelines on diagnosis and practice of male infertility. Open Reprod Sci J 2011; 3: 7-15.
[2] Esteves SC, Agarwal A. What is new in the clinical assessment and treatment of the infertile male? Open Reprod Sci J 2011; 3: 16-26.
[3] Hamada A, Esteves SC, Agarwal A. The role of contemporary andrology in unraveling the mystery of unexplained male infertility. Open Reprod Sci J 2011; 3: 27-41.
[4] Dada R, Thilagavathi J, Venkatesh S, Esteves SC, Agarwal A. Genetic testing in male infertility. Open Reprod Sci J 2011; 3: 42-56.
[5] Rajender S, Agarwal A. Aberrant epigenetic modifications in male infertility. Open Reprod Sci J 2011; 3: 57-64.
[6] Singh A, Agarwal A. The role of sperm chromatin integrity & DNA damage on male infertility. Open Reprod Sci J 2011; 3: 65-71.
[7] Esteves SC, Verza Jr S. Relationship of *in vitro* acrosome reaction to sperm function: an update. Open Reprod Sci J 2011; 3: 72-84.
[8] Mathur P, Francispillai M, Selvaraju V, Agarwal A. NF-kB in male reproduction: a boon or a bane? Open Reprod Sci J 2011; 3: 85-91.
[9] Du Plessis S, Kashou A, Agarwal A. The advent of sperm proteomics has arrived. Open Reprod Sci J 2011; 3: 92-7.
[10] Singh R, Agarwal A. Thyroid hormones in male reproduction and fertility. Open Reprod Sci J 2011; 3: 98-104.
[11] Du Plessis S, Kashou A, Gupta A, Agarwal A. Is there a link between exercise and male factor infertility? Open Reprod Sci J 2011; 3: 105-13.
[12] Mathur P, Huang L, Kashou A, Vaithinathan S, Agarwal A. Environmental toxicants and testicular apoptosis. Open Reprod Sci J 2011; 3: 114-24.
[13] Hamada A, Singh A, Agarwal A. Cell phones and their impact on male infertility: fact or fiction. Open Reprod Sci J 2011; 3: 125-37.
[14] Esteves SC, Schneider DT. Male infertility and assisted reproductive technology: lessons from the IVF laboratory. Open Reprod Sci J 2011; 3: 138-53.
[15] Kashou A, Agarwal A. Oxidants and antioxidants in the pathogenesis of HIV/AIDS. Open Reprod Sci J 2011; 3: 154-61.