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EDITORIAL

The azoospermic male: current knowledge and future perspectives

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This special issue is fully dedicated to the topic of azoospermia and contains the seminal work of renowned scientists and clinicians from seven countries on three continents. In seventeen chapters, a comprehensive review of the epidemiology, genetics, physiopathology, diagnosis, and management of azoospermia addresses our current knowledge on the topic. The clinical results of assisted reproductive techniques applied to this category of male infertility and the health of offspring originating from such fathers are critically analyzed. In addition, the challenges and the future biotechnological perspectives for the treatment of azoospermic males seeking fertility are discussed.

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Two major breakthroughs revolutionized the field of male infertility in the last three decades. The first was the development of intracytoplasmic sperm injection (ICSI) for the treatment of male factor infertility, and the second was application of ICSI to azoospermic males, with the demonstration that spermatozoa derived from either the epididymis or the testis were capable of normal fertilization and pregnancy. Azoospermia, defined as the complete absence of spermatozoa in the ejaculate, invariably results in infertility but does not necessarily imply sterility. In fact, azoospermia has been recognized as one of the most intriguing topics in male infertility. Due to the true nature of research involving the classical disciplines of physiology, biochemistry and molecular biology, a rapid rise in the volume of scientific knowledge regarding azoospermia has been obtained. Invariably, this has led to a better understanding of the multi-faceted aspects of azoospermia. However, there is still relatively little data within the literature supporting common clinical practices. The reproductive potential of azoospermic males with different etiologies is unclear; furthermore, the association of an increased risk of birth defects and potential iatrogenic transmission of genetic abnormalities with ICSI using sperm retrieved from these patients is still under debate.

The scope of azoospermia related-infertility now covers a wide spectrum, including genetic studies, hormonal control, microsurgical and medical therapy, assisted reproduction techniques, and innovative stem cell research that aims to create artificial gametes. In this special issue of *Clinics*, we invited leading, internationally recognized scientists and clinicians from the various sub-specialties to compile a collection of high-quality and comprehensive reviews highlighting the most current advances and contentious issues in azoospermia. Our aim is to provide readers with a thoughtful and wide-ranging review of the epidemiology, genetics, physiopathology, diagnosis, and management of azoospermia. The text is the first of its type and represents an invaluable tool for both basic scientists with an interest in sperm biology and clinicians (urologists, gynecologists, reproductive endocrinologists, and embryologists) working in the field of infertility. The selection of topics demonstrates the exciting breadth of this category of male infertility and the opportunity that research in this area holds for both understanding and improving the reproductive health of azoospermic males.

This Special Issue commences with provocative insights into the genetic and epigenetic paternal contribution to the human embryo (1). Dada and colleagues from New Delhi present the current knowledge on the role of spermatozoa as highly specialized cells with the purpose of not only delivering competent paternal DNA to the oocyte but also providing a robust epigenetic contribution to embryogenesis. The paternal epigenetic contribution to embryogenesis requires that both the sperm DNA and the 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 the epigenome are now known to affect gene expression, and several genes participating in spermatogenesis have been demonstrated to be epigenetically regulated.

The second article by Marcello Cocuzza and colleagues from the University of São Paulo is a comprehensive review of the epidemiology and etiology of azoospermia. According to these authors, azoospermia is identified in approximately 1% of all men and 10% to 15% of infertile males (2). With a population of approximately 3 billion

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people at reproductive age, a gross estimate indicates that approximately 10 million men worldwide are azoospermic. The authors explore several conditions that may lead to azoospermia and didactically divide them into pre-testicular, post-testicular, and testicular causes. Despite the advances in the diagnostic tools that identify men with genetic-origin azoospermia, many men are still classified as having idiopathic azoospermia because the specific etiological factor remains unidentifiable. Therefore, determining the etiology of azoospermia remains one of the main challenges in this field.

Drs. Gudeloglu and Parekattil from the United States follow the theme by highlighting the importance of the clinical exam in the investigation of the azoospermic male. The authors detailed review defines why and how the clinical evaluation should be undertaken. They also discuss the usefulness and limitations of testis biopsies and imaging studies in the context of azoospermia and the importance of genetic counseling before using the spermatozoa from men with nonobstructive azoospermia (NOA) for assisted reproductive techniques (3). Dr. Aziz provides a laboratory perspective from the United Kingdom by cleverly defining azoospermia as a descriptive term for ejaculates that lack spermatozoa without implying a specific underlying cause (4). The author stresses that proper techniques are needed to reduce the amount of analytical error and enhance sperm count precision when evaluating semen specimens. The correct assessment of an initially azoospermic semen specimen should be followed by an examination of the pelleted semen to exclude cryptozoospermia, which is defined by the presence of a very small number of live sperm in a centrifuged pellet. An accurate assessment of very low sperm counts aims to avoid labeling severely oligozoospermic men as azoospermic, which is particularly important in the current era of assisted reproduction technology. Dr. Azizs chapter provides insightful information on the seminal plasma biomarkers that may aid in determining the causes of azoospermia. Laboratory seminology is clearly moving from the assessment of conventional semen profiles into the assessment of sperm function. This strategy is likely to aid in the understanding of the underlying pathophysiology of male infertility, and noninvasive biomarkers may be useful in discriminating NOA cases from obstructive azoospermia (OA).

This Special Issue contains an authoritative article that provide the current knowledge on the genetic aspects of azoospermia and the testing available for clinical use. Dr. Hamada and his co-authors from the Cleveland Clinic provide an immense summary of results on the genetic aspects of male infertility and implication of these results on the diagnosis and treatment of azoospermic males (5). Molecular biology genetic testing involving the Y-chromosome can now correctly identify azoospermic men misdiagnosed as having idiopathic infertility. Moreover, Y-chromosome testing is of prognostic value for sperm retrieval in NOA. The authors also present practical recommendations for testing, and they discuss the possible implications of using spermatozoa from men with genetic abnormalities for assisted conception.

In the clinical setting, azoospermic patients are diagnosed as having obstructive or nonobstructive azoospermia. Obstructive azoospermia has been attributed to a mechanical blockage that can occur anywhere along the reproductive tract, including the vas deferens, epididymis, and ejaculatory duct. OA is considered to be one of the most favorable prognostic conditions for male infertility because spermatogenesis is not disrupted, unlike in NOA. Drs. Baker and Sabanegh from the Cleveland Clinic discuss the current indications, techniques and results of reconstructive procedures in OA (6). These authors highlight the refinements in microsurgery that have optimized the success of reconstructive procedures; additionally, the authors indicate that the use of optical magnification is now the gold standard for vasal reconstruction. While the results of reconstructive procedures are excellent following vasectomies, other complex repairs may be required, especially in other etiological categories of OA. Despite being highly successful, microsurgical reconstruction may not be indicated in all men with OA, such as in patients with congenital bilateral absence of vas deferens (CBAVD) and certain cases of post-infectious obstructions or failed vasectomy reversals. In such cases, sperm retrieval can be performed for use with ICSI.

Nonobstructive azoospermia, on the other hand, poses a different challenge. From the management standpoint, men with NOA are the most difficult to treat, and extensive debate exists on the benefit of intervention for this category of male infertility. Various conditions may cause NOA, including genetic and congenital abnormalities, post-infectious issues, exposure to gonadotoxins, medications, varicocele, trauma, endocrine disorders, and idiopathic causes. This Special Issue contains three articles that critically explore the role of medical and surgical therapy in nonobstructive azoospermia.

Renato Fraietta and colleagues from the Federal University of São Paulo provide a timely review on hypogonadotropic hypogonadism (HH), which is a failure of spermatogenesis due to a lack of appropriate stimulation by gonadotropins (7). This category of patients includes not only congenital forms of HH but also a subset of men whose spermatogenic potential has been suppressed by excess androgens or steroids. These patients benefit from specific hormonal therapy and often show remarkable recovery of spermatogenic function with exogenously administered gonadotropins or gonadotropin releasing hormone. Unfortunately, not all men with NOA have HH. In fact, the larger category of NOA consists of men with intrinsic testicular impairment in which empirical medical therapy shows little benefit, as noted by Dr. Rajeev Kumar from the All India Institute of Medical Sciences in New Delhi (8). However, due to the developments in ART, a renewed interest in the role of interventions in this subset of NOA patients has developed. The author expertly discusses the role of medical therapy in these men to improve the quantity and quality of sperm that can be eventually retrieved from their ejaculates or from their testes for use in ICSI. In this sense, gonadotropins, aromatase inhibitors and non-steroidal antiestrogens show promise in achieving this endpoint. Lastly, Dr. Kubilay Inci from Turkey provides a critical appraisal on the role of varicocele repair for men with NOA (9). His authoritative review discusses the pathophysiology of varicocele-related infertility, and the discussion is supported by his own experience on the impact of varicocele repair in azoospermic patients. From the limited published data, it has been suggested that varicocele repair may not only allow small quantities of sperm to appear in the ejaculate but also may enhance the chances of retrieving sperm from the testis of these patients.

Even a minimal restoration of sperm production facilitates sperm injection procedures.

Currently, ART is the only option for most men with azoospermia-related infertility to have their biological offspring. Success has been achieved with ICSI in both obstructive and nonobstructive azoospermia, and the use of non-ejaculated sperm coupled with ICSI has become a worldwide established procedure. Surgical methods have been developed to retrieve spermatozoa from the epididymides and testes. After sperm retrieval, ICSI is used rather than standard IVF because ICSI has been shown to result in a significantly higher fertilization rate. A section of this Special Issue comprised of seven articles is fully dedicated to the use of assisted conception in azoospermia-related infertility. The authors of these selected titles have extensive publication records and more than a decade of clinical and/or laboratory experience in the management of azoospermic males using assisted conception. In the first article of this section, the authors prepared a comprehensive summary of the current methods for sperm retrieval and critically analyzed the advantages and disadvantages of each method (10). The authors note that the sperm retrieval (SR) method of choice is often based on the type of azoospermia and the attending surgeon's preferences. However, SR should aim to both minimize damage to the reproductive tract, thereby preserving the chance of repeated retrieval attempts, and to obtain an adequate number of good quality sperm that can be immediately used for ICSI or alternatively cryopreserved for future ICSI attempts. Following this theme, two articles from Brazilian groups discuss the key elements for the success of sperm retrieval in obstructive and nonobstructive azoospermia (11,12). Dr. Miyaoka and colleagues summarize the current knowledge on the impact of several factors on sperm injection outcomes using surgically retrieved sperm from men with OA. The authors conclude that SR in OA is highly successful and that causes of obstruction and retrieval methods have little impact on SR success rates. Moreover, current evidence suggests that similar pregnancy outcomes are achieved by ICSI in OA using either fresh or frozen-thawed epididymal or testicular sperm (11). While a successful retrieval attempt is obtained in virtually all cases of OA, Drs. Glina and Vieira highlight the uncertainty of sperm acquisition in cases of NOA, thus making it desirable to determine the prognostic factors. The authors review several clinical and laboratory prognostic markers, such as the etiology of NOA, paternal age, testicular volume, serum levels of pituitary gonadotropins, genetic testing results, method of collection, testicular histopathology results and the impact of the laboratory tissue processing method; the authors concluded that the only unfavorable indicator for SR is the presence of microdeletions in the AZFa and/or AZFb regions of the Y chromosome long arm (12). Another key message from this review is that men with NOA are no longer considered sterile, even with elevated follicle-stimulating hormone levels and small testes, because modern retrieval techniques can be used to collect testicular sperm and produce a healthy biological offspring via assisted conception.

The laboratory management of surgically retrieved gametes requires special attention because spermatozoa collected from azoospermic men are often compromised in quality and more fragile. Drs. Popal and Nagy from Atlanta provide strategies for handling such gametes inside the laboratory and discuss potential dangers (13). Adherence to state of the art laboratory techniques and quality control are recommended to avoid jeopardizing the fertilizing potential of the sperm and chances of achieving a live birth. Several techniques are described for optimizing the chances of harvesting spermatozoa from epididymal fluid and testicular tissue of azoospermic men. The concept of cryopreservation may also be used in association with sperm retrieval procedures. Some centers prefer to retrieve and intentionally cryopreserve sperm for future use. This strategy offers the advantage of avoiding ovarian stimulation when no sperm is obtained from testicular specimens. If sperm is retrieved and frozen, it can be thawed at any time, thereby avoiding the need to organize two operations (oocyte and sperm retrieval) on the same day. Additionally, cryopreservation may spare unused specimens that would be discharged after ICSI, which may be useful if the treatment cycle does not result in a pregnancy. Therefore, future ICSI attempts could be conducted without repeated surgical retrievals. In most cases of epididymal retrievals, motile sperm will be available after thawing, and ICSI outcomes using fresh motile or frozen-thawed epididymal sperm do not seem to differ. Cryopreservation of testicular sperm is also advisable, especially for men with NOA who often require multiple ICSI attempts to conceive but may not have an adequate number of sperm available for repeated retrieval attempts. These important aspects are discussed by Dr. Gangrade from Orlando, who also presents laboratory protocols for the cryopreservation of epididymal and testicular sperm and discusses the reproductive outcomes of using frozen-thawed gametes for sperm injections (14).

The closing articles of the section dedicated to assisted reproduction are authored by the guest editors and Drs. Veerle and colleagues from the Centre of Reproductive Medicine in Brussels, who pioneered the introduction of ICSI and revolutionized the treatment of male infertility in the 90s. In our article, we summarized the data that have been generated on the reproductive potential of azoospermic men undergoing assisted conception (15). We performed a systematic review of the literature focusing on studies that directly compared pregnancy outcomes after sperm injections between couples whose male partner had OA or NOA. We also analyzed a personal database (SCE) of 370 couples who underwent ICSI according to the above-cited categories and compared the outcomes with a group of 465 non-azoospermic infertile males. In our series of 1,092 ICSI cycles performed in 835 male infertility patients, live birth rates were lowest in the NOA group. Miscarriage, ectopic pregnancy, and multiple pregnancy rates did not differ between clinical pregnancies achieved using ejaculated or non-ejaculated sperm from men with OA or NOA. In our series of 427 babies born with ICSI using sperm from non-azoospermic infertile fathers and azoospermic fathers with OA and NOA, the short-term neonatal outcomes were similar among groups, despite a tendency towards higher preterm birth in both azoospermia categories and lower gestational age for twins in OA. The overall perinatal death and malformation rates were 2.8% and 1.6%, respectively, and our results did not differ between deliveries that resulted from ICSI using ejaculated or non-ejaculated sperm from men with OA or NOA. In our review, we critically compare our results with other publications. We note that most published studies, including our data, suffer from methodological shortcomings. For instance, these studies were not designed to detect differences in live birth rates and not powered to detect differences in less frequent outcomes, such as malformations and other complications. Moreover, no follow-up study has yet compared the long-term

physical, neurological and developmental outcomes of children born with ICSI using sperm from azoospermic men with OA and NOA. We conclude that for now, the limited evidence on pregnancy and postnatal outcomes of ICSI using surgically derived sperm from azoospermic men is reassuring; however, a call for continuous monitoring is of utmost importance to support the recommendation of sperm retrieval and ICSI in azoospermia-related male infertility. Following this theme, an authoritative review by Dr. Veerle and co-authors provides an analysis of the results of immature germ cells used for ICSI (16). This strategy has been proposed in cases of NOA in which no spermatozoa can be retrieved. After the initial disappointing results, the in vitro culture of immature germ cells to more mature stages has been proposed as an approach to improve this poor outcome. More than a decade has passed since the introduction of ICSI with elongating and round spermatids; there is still a lot of uncertainty regarding the safety of this treatment option. The authors outline the clinical and scientific evidence for ICSI using immature germ cells and in vitro matured germ cells and describe the physiopathological mechanisms involved in fertilization. In addition, these authors suggest that despite reports of deliveries of healthy offspring, the method has very low efficiency; furthermore, most IVF programs worldwide have stopped spermatid injection. Several ethical and safety concerns related to the potential transmission of genomically imprinted disorders have been raised, leading to the ban of spermatid injection in countries such as the United Kingdom.

Research toward the development of artificial gametes is timely due to the prevalence of NOA and inability of harvesting mature sperm from the testes in approximately half of patients. In addition, the overall efficiency of spermatid injection is disappointing, and the reproductive potential after ICSI using testicular sperm retrieved from azoospermic men with dysfunctional spermatogenesis is only fair. A recent breakthrough report by Japanese scientists at Kyoto University used stem cells from mouse embryos to create primordial germ cells, which were then able to differentiate in spermatozoa after testis transplantation in mice. This topic is the theme of the closing article of this Special Issue, namely the challenges and perspectives of biotechnology and stem cell research to treat the most severe cases of azoospermia and potentially 'cure' male sterility (17). This article is authored by the group led by Dr. Franca from the Federal University of Minas Gerais, Brazil, in collaboration with Dr. Schlatt from Munster, Germany. Dr. Franca is a leading authority in this field, and his provocative insights on cell biology call for a profound reflection. In their article, Dr. França's group proposes that men with incomplete spermatogenesis are collectively classified as aspermatogenic to indicate a highly severe testicular pathology with complete absence of spermatids and spermatozoa. The authors explore the novel biotechnological methods to rescue fertility while maintaining biological fatherhood. Human haploid-like cells have already been obtained from pluripotent stem cells of somatic origin using the novel technique of in vitro sperm derivation. Germ cell transplantation as a form of grafting is a promising method that may restore the fertility of prepubertal boys who previously received cancer treatments. Haploidization is being investigated as an option to create gametes based on biological cloning technology. Although promising, these methodologies are experimental, and the production of human gametes in the laboratory is a highly complex process that has yet to be translated to reproductive medicine.

This Special Issue of *Clinics* aims to be a landmark treatise on azoospermia. Scientists and clinicians from seven countries on three continents have contributed generously to the current scientific knowledge involving human azoospermia and its role in male reproductive health. We recommend its contents not only to students and researchers in the biological, veterinary and medical sciences but also to clinicians involved in the management of infertile couples and urologists, andrologists, gynecologists, embryologists and reproductive specialists interested in following the exponential growth in the knowledge of azoospermia. Due to the multidisciplinary nature of this category of male infertility, unsolved problems present themselves, and the opportunities for advancement continue to expand. We hope that readers will appreciate this Special Issue of *Clinics* and share our excitement in the study of azoospermia.

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