

What is New in the Clinical Assessment and Treatment of the Infertile Male

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Abstract: Male infertility is a fast-evolving area. Recently published evidence has challenged several established concepts and added to the knowledge in the field. Traditionally, the clinical assessment of the subfertile male includes a history and physical examination, hormonal evaluation and semen analyses. Regarding the latter, the World Health Organization has established new reference values that in general include lower limits than those of previous editions. Specialized sperm function assays, such as sperm DNA integrity and measurements of oxidative stress, are now clinically available and may be integrated to the conventional semen analysis to provide a more comprehensive evaluation of a man's fertility status. Also, molecular biology genetic testing involving the Y-chromosome can correctly identify oligozoospermic and azoospermic men misdiagnosed as having idiopathic infertility and be of prognostic value for sperm retrieval in non-obstructive azoospermia. In the field of treatment, emerging evidence suggests that antioxidant therapy along with life-style modifications improve the male reproductive health. Microsurgery has increased the success rates for both varicocele treatment and sperm retrieval for assisted conception. Microsurgical treatment of clinical varicoceles may optimize reproductive outcome of couples undergoing intracytoplasmic sperm injection or microsurgical testicular sperm extraction. Men with non-obstructive azoospermia, elevated follicle-stimulating hormone levels and small testes may no longer be considered sterile. Modern retrieval techniques are used to collect testicular sperm that can be used to produce a healthy biological offspring via assisted conception. This article reviews what is new in the clinical assessment and treatment of the infertile male, including the implications of this increased knowledge in the management of the infertile couple and assisted reproduction technology outcomes.

Keywords: Male infertility, diagnosis, treatment, microsurgery, azoospermia, varicocele, assisted reproduction technology, sperm function tests.

INTRODUCTION

Infertility complaint is common in the urologic office. Approximately 8% of men in reproductive age seek medical assistance for fertility-related problems. Of these, 1-10% carries conditions that compromise the reproductive potential, and varicocele accounts for 35% of the cases [1]. Most infertility conditions are treatable, but in few cases they are associated with life-threatening diseases such as testicular, brain or spinal cord cancers, genitourinary malformations, genetic syndromes or systemic diseases. Therefore, comprehensive male workup should be an integral step in the evaluation of the infertile couple.

Recently published studies have added new evidence to this fast-evolving area. Novel diagnostic tools using molecular biology and DNA integrity or oxidative stress assays allow the clinician to better diagnose a significant proportion of men previously classified as having infertility of unknown etiology. Emerging evidence suggests that life-style and environmental conditions are of utmost importance in male fertility and subfertility. There are new opportunities for men with azoospermia or clinical varicoceles requiring assisted conception. Microsurgery has increased success rates in both

reconstruction of the reproductive tract and retrieval of spermatozoa for assisted conception. The present article reviews the current concepts in the clinical assessment and treatment of the infertile male.

WHAT IS NEW IN THE CLINICAL ASSESSMENT

World Health Organization Reference Values for Human Semen Characteristics

Semen analysis is of great value on the male initial investigation and its results are often taken as a surrogate measure of male fecundity and pregnancy risk. It provides information on the functional status of the germ epithelium, epididymides and accessory sex glands. Reference ranges for semen parameters from fertile populations may provide data from which prognosis of fertility or diagnosis of infertility can be extrapolated. Nonetheless, the prognostic value of semen components such as sperm number, motility and morphology, as surrogate markers of male fertility, is confounded in several ways; the fertility potential of a man is influenced by sexual activity, the function of accessory sex glands and other, defined as well as yet unrecognized, conditions. Routine semen analysis itself has its own limitations, and does not account for putative sperm dysfunctions such as immature chromatin or a fragmented DNA. Results from at least two, preferable three, separate seminal analyses must be

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obtained before a definitive conclusion can be drawn as wide biological variability exists within the same individual. Routine seminal analysis should include: a) physical characteristics of semen, including liquefaction, viscosity, pH, color and odor; b) specimen volume; c) sperm count; d) sperm motility and progression; e) sperm morphology; f) leukocyte quantification; and g) fructose detection in cases where no spermatozoa is found and ejaculate volume is low. The criteria used for normality according to the World Health Organization (WHO) have been recently updated, as shown in Table 1 [2]. Approximately 2,000 men from eight countries whose partners had a time-to-pregnancy of ≤ 12 months were chosen to provide reference distributions for semen parameters. One-sided lower reference limits (the fifth centile) were generated and have been proposed to be considered the lower cut-off limits for normality. Apart from total sperm number per ejaculate, the lower limits of these distributions are lower than that of previously presented 'normal' or 'reference' values [3-5] but are in agreement with recent observations [6-8].

The morphometric description of spermatozoa according to the strict criteria, described by Kruger *et al.* [9], has been incorporated in the current version of the WHO manual. The low proportions of normal spermatozoa, as defined by those selected from the endocervical mucus, produced very low reference limits for the fertile population. With such method, low values of 3-5% normal forms have been found to be optimal cut-off values to discriminate between fertile and infertile men whose spermatozoa were used for *in vitro* fertilization [10], intrauterine insemination [11] and spontaneous pregnancies [12]. Interpretation of the reference ranges requires an understanding that they provide a description of the semen characteristics of recent fathers. The reference limits should not be over-interpreted to distinguish fertile from infertile men accurately, but they do represent semen characteristics associated with a couple's achieving pregnancy within 12 months of unprotected sexual intercourse; as such, the limits provide only a standardized guide regarding a man's fertility. None of these values were able to solely distinguish fertile from infertile men, although morphology has been suggested to be of utmost importance [10-12]. The coexistence of more than one altered seminal parameter significantly increases the risk for infertility [7].

Genetic Testing for Y-Chromosome Infertility

The long and short arms of Y-chromosome are respectively related to spermatogenesis and testicle development. The Y-chromosome region related to fertility is named azoospermia factor locus (AZF - azoospermia factor). Originally, three AZF regions have been defined: AZFa, AZFb, and AZFc, which map on the long arm (Yq) in order from the centromere to the telomere. However, it has been demonstrated that AZFb and AZFc regions overlap [13]. A fourth region, named AZFd, located between AZFb and AZFc has been also reported. These subregions contain multiple genes controlling different steps of spermatogenesis. The locus can present complete or partial microscopic deletions, isolated or in combination.

Clinically available molecular testing may reveal microdeletions in the long arm of the Y chromosome. The molecular diagnosis of Y chromosome deletion consists of a

series of PCR (polymerase chain reaction) amplifications within relatively broad regions of the Y chromosome. Interstitial Yq microdeletions can remove various portions of the long arm, depending on breakpoints. Specific genes (i.e., USP9Y, DDX3Y, HSFY1, RBMY, CDY1, and DAZ) located along the Y chromosome should be included in the analysis to assess Y chromosome integrity [14]. The most common Y-chromosome deletion in infertile men is the one affecting the DAZ gene (*deleted in azoospermia*) located in AZFc region. The relative frequency of individual microdeletions are 60%, 5% and 16% for AZFc, AZFa and AZFb regions, but combined deletions occur in about 15% of the cases [15]. Since deletions tend to occur between large palindromic repeats, Repping *et al.* (2002) proposed a more appropriate nomenclature using the name of the flanking repeats for the types of recurrent deletions [13] (Fig. 1).

Y chromosome infertility is characterized by azoospermia or severe ($<1 \times 10^6$ sperm/mL semen) to moderate oligozoospermia ($1-5 \times 10^6$ sperm/mL semen), but rare cases of mild oligozoospermia ($5-20 \times 10^6$ sperm/mL semen) may occur. Males with Y chromosome infertility usually have no obvious symptoms, although physical examination may reveal small testes and/or cryptorchidism or varicoceles. A single study suggested that one of the deletions of the Y chromosome in AZFc region (designated gr/gr) also causes a slight increase in susceptibility to testicular germ cell tumors [16]. The prevalence of Y chromosome microdeletions is estimated to be about 1:2000 to 1:3000 males. Overall, the frequency of Yq microdeletions in infertile males ranges between 5%-12%. Microdeletions can be found in 6% of men presenting with severe oligozoospermia and 15% of those with azoospermia [17]. For sperm counts between 1 and 5 million/mL the detection rate drops down to 1.7% [18]. A marked variation in the reported worldwide frequency of AZF deletions among patients with non-obstructive (NOA) azoospermia from different regions of the world, i.e., 10-12% in USA, Australia and Asia, 2-10% in Europe, and 7% in Brazil suggests that an ethnic variability may exist [14].

Pregnancies may be achieved by *in vitro* fertilization using intracytoplasmic sperm injection (ICSI) in males with Y-chromosome infertility exhibiting oligozoospermia or azoospermia with retrievable testicular sperm. It seems that the presence of Yq chromosome deletion has no apparent negative effect on fertilization and pregnancy rates, and it does not increase the risk for birth defects in children conceived *via* assisted reproduction [18, 19]. The presence or absence of sperm (ejaculated or retrieved) in men with Yq microdeletions varies depending on the specific deletion. Detection of Y-chromosome microdeletions provides predictive information on the success of obtaining spermatozoa from the testicle of azoospermic individuals for ICSI. Testicular phenotypes (sperm counts and testicular histology) associated with microdeletions in the AZFa region are the most severe and include azoospermia and germ cell aplasia (Sertoli cell-only) pattern on histology while microdeletions in the AZFb subregion are associated with maturation arrest [20]. Testicular phenotypes associated with microdeletions restricted to AZFc ranged from azoospermia to moderate oligozoospermia whereas AZFd microdeletions manifest the broadest range of testicular phenotypes (from azoospermia to normal sperm counts). Large deletions, which involve multi-



Fig. (1). Illustration of the Y chromosome in humans and the regions involved in fertility and infertility (Adapted from *Am J Hum Genet* 71(4), Repping S, et al., *Recombination between Palindromes P5 and P1 on the Human Y Chromosome Causes Massive Deletions and Spermatogenic Failure*, pages: 906-22, Copyright 2002, with permission from Elsevier). Interstitial or terminal deletions that include AZFa, often mediated by recombination between the HERV15yq1-HERV15yq2 repeats, usually produce the severe phenotype of Sertoli-cell-only syndrome. Interstitial or terminal deletions that include AZFb and/or AZFb+c are mediated by recombination between palindromic repeats, either P5/proxP1, P5/distP1, or P4/distP1. These deletions usually result in azoospermia. Interstitial or terminal deletions that include AZFc only are mediated by recombination between the b2/b4 palindromic repeats and result in a variable phenotype, ranging from azoospermia and SCOS to severe or mild oligozoospermia. This type of deletion can occasionally be associated with normal fertility in younger males, with the phenotype worsening with age. Such individuals should consider cryopreservation of ejaculated sperm in early adulthood. Two partial deletions of AZFc, called b1/b3 and gr/gr, are considered polymorphisms.

ple AZF regions generally present with testicular phenotypes similar to those found in individuals with microdeletions restricted to AZFa [14]. In partial and complete AZFc deletion azoospermic patients, sperm can be retrieved from the testis in about 70% of the cases. In contrast, the chance of finding sperm in azoospermic men with complete AZFa or AZFb deletions is unlikely [18, 19].

Y chromosome infertility is inherited in a Y-linked manner. Because males with deletion of the AZF regions of the long arm of the Y chromosome are infertile, the deletions are usually *de novo* and therefore not present in the father of the proband. Rarely, within a family, the same deletion of the Y chromosome can cause infertility in some males but not in others; hence, some fertile males with deletion of the AZF regions have fathered sons who are infertile [21]. In pregnancies achieved from males with infertility caused by deletion of the AZF regions using ICSI, male offspring have the same deletion as their father, with a high risk of male infertility. Female fetuses from a father with a Y chromosome-deletion have no increased risk of congenital abnormalities or infertility. In pregnancies conceived *via* assisted reproduction and known to be at risk of resulting in a male with Y chromosome-deletion, specific prenatal testing or pre-implantation testing could be performed to determine the sex of the fetus and/or the presence of the Y chromosome-deletion.

Sperm DNA Integrity Assays

Sperm DNA fragmentation seems to be one of the most important causes of reduced fertility potential. Abnormalities in the male genome characterized by damaged sperm DNA

may be indicative of male subfertility regardless of routine semen parameters which do not reveal DNA defects. An abnormal proportion of spermatozoa with fragmented DNA can be found in 5% of infertile men with normal semen analyses and 25% of infertile men with abnormal semen analyses but is rarely seen in fertile men [15]. Advanced paternal age, inadequate diet intake, drug abuse, pesticide environmental exposure, tobacco use, varicocele, medical disease, hyperthermia, air pollution, genital inflammation or infectious diseases can be cited as possible causes, some of which are reversible. Fragmentation can be secondary to internal factors such as apoptosis and oxidative stress (a physiological mechanism secondary to a high concentration of free radicals), or external factors such as the presence of leukocytes.

Among the available methods used to assess sperm DNA integrity, the most common are TUNEL, *Comet*, Acridine Orange test and SCSA (Sperm Chromatin Structure Assay) [22]. TUNEL technique (*Transferase-mediated dTUP nick-end labeling*) seems to be the most adequate as it offers the possibility to precisely identify all existing endogenous breaks in sperm DNA. It combines both enzymatic and immuno-histochemical techniques for direct observation of DNA fragmentation using a fluorescence microscope or flow cytometry.

Sperm DNA damage levels are significantly different between fertile and infertile men, and therefore have important clinical significance [23]. The probability of fertilization *in vivo* and by intrauterine insemination (IUI) seems to be close to zero if the proportion of sperm cells with DNA damage exceeds 30% and 12%, as detected

respectively by SCSA or TUNEL [24, 25]. Sperm DNA damage is negatively correlated with embryo quality and blastocyst formation in *in vitro* fertilization (IVF), and with fertilization rates in both IVF and ICSI [26]. However, successful pregnancies in IVF/ICSI can be obtained using semen samples with a high proportion of DNA damage. Bungum *et al.* (2004) demonstrated that significantly higher clinical pregnancy rates (52.9 % vs. 22.2 %) and delivery rates (47.1 % vs. 22.2 %) were obtained after ICSI as compared to IVF when semen samples with high levels of sperm DNA damage were used, as previously suggested [27].

The activation of embryonic genome expression occurs at the four- to eight-cell stage in human embryos. The paternal genome may not be effective until that stage and it is speculated that elevated levels of sperm DNA strand breaks negatively impact the later stages of embryonic development. Aitken and Krausz (2001) suggested that sperm DNA damage is promutagenic and can give rise to mutations after fertilization, as the oocyte attempts to repair DNA damage prior to the initiation of the first cleavage. Mutations occurring at this point will be fixed in the germline but may be responsible for the induction of infertility, childhood cancer in the offspring and higher risk of imprinting diseases [28]. So far, however, follow-up studies of children born after ICSI compared with children born after conventional IVF have not been conclusive regarding the risks of congenital malformations, imprinting diseases and health problems. *In vitro* fertilization, in general, is associated with multiple gestations and increased risk of congenital abnormalities (including hypospadias) [29]. ICSI, in particular, carries an increased risk of endocrine abnormalities, as well as epigenetic imprinting effects [29]. Although the absolute risk of any of these conditions remains low, current data is limited and study populations are heterogeneous [29-32]. It is therefore recommended that well-defined groups of ICSI with ejaculated sperm, ICSI with epididymal sperm and ICSI with testicular sperm, and a control group of naturally conceived children are closely followed up.

Oxidative Stress Measurement

The oxidative stress-induced sperm damage has been suggested to be a significant contributing factor in 30-80% of all cases of male infertility [33]. Reactive oxygen species (ROS) generation of can be exacerbated by environmental, infectious and lifestyle conditions. For example, exposure to phthalate, which is found in plastics used in food packaging, as well as beauty products, has been shown to cause DNA damage in sperm, as well as impaired spermatogenesis [34]. Cadmium and lead have been shown to increase testicular oxidative stress; in fact, increased rates of infertility and miscarriage have been found in welders as well as in workers of paint and battery manufacturers [35]. The food preservative sulfur dioxide directly increases intratesticular oxidative stress and diesel exhaust particulates have been shown to activate leukocyte ROS production [36]. Exposure to cigarette smoke increases both seminal leukocyte concentrations and seminal ROS generation, and decrease seminal levels of the antioxidant enzyme superoxide dismutase. Smoking has been noted to decrease concentrations of the seminal plasma antioxidants Vitamin C and E thereby reducing the oxidant scavenging capacity of spermatozoa and seminal fluid [37].

Oxidative stress (OS) can be measured during the clinical evaluation of the infertile male using direct and indirect assays [22]. Indirect assays measure the net oxidative sum of the balance between ROS production and the intra- and extracellular antioxidants that scavenge ROS, by assessing oxidation of the sperm cell membrane. The most widely used assay measures malondialdehyde (MDA), one of the final products of sperm cell membrane lipid peroxidation. Quantification of sperm DNA damage has also been used as an indirect assay of intracellular ROS-induced oxidant injury, although it is unable to distinguish oxidant-induced DNA damage from non-oxidative mechanisms. One possible solution is to measure oxidized deoxynucleotide, 8-oxo-7,8-dihydro 2'-deoxyguanoside (8-OHdG), the specific byproduct of oxidant-induced DNA damage [38]. The most common direct method of measuring seminal ROS is via chemiluminescence. Luminol or lucigen probes can be used for quantification of redox activities of spermatozoa. Quantification of oxidative stress using luminol can be performed using a luminometer, which is less expensive and easier to use compared to the instruments for the indirect assays. Furthermore, luminol chemiluminescence has well-established ranges for fertile and infertile populations, bringing clinical relevance to its use [37].

WHAT IS NEW IN TREATMENT

Antioxidant Therapy

Recent reports have focused on the therapeutic management of oxidative stress in male infertility. Most studies, however, demonstrate a suboptimal experimental design making effective interpretation difficult. When considering therapeutic measures to decrease oxidative stress, counseling toward lifestyle modifications must be considered. An important challenge to proper counseling is the paucity of evidence supporting objective improvement in infertility following lifestyle modification [22]. Varicoceles have been shown to increase oxidative stress levels in the testes as well as in the semen. Varicocelectomy, in the other hand, may decrease seminal oxidative stress, increase seminal concentrations of antioxidants and also improve sperm quality [39]. In recent years, interest has increased regarding the role of antioxidants and B-vitamins as modulators of fertility [40-43]. The antioxidants α -tocopherol (Vitamin E), ascorbic acid (Vitamin C) and the retinoids (Vitamin A) are potent scavengers of ROS. Deficiencies in vitamin B cause elevated homocysteine concentrations and impair the remethylation cycle. Vitamin B is involved in the methylation of phospholipids, proteins, DNA and RNA, and in the synthesis and repair of DNA. These processes are essential for normal spermatogenesis; therefore, derangements in these pathways may be detrimental for reproduction. Many studies have investigated the role of these and other vitamins and antioxidants in improving sperm parameters. Wong *et al.* (2002) demonstrated, in a randomized and placebo-controlled trial, that a 6-month administration of folic acid (5mg) and zinc phosphate (66mg) increased sperm counts in subfertile men [40]. Boxmeer *et al.* (2009) reported for the first time that low folate concentrations in the seminal plasma are associated with higher susceptibility of sperm DNA damage

[41]. This novel finding is in line with the role of folate in both DNA synthesis and DNA and protein methylation processes. Folate shortage increases DNA fragility due to the misincorporation of uracil instead of thymine. During normal repair processes, when the removal of the misincorporated uracil fails, double-strand breaks resulting in chromosome instability may occur. Greco *et al.* (2005), studying a large cohort of infertile men with >15% DNA-fragmented spermatozoa treated with either 1 gram of Vitamin C and E daily or placebo for two months, demonstrated beneficial effects of antioxidants in reducing the percentage of DNA-fragmented spermatozoa; however, no effects were observed on the sperm parameters [42]. The authors further went on by demonstrating that supplementation with vitamins E and C significantly increased the rates of clinical pregnancy and implantation following ICSI, although a dose-response association was not seen between DNA fragmentation index (% DFI) results and antioxidant intake [43]. Recently, a case series study involving couples with history of recurrent embryo losses suggested that an increased intake of antioxidant-rich food or antioxidant supplements by men with high levels of sperm DNA fragmentation or lipid peroxidation may optimize gestational outcomes [44].

Microsurgical Repair of Clinical Varicocele

Several studies have demonstrated that the surgical treatment of clinical varicoceles is highly effective to decrease seminal oxidative stress, to increase seminal concentrations of antioxidants and to improve sperm quality [39, 45-47]. However, a meta-analysis of seven randomized, controlled trials of varicocele repair for male subfertility concluded that treatment of varicocele in men from couples with otherwise unexplained subfertility could not be recommended [48]. The study by Evers and Collins [48] was challenged by Ficcaro *et al.* (2006), who argued that less than half of the studies of this Cochrane meta-analysis included patients with abnormal semen analysis and palpable varicocele. Moreover, Ficcaro and colleagues [49] demonstrated that the methodology and statistical power of the Cochrane review were poor, thereby minimizing the significance of the conclusions against varicocele repair. The most recent meta-analysis on varicolectomy by Marmar and colleagues (2007) unequivocally demonstrated the benefit of the surgical treatment of clinical varicoceles in infertile men with abnormal semen analyses [50]. The authors showed that the chances of obtaining a spontaneous conception were 2.8 times higher in

Microsurgical Subinguinal Varicocele Repair

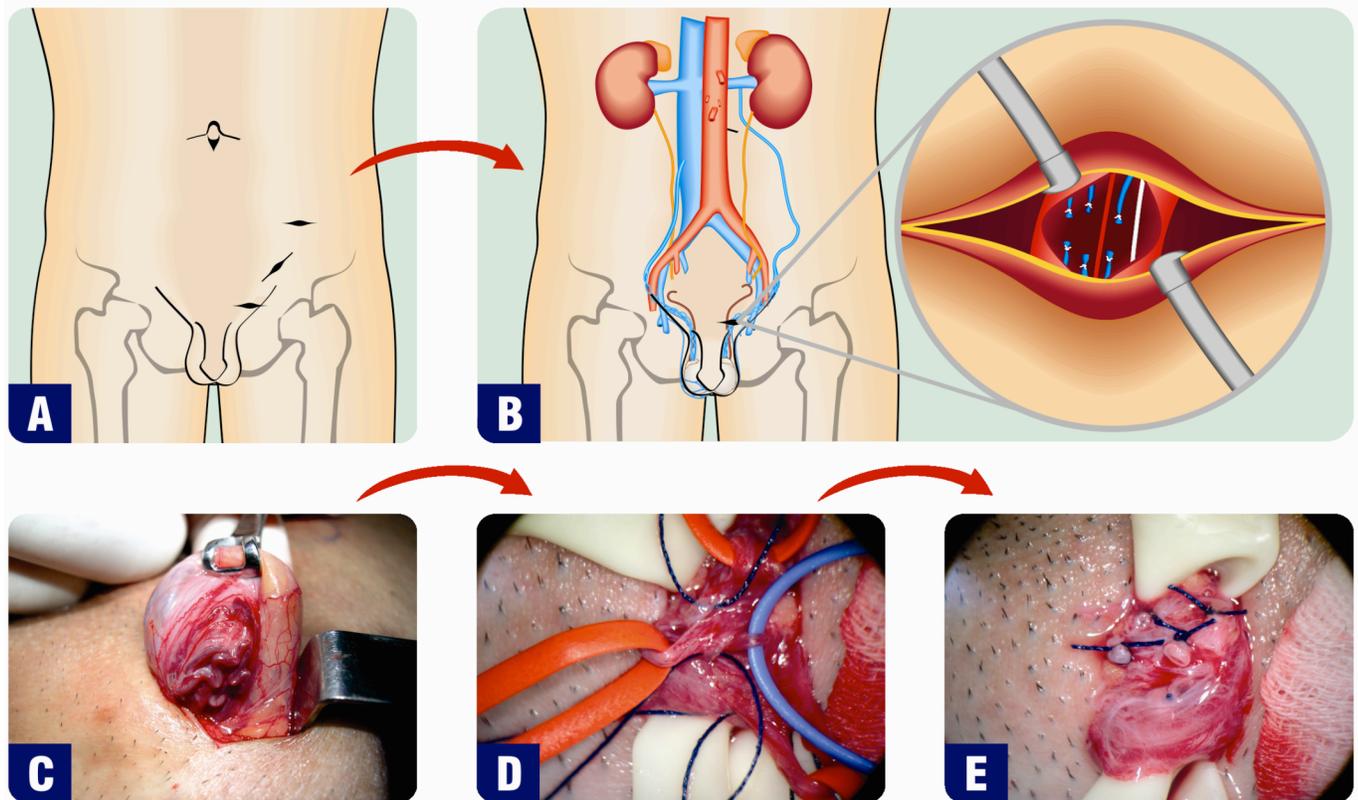


Fig. (2). Microsurgical Subinguinal Varicocele Repair. **A)** Incision sites commonly used for subinguinal, inguinal and retroperitoneal varicocele repair. **B)** In the subinguinal approach, a transverse incision is made just below the level of the external inguinal ring. **C to E)** Intraoperative photographs of the spermatic cord. **C)** Dilated cremasteric veins are identified by elevating the spermatic cord with a Babcock clamp. **D)** Testicular artery (blue vessel loop), lymphatics (blue cotton suture) and dilated varicose veins (red vessel loops) are demonstrated. **E)** Final surgical aspect of varicose veins transected and ligated with non-absorbable sutures.

the varicocelectomy group as compared to the group of patients who received either no treatment or medication. Recently, it has been shown that microsurgical varicocelectomy may also improve the outcomes of assisted reproduction in couples with varicocele-related infertility. Esteves *et al.* (2010) studied 242 infertile men with treated and untreated clinical varicoceles who underwent ICSI. The authors observed significantly higher pregnancy rates after ICSI in the group of men who underwent microsurgical varicocele repair before assisted conception. The clinical pregnancy (60.0% vs. 45.0%) and live birth (46.2% vs. 31.4%) rates after ICSI were significantly higher in the treated compared to the untreated group. The chances of achieving clinical pregnancy (odds-ratio [OR]: 1.82; 95% confidence interval [CI]: 1.06-3.15) and live birth (OR: 1.87, 95% CI: 1.08-3.25) by ICSI were significantly increased while the chance of miscarriage was reduced (OR: 0.433, 95% CI: 0.22-0.84) after varicocele repair [51]. Inci *et al.* (2009) reported that varicocele repair prior to sperm retrieval significantly increased the chance of obtaining testicular sperm using microdissection testicular sperm extraction (micro-TESE) in a group of men with non-obstructive azoospermia (NOA) and clinical varicoceles. In their retrospective study, sperm retrieval rates were 53% and 30% in the treated and untreated men, respectively (OR: 2.63; 95% CI: 1.05-6.60) [52].

Currently, varicoceles are treated either by surgery (open with or without magnification and laparoscopy) or percutaneous embolization of the internal spermatic vein. Although techniques vary, the main concept is the occlusion of the dilated veins of the pampiniform plexus. The high retroperitoneal (Palomo), radiologic, and laparoscopic approaches are performed for internal spermatic vein ligation. The inguinal (Ivanissevich) and subinguinal approaches also allow the ligation of the external spermatic and cremasteric veins that may contribute to the varicocele. A recent systematic review including 4,473 infertile individuals was performed to define the best treatment modality for varicocele [53]. The authors concluded that open microsurgical inguinal or subinguinal varicocelectomy techniques resulted in higher spontaneous pregnancy rates and fewer recurrences and postoperative complications than laparoscopic, radiologic embolization and macroscopic inguinal or retroperitoneal varicocelectomy techniques (Fig. 2).

Sperm Retrieval: Predictive Factors and Techniques

The concept that elevated FSH levels are associated with male sterility has been challenged [54]. Serum FSH is an indirect reflection of the global (spermatogenic) function and histology of the testis. In cases of diffuse maturation arrest, for instance, adequate control feedback from germ cells and Sertoli cells exists despite the absence of sperm production; as a result, normal FSH levels are observed. Sperm retrieval techniques are able to obtain sperm from the testicle even in the cases of elevated serum FSH. However, it seems that the technique used for sperm retrieval plays a major role. Bromage *et al.* (2007) reported that the probability of testicular sperm retrieval using random TESE is decreased in NOA men with elevated FSH levels [55]. Conversely, Ramasamy *et al.* (2009) demonstrated that retrieval rates

were nearly identical (~60% success rate) regardless of FSH levels by using micro-TESE; these authors showed that preoperative FSH level of ≥ 15 IU/L resulted in greater odds of finding sperm with micro-TESE compared with normal FSH values [56]. Since its first description by Schlegel in 1998, microdissection testicular sperm extraction (micro-TESE) has become a recognized procedure for men with NOA [57]. Micro-TESE is more successful in sperm retrieval compared to single or multiple random biopsies in such cases [58]. Additionally, micro-TESE is associated with less effect on testicular function because it spares vessels during dissection and removes less tissue than random biopsies [59] (Fig. 3).

To date, there are no absolute predictors of successful sperm retrieval in men with NOA. Even the combination of the results of testicular histopathology and FSH levels provides only a “fair” prediction model (accuracy: 0.74) [60]. Nonetheless, Esteves *et al.* (2006) demonstrated that testicular histopathology is predictive of the chances of retrieving testicular sperm using micro-TESE. Sperm retrieval rates were significantly higher in the cases of hypospermatogenesis or maturation arrest (100% and 75%, respectively) as compared to Sertoli-cell only (32%) [61]. These findings also highlight the concept that even the more adverse pattern on testicular histology does not determine if sperm are present elsewhere in the testis. However, it is still controversial whether invasive examination such as testicular biopsy should be performed, because it may cause inflammatory changes, hematoma, parenchymal fibrosis, or permanent devascularization of the testes.

Recently, the importance of surgical and medical treatment prior to sperm retrieval in NOA men has been highlighted. As already mentioned, treatment of clinical varicoceles prior to sperm retrieval may increase the chance of obtaining testicular sperm using micro-TESE in NOA men with clinical varicoceles [52]. Ramasamy and colleagues (2009) showed that men with Klinefelter syndrome (KS) with either normal or low baseline testosterone who responded to medical therapy had a better chance of sperm retrieval. In their study, men with serum testosterone levels less than 300 ng/dL received medical therapy (aromatase inhibitors, clomiphene or human chorionic gonadotropin) prior to micro-TESE. Patients who required medical therapy and responded to that treatment with a resultant testosterone of 250 ng/dL or higher had higher SR rate than men in whom post-treatment testosterone was less than 250 ng/dL (77% vs. 55%) [62]. Sperm retrieval rates were similar irrespective of the type of preoperative hormonal therapy, but the best SR rates (86%) were achieved in men with normal baseline testosterone levels. Klinefelter syndrome is a common condition among men with NOA, accounting for about 11% of the cases [63]. The sterility of KS is due to the high prevalence of azoospermia, present in 92% of KS men able to provide a semen sample with the remainder having a median of 0.1 million sperm/mL. Nevertheless, sperm are found in 50% of cases on testicular exploration and in the couples who undergo ICSI, pregnancy rates range from 30% to 50% [63]. In KS, the births of more than 60 children have been reported and ~50 had karyotype analysis, all of which were normal [63, 64].

Microsurgical Testicular Sperm Extraction

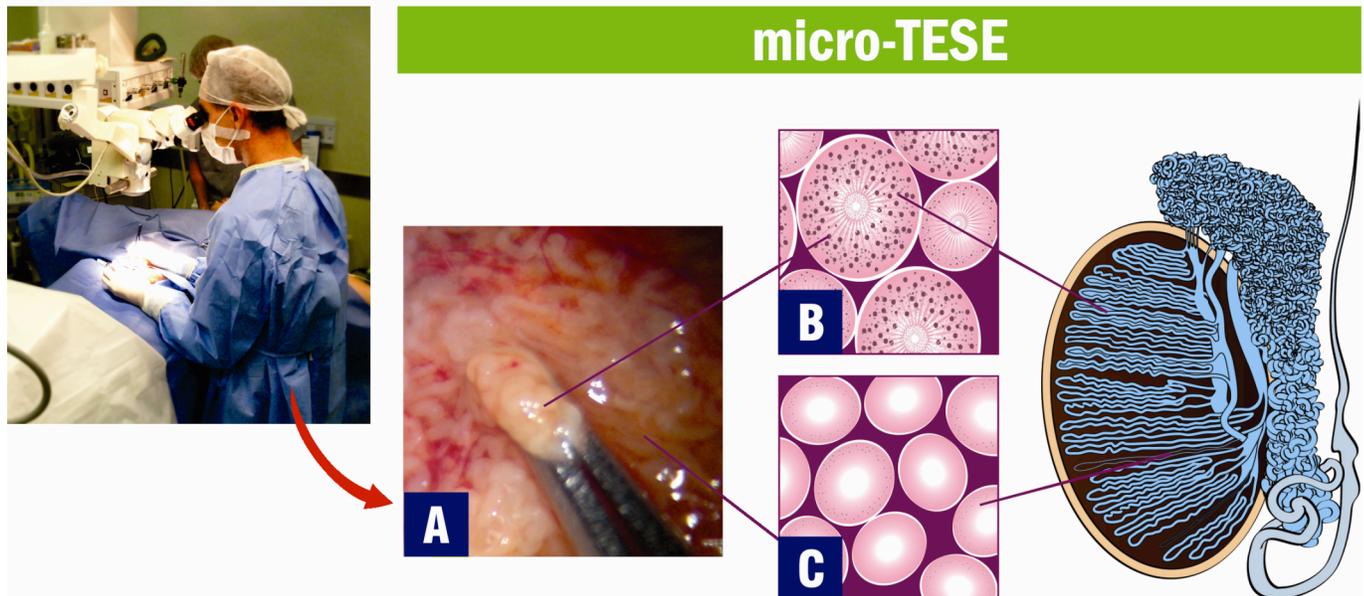


Fig. (3). Microsurgical testicular sperm extraction (micro-TESE). **A)** After the testicle is exteriorized, a single and large incision is made in an avascular area of the albuginea to expose the seminiferous tubules. **B)** Dilated tubules are identified and removed using the operating microscope and microsurgical technique (intraoperative photograph at x40-magnification). **C)** Illustration of the histopathology cross-section of a dilated seminiferous tubule with active spermatogenesis. **D)** Illustration of the histopathology cross-section of a thin tubule with Sertoli-cell only.

EXPERT COMMENTARY

Semen parameters within the reference interval do not guarantee fertility nor do values outside those limits necessarily imply male infertility or pathology. A man's semen characteristics need to be interpreted in conjunction with his clinical information. The reference limits provided by the WHO manual are from semen samples initiating natural conceptions; as such, they may indicate the need for infertility treatment but should not be used to determine the nature of that treatment.

Y-chromosome screening is indicated to infertile men presenting with moderate to severe (sperm count of <5 million/mL) oligozoospermia and NOA of unknown origin. It is debatable, however, if screening is valid when well-defined pathologies, such as radiation/chemotherapy or orchitis or trauma, are identified as the cause of oligo-/azoospermia. In other conditions, such as varicocele and cryptorchidism, Yq microdeletion screening is advisable since occasional cases of an association have been reported [65].

Sperm chromatin integrity, evaluated on neat semen, becomes particularly relevant when contact between the two gametes occurs in a more natural way and selective pressures operate to avoid the development of an embryo derived from sperm with a high load of genetic damage. ICSI can lead to pregnancy regardless of traditional sperm quality parameters and sperm chromatin structural integrity, but the potential adverse effects of using gametes with high loads of DNA damage are still unclear. Existing data justify the introduction of sperm DNA damage assessment to the male infertility workup, but evidence seems not to be strong

enough to provide a clinical indication for routine use of these tests in infertility evaluation of men. Nonetheless, sperm DNA damage testing is being clinically utilized in at least four distinct clinical scenarios: i) to assess cases of unexplained infertility when traditional semen analysis falls into normal range and no evident female reproductive system pathologies can be revealed, ii) in selected cases of recurrent miscarriage, iii) to aid in the selection of the best assisted reproduction technology (ART) method, and iv) to define the sperm source, i.e. ejaculated or testicular sperm, for ICSI. However, the widespread use of sperm DNA testing and its inclusion as a routine testing into the clinical andrology laboratory is currently limited to the absence of robust standardized methods with rigorous quality control and high quality comparative clinical data. The methods used for detecting DNA damage differ in their mechanism of action and as a consequence what they ultimately reveal about the nature of that damage. Some of the assays are difficult to incorporate in most clinical andrology and ART laboratories due to equipment size/expense and hazardous reagents. Moreover, the ability to establish reliable positive and negative predictive values to be used for diagnosing fertility potential and ART outcome is still inadequate due to the lack of large, prospective adequately powered studies. Whether sperm DNA damage can be decreased by some treatment modalities, allowing these couples to switch from ICSI to IVF/IUI or even achieve a pregnancy in a natural way, remains to be elucidated. It is plausible to believe that administration of antioxidants, varicocelectomy and modifications in both the life-style and environmental exposure may play a role to infertile men with high sperm DNA damage.

According to our experience involving approximately 200 individuals with NOA, successful SR rates of 55.7% have been achieved and results were similar in etiology categories of cryptorchidism, orchitis, genetic, radio-/chemotherapy and idiopathic [66]. Testicular histopathology results were predictive of sperm collection using both testicular sperm aspiration (TESA) and micro-TESE. Sperm retrieval by TESA was 100% and 82.3% in our group of NOA men presenting with either hypospermatogenesis or a history of previous successful TESA attempt. Using micro-TESE, SR rates were significantly higher than TESA in cases of SCO and maturation arrest (39.2% vs. 22.8%). Both methods yielded similar SR rates of 100% in cases of hypospermatogenesis. According to our data, the chances of retrieving spermatozoa and of achieving a live birth by ICSI are significantly increased in couples whose male partner had obstructive rather than non-obstructive azoospermia, thus indicating that the reproductive potential of infertile men undergoing ART is related to the type of azoospermia. [67].

FIVE-YEAR REVIEW

Y-chromosome infertility is associated with an intriguing AZF region which contains multiple genes, each of which is present in multiple copies. It is generally accepted that the chances of retrieving testicular spermatozoa in men presenting with complete deletions involving AZFa and/or AZFb regions is virtually zero. Deletion of the entire AZFb interval, for instance, has been associated with azoospermia arising from maturation arrest during meiosis. However, data are inconsistent with the phenotype typically seen in complete AZFb microdeletion [68, 69]. Longepied *et al.* (2010) described an exceptional case of one oligozoospermic man with a complete AZFb+c (P5/distal-P1) deletion. Ploton *et al.* (2010) reported a case of an infertile man also presenting with oligozoospermia (3.6 million sperm/mL), small testes and slightly elevated FSH levels, and a microdeletion spanning a region between AZFb and AZFc. The authors showed that the microdeletion was transmissible over at least three generations. Genomic DNA from the patient's father gave the same pattern and during the course of investigation a pregnancy occurred in the patient's wife who delivered a healthy boy also showing the same microdeletion. In light of these recent observations, and since few patients with NOA and AZFa or AZFb deletions have undergone sperm retrieval, caution should be applied when counseling such individuals against testicular collection techniques.

Human sperm nuclei contain considerably fewer protamines than those of several mammalian species. Human sperm chromatin, therefore, is less regularly compacted and frequently contains DNA strand breaks. DNA fragmentation is characterized by both single (STB) and double DNA strand breaks (DSB), and is particularly frequent in the ejaculates of subfertile men [22]. Oocytes and early embryos may repair sperm DNA damage. Consequently, the biological effect of abnormal sperm chromatin structure depends on the combined effects of sperm chromatin damage and the capacity of the oocyte to repair it. Although the assessment of sperm DNA integrity seems to be an useful and

potentially independent marker of fertility on both natural and assisted conception, recent reviews concluded that the clinical significance of sperm DNA integrity assessment remains unclear [70-72]. A relatively recent meta-analysis involving 22 relevant studies with infertile couples undergoing IVF and ICSI failed to support the concept that DNA integrity testing was more predictive for IVF than ICSI, and even that such testing is predictive of pregnancy outcome either in IVF or ICSI [70]. It is suggested that key objectives need to be urgently achieved for sperm DNA assessments to be widespread as effective clinical tools. These include: i) continuation of basic and translational research, ii) robust standardization of clinical assays and incorporation of systems for training staff and quality control; iii) high quality comparative clinical data and long-term follow-up of children resulting from assisted reproductive technology (ART) using sperm with defective sperm DNA damage [73, 74].

For men with NOA undergoing assisted conception treatments, follow-up of children born and identification of reliable predictors for successful sperm retrieval are of utmost importance. Recently, Sciarano *et al.* (2009) have shown that seminiferous tubuli with germ cells may be found in men with non-mosaic Klinefelter syndrome. Using fluorescence *in situ* hybridization (FISH) with probes for the X-centromere and immunolocalization of meiotic proteins, they showed that all meiotic spermatocytes analyzed with FISH were euploid (46,XY) and thus could form normal, haploid gametes. On the other hand, Sertoli cells showed two marks for the X chromosome, meaning that they were 47,XXY [75]. These new findings may explain the high rate of normal children born after testicular sperm extraction plus ICSI when applied to Klinefelter patients. The authors speculated that these spermatogenic foci most probably originated from clones of spermatogonia that have randomly lost one of the X chromosomes, probably during periods of life when high spermatogonial mitotic activity occurred. Encouragingly, studies are now appearing addressing novel potential biomarkers for the prediction of sperm production within the testes. Magnetic resonance spectroscopy has been recently proposed as a possible tool to identify metabolic signatures associated with various histological states in infertile men. Based on *ex-vivo* analysis of testicular biopsy specimens, concentrations of several tissue metabolites were acquired and then reassessed in men with a diagnosis of NOA. A singular pattern could be determined for two testis histological states: normal and Sertoli cell-only (SCO) syndrome. Proliferating germ cells are related to high phospholipid synthesis and with elevated phosphocoline. Normal spermatogenesis spectroscopic pattern presented high peaks of phosphocoline as opposed to SCO. Further research in this area may aid in the identification of a distinct metabolic signature for sperm presence, regardless of testis histopathology [76].

KEY ISSUES

- World Health Organization established new reference values for human characteristics. Men whose partners had a time-to-pregnancy of ≤ 12 months were chosen

as individuals to provide reference distributions for semen parameters, as follows: semen volume of 1.5 ml; sperm numbers of 39 and 15 million per ejaculate and per mL, respectively; vitality of 58% live; progressive motility of 32% and morphologically normal forms (strict criteria) of 4.0%. Semen parameters within the reference interval do not guarantee fertility nor do values outside those limits necessarily imply male infertility or pathology. A man's semen characteristics need to be interpreted in conjunction with his clinical information.

- About 10% of the infertile male population previously misdiagnosed as idiopathic have *de novo* Yq microdeletions. The presence or absence of sperm (ejaculated or retrieved) varies depending on the specific deletion. Y-chromosome screening is indicated to infertile men presenting with moderate to severe (sperm count of <5 million/mL) oligozoospermia and non-obstructive azoospermia of unknown origin or with associated conditions such as varicocele and cryptorchidism. Although no treatment exists, ICSI may be effective but the male offspring will inherit the same deletion as their father.
- Assessment of sperm DNA integrity seems to be an useful and potentially independent marker of fertility on both natural and assisted conception. However, robust standardization of clinical assays and quality control as well as comparative clinical data are key elements that need to be urgently addressed for the widespread of sperm DNA assessments as effective clinical tools.
- Oxidative stress is associated with sperm quality and infertility. Therapeutic measures to decrease oxidative stress show promising results, and include lifestyle modifications, varicocelectomy in selected cases as well as use of vitamins and antioxidants. However, most studies still demonstrates a suboptimal experimental design, making effective interpretation difficult.
- Spontaneous conception is significantly increased after varicocele ligation in infertile men with clinical varicoceles and abnormal semen analyses. Recent data suggest that microsurgical treatment of clinical varicoceles optimize reproductive outcome of couples undergoing ICSI or micro-TESE sperm retrieval. The microsurgical approach is associated with lower recurrence and complication compared to conventional surgical modalities and embolization.
- In the subset of men with NOA, FSH levels are not predictive of successful sperm retrieval or sterility. Testicular microdissection sperm extraction yields the best SR rates for these patients, and success may be optimized by surgical treatment of clinical varicoceles or by medical therapy in non-mosaic KS men. In about 50% of non-mosaic Klinefelter syndrome azoospermic men testicular sperm are found by micro-TESE. Children born after testicular sperm extraction plus ICSI in KS have normal karyotype due to the fact that the minor foci of germ cells into the seminiferous tubuli are euploid.

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CONFLICT OF INTEREST

None Declared.

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