A Schematic Overview of the Current Status of Male Infertility Practice

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The practice of male infertility has dramatically evolved over the past few decades. This has been achieved after the realization of the important contribution the male counterpart holds in the couple’s infertility. It is also supported by a number of breakthroughs in the diagnosis and treatment of this medical condition. Several tests to investigate sperm quality and function were introduced along with refinements and/or institution of novel surgical techniques that can correct several causes of infertility. This manuscript is aimed at highlighting the current state of male infertility practice in a robust, schematic method addressing a broader audience involved in the treatment of male infertility.

Keywords: Andrology; Hormones; Infertility, male; Oxidative stress; Semen analysis

INTRODUCTION

After the birth of Louise Brown in 1978, the first successful delivery from an in vitro fertilization procedure [1], the fields of human reproduction and embryology have witnessed major breakthroughs that allowed couples previously regarded as sterile to conceive their own biologic children. This was made possible through the enhanced understanding of the physiology of fertilization and pathophysiology of infertility allowing a cause-directed treatment approach to a variety of etiologies. Subsequent advances in assisted reproductive technologies (ART) have revolutionized our comprehension of the sperm/egg interaction at the molecular level.

In recent years, a plethora of research was conducted on the study of male infertility, primarily as a result of the perceived decline in semen quality among healthy men worldwide, the growing public awareness and the psychological impact of this condition on men’s health and wellbeing. Advances in diagnostic and treatment modalities of the infertile man have emerged, including testing of sperm function, genomic and proteomic expression of seminal plasma proteins and refinement of microsurgical reconstructive surgeries. Training programs were developed worldwide to aid in the diagnosis and treatment of male reproductive conditions, allowing for the delivery of subspecialized care to this group of patients. Unfortunately, access to male infertility providers is highly variable both within the
United States and around the globe. Several studies have highlighted this growing issue. For example, as of 2010, 1.5 million adults in Kentucky had access to a single infertility treatment center in the state. However, in the District of Columbia, there is one infertility treatment center for every 65,000 adults [2,3]. Nangia et al [2] further demonstrated this unequal distribution of male infertility providers across the United States [4]. As of 2010, there were only 197 male infertility specialists in the United States [2]. Moreover, 13 states had no male infertility provider and many more infertility treatment centers could not refer men to a male infertility specialist within a one hour driving distance. This disparity has required general urologists, who may not have had the training or harbor any interest in the subject, managing men with male factor infertility. This may potentially result in inadequate treatment of the male or unnecessarily proceeding with ART with its associated risks to the female partner.

In the United Kingdom, only 3.6% of urology residents cited exposure to training in male factor infertility. While the majority of residents reported a desire to treat male factor infertility in their future practice, only 27% felt prepared to do so [5]. In the United States, the number of clinical andrology fellowships has been steadily growing, improving resident exposure. However, the Accreditation Council of Graduate Medical Education does not require learning procedural skills or core competencies in order to manage patients with male factor infertility [6]. Therefore, the disparity in access to fellowship trained male fertility specialists is likely to continue to grow.

Clearly, there is a need for a more comprehensive approach and lessening of the gap of care between patients and fertility specialists. Globally, male infertility management is shared with gynecologists, endocrinologists, dermatologists, internists and embryologists, especially in the setting of assisted reproduction (Fig. 1). This fact justifies the writing of manuscripts such as this one, which is aimed at highlighting the current state of male infertility practice in a simplified, easy to follow schematic method addressing a broader audience involved in the treatment of male infertility. This article will (1) review the key principles of male fertility evaluation; (2) explore important diagnostic tests involved in the work-up; and (3) elaborate on several treatments utilized in a number of presentations.

MALE INFERTILITY: GENERAL CONSIDERATIONS AND THE INITIAL ASSESSMENT

Infertility is defined as the inability of a couple to conceive after at least one year of regular unprotected intercourse. This condition has become a global health concern affecting about 187 million couples worldwide or one out of every six couples of reproductive age [7,8]. Among other complications, male infertility can lead to psychological problems, ridicule or domestic violence [9]. Particularly, in male driven societies, it can even force the female partner into extramarital relationships and thereby increase the risk of contracting sexually transmitted diseases, such as human immunodeficiency virus/acquired immune deficiency syndrome [10]. Since the male is partially or completely responsible for half the cases of infertility (Fig. 2) among couples, this underscores the importance of delivering a specialized male fertility evaluation for those seeking fertility [11].

The American Urological Association (AUA) and American Society of Reproductive Medicine (ASRM) recommends that at a minimum, the infertility workup includes a detailed history, physical exam and two separate semen analyses to identify risk factors for male infertility (Fig. 3, 4) [12,13]. Moreover, numerous causes of male infertility have been recognized and are
classified at pre-testicular, testicular or post-testicular (Fig. 5). The vast majority of these causes can be recognized with great certainty provided that a proper assessment has been conducted. Specific details regarding the couple’s sexual activities are important to identify modifiable causes of delayed conception, such as timing and frequency of intercourse, ejaculation practices and/or lubricant use and presence of male sexual dysfunction. A checklist of risk factors should be directly solicited from patients including medical conditions, such as history of genitourinary infections, trauma or prior surgery; medication history, specifically the use of exogenous testosterone and chemotherapeutic medications; and environmental and lifestyle risk factors that are known to negatively impact fertility. These include smoking [14], obesity [15], alcoholism [16], exposure to heavy metals (lead, cadmium) [17], ionizing radiation [18], and high temperatures [19]. Furthermore, in roughly 1% of men who present for male fertility evaluation, a comprehensive evaluation has the ability...
to uncover more serious or potentially life-threatening conditions. Normal semen parameters and reproductive history should not preclude men from being fully assessed, approximately 0.16% of men can still harbor a significant medical condition [20].

The next step is a focused physical examination. The patients’ general appearance can partly foreshadow their endocrine status, for example, hypogonadism may be suspected in patients with reduced facial hair, gynecomastia and obesity. Genital examination is performed in both the standing and supine positions. The testes, epididymides, vasa deferentia and the spermatic cord are individually palpated looking for abnormalities. The size and consistency of the testes can roughly estimate its spermatogenic function. Care must be taken to look for testicular masses as infertile men are 3 times more prone to develop testicular cancer [21].

Fullness of the epididymis may indicate obstruction, while an underdeveloped epididymal tail together with unilateral or bilateral absence of the vas deference is seen in cystic fibrosis patients or carriers. In addition, hard swelling of the epididymal tail and beading of the vas deference can occur as a sequela of granulomatous inflammation secondary to tuberculosis infection. The cord is then examined for a varicocele which is clinically classified into three grades [22]. Grade 1: varicocele palpation is only demonstrated through valsalva maneuver; grade 2: palpation is felt regardless of valsalva maneuver; and grade 3: varicocele is visible without palpation.

Digital rectal examination is usually indicated in patients with low ejaculate volume, or above 50 years of age with lower urinary tract symptoms. The prostate is assessed for size and consistency while palpation of the seminal vesicles may indicate ejaculatory duct obstruction or a seminal vesicle pathology. A comprehensive initial assessment is a key component of the male fertility workup, it may indicate underlying causes of infertility, but also factors that could pose a significant risk to overall male health and potential progeny conceived naturally or with ART [23].

**MALE INFERTILITY: INVESTIGATIONS**

A number of laboratory and radiologic investigations are available for the evaluation of male infertility. Their respective indications are presented in Fig. 6. Semen analysis is the single most important investigation during the evaluation of infertile men. Despite its value as the cornerstone test of a man’s fertility potential, it fails as a true predictor of fecundity [24]. To ensure
accurate and homogeneous results, samples are better analyzed at laboratories implementing quality control programs for semen analysis and adopting the latest World Health Organization protocols for semen testing and reporting of reference values [25]. The shortcoming of conventional semen analysis to accurately predict fecundity triggered the search for advanced tests of sperm function. While several tests have been explored, measurement of seminal oxidative stress and levels of sperm DNA fragmentation (SDF) are currently the most commonly utilized.

1. Oxidative stress

Reactive oxygen species (ROS) are highly reactive, oxygen containing molecules that, at physiologic levels, augment key steps, such as sperm capacitation and maturation necessary for optimal function [26,27]. Nevertheless, unphysiologically elevated amounts of ROS can have an opposite effect impairing and damaging the function of several enzymes and inducing lipid peroxidation and SDF [28,29]. Antioxidants, on the other hand, through their ability to neutralize or scavenge excessive ROS help in maintaining a natural redox balance [30]. However, when this balance is disrupted due to excessive production of ROS or reduced quantities of antioxidants, a state of oxidative stress will ensue (Fig. 7). Studies have confirmed the impact of oxidative stress on male fertility through finding significantly higher levels of ROS in semen samples obtained from infertile patients compared with fertile men [28,31,32].

Several direct and indirect measures of oxidative stress are available. The chemiluminescence assay which directly measures the interaction between ROS and certain reagents is the most commonly utilized method for oxidative stress measurement [33]. The thiobarbituric acid assay is an example of indirect tests for oxidative stress, which measures the levels of malondialdehyde, a byproduct of lipid peroxidation [34]. Recently, measurement of the oxidation-reduction potential with MiOXSYS (Male Infertility Oxidative Stress System) has been introduced, which directly assesses the balance between oxidants and reductants in seminal fluid [28,35]. The advantages of oxidative stress testing are perhaps more pronounced in men with idiopathic infertility as the test result can direct treatment decisions [36]. Examples of treatments that can be offered include the application of risk reduction strategies, treatment of genital tract infections or varicocele and the monitored use of oral antioxidants.

2. Sperm DNA fragmentation

Sperm DNA structure is unique, as the DNA is bound to protamines allowing it to be present in a compact, array-type state and shielding it from potential damage occurring during sperm transport [37]. However, sperm DNA damage may still occur due to errors in chromatin condensation or excessive ROS levels thereby affecting fertility potential [38]. In addition, since the enzyme topoisomerase II is cleaving DNA strands during the process of sperm chromatin condensa-
sation with the replacement of histones by protamines as nuclear proteins in order to achieve higher compaction, this opening of the DNA structure also makes the DNA more susceptible to damage through oxidative assaults. Apart from that, linking up these nicks, also by topoisomerase II, is also not been done in a 100% correct manner. Consequently, DNA strand breakages might occur leaving open nicks behind. Sperm with fragmented DNA can significantly impair fertilization, early embryo development, implantation, overall pregnancy rates and can even be the cause of early childhood cancer, particularly in male offspring [39].

Several methods for testing SDF have been identified with the four most commonly used tests being the terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling, single cell gel electrophoresis, sperm chromatin dispersion test and sperm chromatin structure assay (Fig. 8) [40].

Recent guidelines have been published highlighting the clinical indications of SDF testing [41]. Unexplained infertility, recurrent spontaneous abortion, varicocele, recurrent ART failure and lifestyle risk factors have been linked with higher SDF levels [42]. Several treatment options are available for patients with high levels of SDF. These include risk reduction, frequent ejaculations [43], treatment with antioxidants [44], varicocelectomy [45] and the use of sperm selection techniques and/or testicular sperm for intracytoplasmic sperm injection (ICSI) [46,47].

New generation diagnostic modalities are emerging such as the proteomic, genomic and metabolomic analyses of sperm and seminal fluid and may hold the key to more accurate diagnosis and treatment of male infertility (Fig. 9). Proteins are essential cell signaling biomolecules and since spermatozoa are translationally and transcriptionally silent, they depend on seminal plasma proteins to function appropriately. Aberrant expression of proteins has been identified in seminal fluid of infertile men and several studies are underway aiming to explore these proteins in various etiologies of male infertility [48]. Metabolomic profiling of human spermatozoa identified a number of metabolites that were deranged in men with asthenozoospermia and may serve as potential target for further investigations [49]. Higher resolution assessment of genetic material using fluorescent in situ hybridization and microarray technologies hold great promise for accurately identifying relevant genetic biomarkers. Copy number variations, single nucleotide polymorphisms, and ribonucleic acids are examples of promising biomarkers that may be evaluated in the near future [50].

**AZOOSPERMIA**

Azoospermia, defined by the absence of sperm in the ejaculate, is a not uncommon presentation occurring...
in up to 15% of men consulting for infertility [51]. Vital information picked up during the history, physical examination and laboratory assessment may point out whether the azoospermia is of obstructive or non-obstructive nature (Fig. 10). Classically speaking, obstruction is generally suspected in patients with normal testicular size, full epididymides and normal hormone evaluation. While non-obstructive azoospermia (NOA) is assumed in patients with small testicular size, flat epididymides, and deranged hormonal assessment. Testicular biopsy can accurately discriminate these two conditions, however, it is rarely performed for diagnostic purposes solely due to its invasive nature and likelihood for negatively affecting the outcome of a possible forthcoming sperm retrieval procedure [52].

The AUA and ASRM recommends that in men with NOA or severe oligospermia (<5 million sperm/mL) karyotype and genetic evaluation should be considered
The hormonal and genetic evaluation of patients with NOA will provide important information that would direct patient preparation for sperm retrieval procedures. Patients with hypogonadotropic hypogonadism will often respond to hormone replacement with gonadotropins and may even witness the return of sperm to the ejaculate and avoid surgical sperm retrieval. On the other hand, the results of a genetic testing may offer important prognostic information before sperm retrieval is performed. Genetic abnormalities are prevalent in about 2% to 7% of men with infertility [53], and in up to 35% of men with azoospermia and severe oligozoospermia, thus, justifying genetic testing in this patient population [54].

Karyotype analysis detects a number of autosomal aberrations such as translocations (Robertsonian and reciprocal), inversions and sex chromosomal abnormalities. A supernumerary X chromosome, or otherwise known as Klinefelter’s syndrome is the most common sex chromosome abnormality detected in men with azoospermia [55]. Microdeletions of the azoospermia factor (AZF) region of the Y chromosome have been linked with defective spermatogenesis and hence should be tested in azoospermic patients [56]. Three non-overlapping regions of the Y chromosome are investigated: the AZFa, AZFb and AZFc. Fortunately, about 2/3rd of patients have their deletion in the AZFc region, which has a better prognosis, in terms of the surgical sperm retrieval rate than the other regions [57]. In fact, many specialists would not offer sperm retrieval to patients with AZFa, AZFb, or AZFa/b microdeletions due to a high failure rate [58,59]; although recent studies have reported sperm recovery from a few cases [60]. Patients with chromosomal abnormalities will also require genetic counselling and the use of pre-implantation genetic sampling after successful sperm recovery and fertilization.

Surgical sperm retrieval is a vital male infertility treatment milestone that significantly impacted the lives of many men who were previously considered sterile. Sperm retrieval was first performed in 1985 from epididymides of patients with obstructive

![Fig. 10. Azoospermia. CFTR: cystic fibrosis transmembrane conductance regulator, ICSI: intracytoplasmic sperm injection, FSH: follicle-stimulating hormone, LH: luteinizing hormone, US: urethrography, TURED: transurethral resection of ejaculatory ducts.](https://www.wjmh.org)
A decade later, Devroey et al [62], showed that sperm can also be retrieved from men with testicular failure and could be used for ICSI. Several refinements were introduced into the sperm retrieval procedure, the most prominent of which is the use of surgical microscopy [63]. Currently, sperm can be retrieved percutaneously (testicular sperm aspiration), conventionally (open testicular sperm extraction, TESE) or through microsurgery (micro TESE). The available evidence favors micro TESE as the “gold standard” approach as it is provides the highest sperm retrieval rate and the lowest risk of postoperative complications (Fig. 11) [64]. Studies have shown that in patients opting for sperm retrieval, pre-treatment with hormonal therapies can positively affect the surgical sperm retrieval outcome. Examples of medications used include antiestrogens [65], aromatase inhibitors [66] and gonadotropins [67].

Patients with obstructive azoospermia, whether it is iatrogenic (e.g., post vasectomy) or from prior infectious or traumatic etiologies can be offered reconstructive surgery or sperm retrieval procedures and ICSI (Fig. 12, 13). Sperm retrieval generally has a high success rate and can be performed percutaneously from the epididymis (percutaneous epididymal sperm aspiration) or with the help of a surgical microscope (microsurgical epididymal sperm aspiration, MESA). While MESA offers some advantages over the percutaneous approach, it is time consuming, more expensive and requires superior surgical skills (Fig. 13) [68]. When epididymal sperm retrieval has failed, testicular sperm retrieval can also be conducted for obstructive azoospermia patients.

The macroscopic characteristics of the seminal fluid can also aid in diagnosing obstructive azoospermia where patients with a low semen volume, acidic pH and absence of fructose are considered to have ejaculatory duct obstruction. In these patients, a confirmatory post ejaculate urine analysis should be performed to rule out the presence of retrograde ejaculation, which is suspected in patients with a medical history suggesting neurologic impairment (diabetes mellitus or spinal/
Fig. 12. Surgical correction of obstructive azoospermia.

Two-needle technique
This technique allows the use of two double-armed sutures to provide a four-point fixation on the vasal end for the anastomosis.

Triangulation technique
Three double-armed microsutures are placed in a triangulated fashion on the epididymal tubule which is opened in the center of the triangle formation. The ends of the sutures are placed on the vasal end to complete the anastomosis.

Modified two layer
Full-thickness 9–0 interrupted nylon stitches incorporating the serosa, muscularis and mucosa are used to approximate the two vasal ends. A second layer of 9–0 stitches incorporating the adventitia and small amount of muscularis is then placed.

Fig. 13. Sperm retrieval procedures in obstructive azoospermia. PESA: percutaneous epididymal sperm aspiration, MESA: microsurgical epididymal sperm aspiration. *Data from the article of Esteves et al (J Urol 2013;189:232-7) [68].
pelvic surgery) [69]. A transrectal ultrasound is then performed to evaluate the prostate and seminal vesicles. Complete or partial ejaculatory duct obstruction is suspected in the presence of dilated seminal vesicles (>1.5 cm in diameter), dilated ejaculatory ducts (>2 mm in diameter) and/or midline prostatic cystic structures.

In order to improve the diagnostic accuracy of transrectal ultrasound in cases of ejaculatory duct obstruction, seminal vesicle aspiration and seminal vesiculography can be performed during the procedure.

Jarow [70] was the first to recognize that sperm are not normally present within the seminal vesicles and that ejaculatory duct obstruction should be suspected when three motile sperm per high-power field are detected in the seminal vesicle aspirate immediately after ejaculation. Patients with ejaculatory duct obstruction are offered transurethral resection of ejaculatory ducts which should improve sperm quality in 70% to 80% of patients with a 20% to 30% pregnancy rate [71]. Complications occur in 10% of cases and include hematuria, watery ejaculate, and epididymitis [71].

VARICOCELE

Varicocele, defined as the dilatation of the pampiniform plexus of veins together with reflux of blood, is a common etiology of male factor infertility. Much controversy surrounds this clinical condition mainly because it is also seen in patients with normal fertility potential. All efforts were directed towards selecting the ideal patient who would benefit from surgical ligation. Varicocele can affect testicular function through a number of mechanisms, the most commonly accepted include hyperthermia and testicular tissue hypoxia or ischemia (Fig. 14) [72]. This results in alteration in conventional semen parameters and sperm function tests (oxidative stress and SDF) [73].

The most accepted approach for varicocele treatment is surgical repair, whereas percutaneous embolization of the refluxing internal spermatic veins remains a primary alternative [74]. Varicocelectomy can be performed through the conventional retroperitoneal, laparoscopic/robotic or microsurgical inguinal or subinguinal approaches [75]. No major differences exist between the different surgical approaches with regards to success rate, however microsurgical subinguinal varicocelectomy has been considered the “gold standard” approach as it is associated with lower risk of varicocele recurrence and lower rate of postoperative hydrocele formation [76]. Varicocele ligation was found to improve conventional semen parameters and revert seminal oxidative stress and SDF [76].

Varicocele treatment has been recently indicated in patients with hypogonadism, regardless of semen parameter results as evidence have linked varicocele to Leydig cell hyperplasia and altered endocrine testicular function [77]. Furthermore, significant improvement in serum testosterone levels were observed following varicocelectomy [78,79]. Interest in varicocele ligation for patients with clinically palpable disease and non-
obstructive azoospermia has re-emerged since the initial report by Tulloch [80] in 1952. Current evidence justifies varicocele ligation in this subset of patients as a good number of them will witness the return of sperm to the ejaculate thereby avoiding testicular sperm retrieval [81]. Moreover, those who remain azoospermic following varicocele ligation will also have a better surgical sperm retrieval outcome [81].

CONCLUSIONS

The field of male infertility has witnessed several advancements in the past few decades. These advancements were the result of the continued recognition of the key role that the male counterpart plays in human fecundity. With continued improvement in biotechnology, new generation tests of sperm function are underway to help understand the various pathophysologies of testicular dysfunction. This should further allow the introduction of novel cause directed treatment modalities capable of restoring fertility potential.

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Conflicts of Interest

The authors have nothing to disclose.

Author Contribution

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