Arch Gynecol Obstet
DOI 10.1007/s00404-012-2274-x

REPRODUCTIVE MEDICINE

# What every gynecologist should know about male infertility: an update

**Sandro C. Esteves · Alaa Hamada · Victor Kondray · Aruna Pitchika · Ashok Agarwal**

Received: 12 January 2012 / Accepted: 21 February 2012


**Abstract**
*Purpose* Our article reviews the evolving concepts in the field of male infertility for gynecologists and other health professionals involved in the care of men and women experiencing difficulty in having a child. The increased knowledge will help in the better management and treatment of infertile couples.
*Methods* Review of literature through Pubmed, Science Direct, Online Library.
*Results* Gynecologists are often the first healthcare providers to assess an infertile couple. Because half of all infertility problems stem from male factors, it is crucial for the gynecologist to remain updated on the main conditions that cause male infertility as well as current diagnostic tools and treatment options, including conventional strategies and assisted reproductive techniques.
*Conclusions* Extraordinary advances have been achieved in the field of male infertility over the past several years and many old concepts are now challenged. Therefore, it is imperative that male infertility physicians should update the gynecologists about the recent advances in the work-up of infertile men in terms of diagnosis and management. Such convention will help improve the standards of care for the infertile couple and enhance the cooperation between male and female reproductive endocrinologists.

**Keywords** Male infertility · Diagnosis · Treatment · Azoospermia · Oxidative stress

## Introduction

Infertility affects approximately 15% of couples worldwide and negatively impacts the quality of life in those who are affected [1]. A common misconception is that female factors account for most causes of infertility. However, the truth is that male factor infertility, alone or in combination with female factors, actually accounts for at least 50% of cases [2]. Male infertility arises from a variety of problems, most notably varicocele, genetic disorders and other factors such as excessive oxidative stress (OS), improper lifestyle habits and sperm DNA fragmentation.

Despite the fact that about half of infertility problems stem from male factors, gynecologists are often the first healthcare providers to perform the initial assessment of the infertile couple. As such, it is crucial that they remain updated on the main conditions that cause male infertility as well as current diagnostic tools and treatment options, including conventional strategies and assisted reproductive techniques.

This article will provide a better understanding of the evolving concepts in the field of male infertility to gynecologists and all health professionals involved in reproductive medicine.

S. C. Esteves
ANDROFERT-Andrology and Human Reproduction Clinic,
Av. Dr. Heitor Penteado, 1464, Campinas,
São Paulo 13075-460, Brazil
e-mail: s.esteves@androfert.com.br

A. Hamada
Center for Reproductive Medicine, Cleveland Clinic,
9500 Euclid Avenue, Cleveland, OH 44195, USA
e-mail: ala1977h@yahoo.com

V. Kondray · A. Pitchika · A. Agarwal (✉)
Center for Reproductive Medicine, Cleveland Clinic,
Desk A19.1, 9500 Euclid Avenue, Cleveland,
OH 44195, USA
e-mail: agarwaa@ccf.org

Published online: 06 March 2012

## The role of the gynecologist in the initial male workup: an overview

A comprehensive history must be taken to gain a greater insight into the possible contributors to male infertility (see below). In addition, a preliminary semen analysis should be ordered before referring the male partner for urological examination. Additional tests may be used to determine the physiological health of the male as well as to identify possible genetic factors that contribute to infertility. Although it is outside the expertise of gynecologists to interpret these specific tests and to treat the conditions affecting male fertility, it is important for them to have an in-depth understanding of the available diagnostic tools and treatments so that they can counsel and refer the patient appropriately.

## History taking of the infertile male patient: unraveling contributing factors to subfertility

Certain historical data may point to male reproductive dysfunction, and a comprehensive review of these aspects can be obtained elsewhere [3], and an outline of the main aspects of history taking for infertile men is provided in Table 1.

In brief, gynecologists should inquire about the duration of infertility, the age of the couple and their sexual, family, childhood, developmental, surgical and fertility history. They should also inquire about the presence of systemic medical conditions, current and past medication use and social, occupational and gonadotoxic exposure history. It has been reported that a long duration of male infertility is usually associated with depression, erectile dysfunction and low self-esteem [4, 5]. As the male partner's age increases, the conception rate decreases and the risk of genetic defects in offspring (e.g., Down syndrome) rises. By age 35, sperm DNA fragmentation rates start to rise whereas germ cell apoptosis rates begin to decrease [6–8]. In addition, semen volume, sperm morphology and motility all start to markedly decline [9–11]. The risk of having a child with autosomal dominant disorders for older men is equal to that of having a child with Down syndrome for women aged >45 years [12]. Antihypertensive drugs such as alpha and beta-blockers, thiazide diuretics and spironolactone may cause erectile and ejaculatory dysfunction. Also, calcium-channel blockers may negatively impact sperm fertilizing ability by blocking the acrosome reaction. Antibiotics such as gentamicin, erythromycin and nitrofurantoin are gonadotoxic [13]. Cimetidine, spironolactone, certain hormonal preparations and anabolic steroids may alter the hypothalamic-pituitary gonadal axis, thus affecting spermatogenesis [14]. Cancer treatment such as radiotherapy and chemotherapy also decreases sperm production. Obesity negatively influences male fertility by causing erectile dysfunction (ED) and impairing semen parameters [15–19]. Corona et al. [15] showed that 96.5% of their patients with metabolic syndrome—a constellation of conditions that usually includes obesity—had ED. The risk of fertility problems is even higher for couples where both partners are obese [16]. Furthermore, reduced sperm DNA integrity is common in infertile obese men [20]. These changes are attributed to high estrogen production from fat stores and/or high accumulation of fertility-jeopardizing environmental

**Table 1** Clinical male infertility history outline

| |
|---|
| 1) Infertility history |
| Age of partners, time attempting to conceive |
| Contraceptive methods/duration |
| Previous pregnancy (actual partner/other partner) |
| Previous treatments |
| Treatments/evaluation of female partner |
| 2) Sexual history |
| Potency, libido, lubricant use |
| Ejaculation, timed intercourse, frequency of masturbation |
| 3) Childhood and development |
| Cryptorchidism, hernia, testicular trauma |
| Testicular torsion, infection (e.g., mumps) |
| Sexual development, puberty onset |
| 4) Personal history |
| Systemic diseases (diabetes, cirrhosis, hypertension) |
| Sexually transmitted diseases, tuberculosis, viral infections |
| 5) Previous surgeries |
| Orchidopexy, herniorraphy, orchiectomy (testicular cancer, torsion) |
| Retroperitoneal and pelvic surgery |
| Other inguinal, scrotal and perineal surgery |
| Bariatric surgery, bladder neck surgery, transurethral resection of the prostate |
| 6) Gonadotoxin exposure |
| Pesticides, alcohol, cocaine, marijuana abuse |
| Medication (chemotherapy agents, cimetidine, sulfasalazine, nitrofurantoin, allopurinol, colchicine, thiazide, $\beta$- and $\alpha$-blockers, calcium blockers, finasteride) |
| Organic solvents, heavy metals |
| Anabolic steroids, tobacco use |
| High temperatures, electromagnetic energy |
| Radiation (therapeutic, nuclear power plant workers), etc. |
| 7) Family history |
| Cystic fibrosis, endocrine diseases |
| Infertility in the family |
| 8) Current health status |
| Respiratory infection, anosmia |
| Galactorrhea, visual disturbances |
| Obesity |

toxins in fatty tissue. Particular attention should be directed toward certain occupations that risk male fertility potential such as bakers, steel workers, welders, painters, printer and dye manufacturers. Sedentary jobs, sauna and hot tubs may increase scrotal temperature and may also affect testicular function [14]. The use of pesticides, radiation exposure from X-ray, excessive use of cell phones [21] and heavy metal intoxication may also potentially affect sperm production and quality.

## The importance of routine semen analysis in the male infertility workup

Semen analysis is of paramount importance in the initial investigation of the male partner and it is often ordered by the gynecologist during the female infertility workup. It provides information on the functional status of the seminiferous tubules, epididymis and accessory sex glands, and its results are often taken as a surrogate measure of his ability to father a pregnancy.

Semen analysis is a complex test which ideally should be carried out in andrology laboratories. Minimum standards for laboratories performing semen analyses include the presence of experienced technicians, internal and external quality control, validation of test systems, quality assurance during all testing processes, and communication with clinicians and patients. Accuracy, the degree to which the measurement reflects the true value, as well as precision, the reproducibility of the results, are vitally important for clinicians who rely upon the values provided by the laboratory to direct the further work-up, diagnosis and counseling of the infertile male. Routine semen analysis should include: (a) physical characteristics of semen, including liquefaction, viscosity, pH, color and odor; (b) specimen volume; (c) sperm concentration; (d) sperm motility and progression; (e) sperm morphology; (f) leukocyte quantification; and (g) fructose detection in cases where no spermatozoa are found and ejaculate volume is low [22]. Results from at least two, preferably three, separate seminal analyses must be obtained following 2–5 days of ejaculatory abstinence before a definitive conclusion can be drawn since wide biological variability exists even within the same individual. Despite being arbitrary, multiple semen analyses are usually taken at 7- to 14-day intervals. Table 2 highlights the cutoff values for semen analysis as published in consecutive World Health Organization (WHO) guidelines [23, 24].

Physicians treating infertile couples should exercise caution when interpreting the reference values for a number of reasons. First, results are normal in approximately 6–27% of infertile men [25]. Second, current semen reference values were obtained primarily from Northern Europe, North America, and Australian men [23], and thus, the values may not apply to all men universally. Third, reference values in the newest WHO guidelines, issued in 2010, are lower than those of previous versions. As a result, patients previously categorized as having an abnormal semen analysis will now be considered 'normal'.

According to Esteves et al. in a critical analysis of the newly released WHO reference values for semen analysis characteristics, "male partner referral for evaluation may be postponed or not undertaken. This deferment poses a potential problem, since it has been exhaustively reported that male and female reproductive age are clearly associated with reproductive outcome. It is unclear at this time, whether this re-classification will result in a more cost-effective evaluation of the infertile couple or in a delay in the male factor evaluation with subsequent delay in the definitive diagnosis and management of the infertile couple" [25].

Although helpful, conventional semen analysis can not alone be used to predict male fertility potential. Therefore, the male infertility evaluation must go far beyond a simple semen analysis, as it has to be complemented with a proper physical examination, a comprehensive history taking, and relevant endocrine, genetic, and/or other investigations.

## Genetic aspects of male infertility

Male infertility can be associated with different genetic factors that include chromosomal aberrations, genetic alterations and Y-chromosome microdeletions. Approximately 6% of infertile men have chromosomal abnormalities; the rate is even higher (~16%) in men with azoospermia [26]. Sex chromosomal aneuploidy (Klinefelter syndrome [KS]; 47,XXY) is the most common chromosomal disorder in infertile men and is generally associated with hypotrophic or atrophic testicles, elevated serum follicle stimulating hormone (FSH) levels and azoospermia and/or severe oligozoospermia. Spermatogenesis can be variably affected in patients with a mosaic karyotype (46,XY/47,XXY). In men with KS presenting with azoospermia, spermatozoa are present in approximately 50% of cases on testicular exploration, and pregnancy rates associated with intracytoplasmic sperm injection (ICSI) range from 30 to 50% [27]. ICSI is an in vitro fertilization procedure in which a single spermatozoon is injected into the oocyte cytoplasm and is largely used in cases of severe male factor infertility. Men with KS can have biological offspring with a normal karyotype because germ cells in men with KS are usually euploid (46,XY) and thus can form normal, haploid gametes [28].

Among genetic disorders, cystic fibrosis is the most common. The condition is caused by a mutation in the gene coding for the protein cystic fibrosis transmembrane conductance regulator (CFTR); the gene is located on the long

**Table 2** Cut-off reference values for semen characteristics as published in consecutive WHO manuals

| Semen characteristics | WHO (1980) | WHO (1987) | WHO (1992) | WHO (1999) | WHO (2010)[a] |
|---|---|---|---|---|---|
| Volume (mL) | ND | ≥2 | ≥2 | ≥2 | 1.5 |
| Sperm count ($10^6$/mL) | 20–200 | ≥20 | ≥20 | ≥20 | 15 |
| Total sperm count ($10^6$) | ND | ≥40 | ≥40 | ≥40 | 39 |
| Total motility (% motile) | ≥60 | ≥50 | ≥50 | ≥50 | 40 |
| Progressive motility[b] | ≥2[c] | ≥25% | ≥25% (grade a) | ≥25% (grade a) | 32% (a + b) |
| Vitality (% alive) | ND | ≥50 | ≥75 | ≥75 | 58 |
| Morphology (% normal forms) | 80.5 | ≥50 | ≥30[d] | (14)[e] | 4[f] |
| Leukocyte count ($10^6$/mL) | <4.7 | <1.0 | <1.0 | <1.0 | <1.0 |

[a] Lower reference limit obtained from the lower fifth centile value

[b] Grade a, rapid progressive motility (>25 μm/s); grade b, slow/sluggish progressive motility (5–25 μm/s); Normal, 50% motility (grades a + b) or 25% progressive motility (grade a) within 60 min of ejaculation

[c] Forward progression (scale 0–3)

[d] Arbitrary value

[e] Value not defined but strict criterion is suggested

[f] Strict (Tygerberg) criterion; *ND* not defined

arm of chromosome 7. Depending on the extension of the mutation, cystic fibrosis can manifest in a full clinical presentation (an autosomic recessive potentially fatal disease) or in a mild form, where congenital bilateral absence of the vasa deferentia (CBAVD) exists and affects approximately 1.3% of infertile men [29]. Approximately 80% of men presenting with CBAVD have a CFTR mutation, and because the genetic testing is not 100% sensitive, these men with CBAVD should be assumed to harbor the genetic anomaly. The female partner should be tested for the mutation as she may also be a carrier (approximately 4% risk). Such testing should be done before the man's sperm is used for assisted conception because the cystic fibrosis gene can be transmitted to offspring [3].

The long arm (q) of Y-chromosome is related to spermatogenesis [30]. The Y-chromosome region related to infertility is called azoospermia factor locus (AZF-azoospermia factor). The locus can present in complete or partial microscopic deletions, isolated or in combination, and in non-overlapping subregions called AZFa, AZFb, AZFc and AZFd. These subregions contain multiple genes that control the different steps of spermatogenesis.(See Fig. 1).

The most common Y-chromosome deletion in infertile men is the one affecting the DAZ gene (deleted in azoospermia), which is located in AZFc region. Severe oligozoospermia or azoospermia can be seen in such cases. Y-chromosome microdeletions are found in 15% of men with azoospermia and in 6% of men presenting with severe oligozoospermia (<1 million/mL) [26, 31, 32]. For sperm counts between 1 and 5 million/mL, the detection rate drops down to 1.7% [33]. As such, screening for Y-chromosome microdeletions is generally recommended for men who have azoospermia or severe oligozoospermia. Y-chromosome infertility may be found in men with other phenotypic conditions affecting fertility such as varicocele and/or cryptorchidism.

To test for Y-chromosome microdeletions, peripheral blood is obtained, and polymerase chain reaction (PCR) is used to detect amplifications on the long arm of the Y-chromosome, which will identify deletions of the AZF regions. Patients with an AZFa microdeletion usually present with germ cell aplasia on testicular histopathology while most patients with an AZFb microdeletion present with maturation arrest [34, 35].

Y-chromosome microdeletion screening is a simple test that offers significant advantages to men with azoospermia or severe oligozoospermia: it may not only identify the cause of male infertility but also predict the chance of sperm retrieval for candidates of assisted conception. In cases involving AZFa and/or b microdeletions, sperm retrieval is currently not recommended because there is no evidence that testicular sperm can be found irrespective of the retrieval method. In cases of an AZFc microdeletion, sperm can be retrieved in approximately 71% of patients [33]. Clinical pregnancy rates are virtually the same as those of idiopathic azoospermic patients [3].

As such, costs, morbidity and stress related to invasive surgical sperm retrievals may be avoided by genetically screening infertile men with severe infertility. In addition, the identification of a genetic basis for male infertility will help couples make more informed decisions and seek adequate counseling. It must be noted that the offspring of a father with a Y-chromosome deletion will inherit the

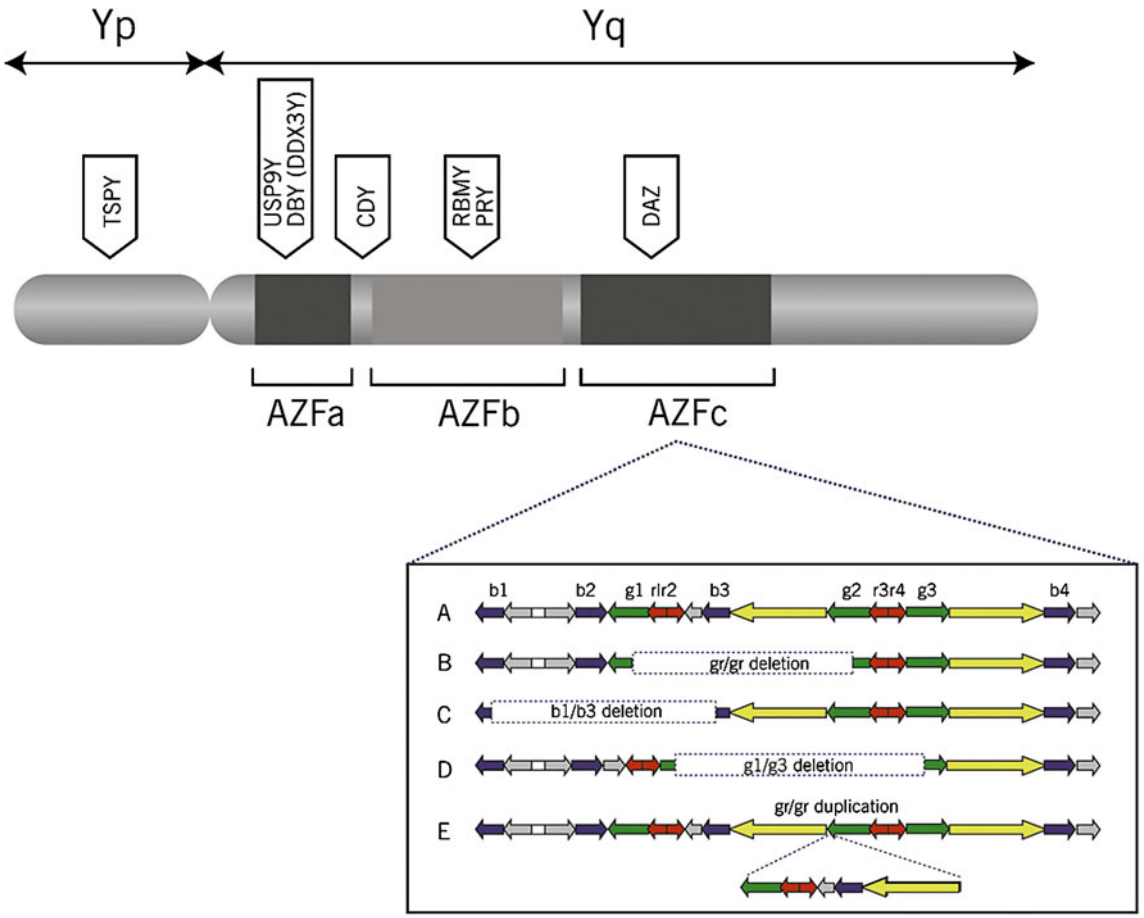


**Fig. 1** Illustration of the Y chromosome and its regions involved in spermatogenesis. (Reprint from Fertil Steril. 93, O'Flynn O'Brien KL, Varghese AC, Agarwal A. The genetic causes of male factor infertility: A review, pages 1-12, Copyright 2010, with permission from Elsevier (103))

same genetic trait. It has been suggested that sperm production seen in men with an AZFc microdeletion may decrease over time. As such, sperm cryopreservation should be considered as an alternative to preserve fertility for such individuals. The main indications for genetic testing in male infertility and the tests used to assess such conditions are highlighted in Table 3.

## The role of varicocele in male infertility

Varicocele is an elongated, dilated and tortuous testicular vein in the spermatic cord. It is a highly prevalent condition—it affects 15% [36] of male adults in the general population and 40% of patients who are unable to initiate a pregnancy [37]. Its epidemiologic features suggest that it is

**Table 3** Main indications for genetic testing in male infertility

| Indications | Recommended tests |
|---|---|
| Men with infertility of unknown etiology and sperm concentration <10 million/mL who are candidates for ART | Y-chromosome microdeletion and G-band karyotype |
| Non-obstructive azoospermia in a male considering testicular sperm retrieval for ART | Y-chromosome microdeletion and G-band karyotype |
| Azoospermic or oligozoospermic men with absence of at least one vas deferens at physical examination | CFTR gene mutation |
| Azoospermic men with signs of normal spermatogenesis (e.g., obstructive azoospermia of unknown origin) | CFTR gene mutation |
| History of recurrent miscarriage or personal/familiar history of genetic syndromes | G-band karyotype |

*ART* assisted reproductive techniques, *G-band karyotype* Giemsa band karyotype, *CFTR* cystic fibrosis transmembrane conductance regulator

a progressive pathology with a genetic predisposition [38, 39].

Varicocele pathophysiology remains poorly understood. Recent data suggest that varicocele causes infertility by inducing ultrastructural testicular changes and oxidative stress, with implications for the seminal antioxidant capacity and sperm chromatin integrity [40–42]. Sperm characteristics of men with varicoceles are highly variable, but the most common features involve abnormalities in count, motility and morphology [43]. The cornerstone of varicocele diagnosis remains the physical examination, although ultrasound may be helpful in certain scenarios.

Even though its presence affects both sperm function and testosterone production, it is not clear whether varicocele repair improves male fertility. Evidence exists both in favor of and against it, but as of now, most specialty societies recognize that varicocele is detrimental to male reproductive health and that its treatment may improve sperm function and chances of conceiving. Surgical treatment is the gold standard, and the subinguinal approach using microsurgery offers the best results with the fewest complications. It should be noted, however, that current evidence does not support treatment of subclinical varicoceles—these should only be treated when associated with a contralateral clinical one [44].

A recent meta-analysis on varicocelectomy by Marmar et al. [45] demonstrated the benefit of surgical treatment in infertile men with abnormal semen analyses. The authors showed that the chances of spontaneous conception were 2.8 times higher in men who underwent varicocelectomy than in patients who received either no treatment or medication [45].

Assisted reproductive technology (ART) is routinely used to treat male factor infertility. Decision analysis-based comparisons of ART and varicocelectomy suggest that varicocele repair is more cost effective than the use of ART in men with impaired semen parameters, which should be taken in consideration in patient care [46].

In addition, the indication of varicocele repair prior to in vitro fertilization/intracytoplasmic sperm injection (IVF/ICSI) may be considered in certain circumstances. In men with non-obstructive azoospermia (NOA) with favorable testicular histopathology, clinical varicocele repair restores the presence of sperm in the ejaculate [47]. This means that IVF/ICSI can be performed without the need to surgically retrieve sperm. It has been shown that for patients who are still azoospermic after varicocelectomy, sperm retrieval success rates using testicular microdissection sperm extraction may increase along with the couple's chance for pregnancy [48].

Varicocelectomy also has the potential to obviate the need for ART or to down stage the level of ART needed to bypass male factor infertility [49]. Recently, it has been shown that treatment of clinical varicocele may also improve the outcomes of assisted reproduction in couples with varicocele-related infertility. Esteves et al. analyzed a group of infertile men with treated and untreated clinical varicocele who, with their partners, underwent ICSI. Live birth rates were significantly higher after ICSI in the group of men who underwent artery and lymphatic sparing subinguinal microsurgical varicocele repair before ART (46.2%) than for the ones undergoing ICSI in the presence of a clinical varicocele (31.4%) [50]. In the aforementioned study, the chance of achieving a live birth by ICSI increased by 1.8 times while the chance of miscarriage after obtaining a pregnancy by ICSI decreased by 2.3 times if the varicocele was treated before ART.

Lastly, the adoption of the newly released 2010 WHO reference values for semen parameters is likely to have a significant impact on varicocele treatment by excluding former candidates for varicocele repair based on the current recommendations for surgery. Current guidelines propose that varicocele should be treated if palpable and in the presence of abnormal semen analyses [51–53]. This recommendation should be analyzed with caution in order not to miss the adequate timing to intervene and prevent testicular damage.

## Azoospermia is not synonymous with sterility

Azoospermia is defined by the complete absence of sperm cells in the ejaculate. It affects approximately 1% of the male population and can be easily detected and diagnosed by using simple microscopy techniques following centrifugation. Although a complete lack of sperm cells in the ejaculate may seem like a daunting prognosis for the patient, it must be noted that azoospermia does not necessarily mean that the male is sterile. This is because a male may either have obstructive or non-obstructive azoospermia, and the type of azoospermia is associated with a distinct prognosis of fertility.

Obstructive azoospermia is a condition where there is a mechanical obstruction in the male fertility track. Such obstructions are usually located between the epididymis and the ejaculatory duct, and they block semen from being expelled into the vaginal tract. Causes of obstructive azoospermia include congenital disease, infection, and vasectomy. In typical cases, the obstruction lies within the epididymal tract thus obstructive azoospermia presents a more optimistic outcome for fertility because spermatogenesis remains largely functional. Surgical reconstructive procedures are available for patients with obstructive azoospermia.

A comprehensive review of surgical modalities for male infertility patients has been discussed elsewhere [54]. It should be stressed, however, that patients must be informed

that the success rate of vasovasostomy decreases as time from the vasectomy increases [55]. Secondary epididymal obstructions increase the failure rate. A cost-effectiveness analysis comparing vasectomy reversal with assisted conception suggests that the former procedure is more advantageous in regard to procedure expenses, pregnancy rates and morbidity [56].

The other class of azoospermia is non-obstructive. Like obstructive azoospermic patients, non-obstructive azoospermic patients do not have sperm cells in their ejaculate. The cause is more troubling, however, because it indicates that there is a possible failure of spermatogenesis or maturation. Poor genetic makeup accounts for many non-obstructive azoospermia cases. While half of non-obstructive azoospermic patients will have mature spermatozoa in their testicles, there are no reliable noninvasive predictive factors to distinguish prospectively which patients will have sperm that can be surgically retrieved [57].

The only exception to this rule is the presence of Y-chromosome microdeletions involving the AZF regions. As already discussed, in case an AZF microdeletion is found, its location may predict the chances of retrieving testicular sperm [58]. Conversely, invasive procedures may be used to collect a testicular sample for histological examinations to determine the likelihood of sperm retrieval [58, 59]. If sperm is likely to be retrievable, sperm retrieval techniques coupled with ICSI should be used to achieve pregnancy. An overview of sperm retrieval techniques and their results can be found elsewhere [60].

Microsurgical testicular sperm extraction (micro-TESE) is considered to be the best option for sperm retrieval in men with non-obstructive azoospermia. Micro-TESE offers the highest probability of finding testicular sperm for fertilization, and it is coupled with the opportunity to both preserve testicular vasculature and to minimize the amount of extracted parenchyma [57].

ICSI is associated with lower fertilization rates per injected oocyte, as well as, clinical pregnancy and delivery rates when testicular spermatozoa from men with non-obstructive azoospermia are used in comparison with epididymal/testicular sperm from men with obstructive azoospermia [61]. Such differences may be explained by the fact that testicular spermatozoa from men with severely impaired spermatogenesis have a higher tendency to carry deficiencies such as the ones related to the centrioles and genetic material, which ultimately affect the capability of the male gamete to activate the egg and trigger the formation and development of a normal zygote and a viable embryo [62]. If there is no worthy sperm for fertilization, then the couple must consider adoption or donor insemination.

## High ROS levels and oxidative stress

An emerging explanation for several types of male infertility arises from oxidative stress (OS) which is believed to be present anywhere from 30 to 80% of infertile men [63].

Free radicals are unstable molecules that lack a valence electron and generally tend to contain a nitrogen or oxygen atom. They are divided into reactive nitrogen species (RNS) and reactive oxygen species (ROS), respectively. There are many examples of RNS and ROS molecules, such as superoxide anions, nitrous oxide, hydroxyl radicals, hydrogen peroxide, and hypochlorous acid. The unstable nature of ROS makes them essential for normal physiological function and chemical reactions in the body. The spermatozoon is a redox cell that is able to generate reactive oxygen species to perform physiological functions. ROS are necessary for sperm function, capacitation, and fertilization.

Although ROS have important physiological roles in fertility, excessive production of ROS disproportionate to the level of natural antioxidants can lead to a condition known as oxidative stress, which is harmful to the reproductive cells [64] and manifests in decreased overall sperm parameters. The unpaired electron in the outer shell of RNS and ROS molecules means that it is highly reactive with other unintended molecules such as polyunsaturated fatty acids (PUFA) which are equally rich in electrons and abundant in sperm membranes; PUFA provide an easy pathway for oxidative molecules to rob their electrons. The initiation of such a reaction could be amplified by the cyclic chain reaction of radical formation resulting in peroxidation of lipid membranes which has detrimental effects on sperm, such as decreased membrane fluidity [65] and sperm head and mid-piece integrity [66]. Consequently, oxidative stress in the sperm membrane could decrease sperm motility, morphology, and other important functions for fertilization [67]. Oxidative stress caused by lipid peroxidation can be detected by measuring levels of seminal malondialdehyde, which is a product of lipid peroxidation. Seminal plasma antioxidants play a vital role in alleviating or preventing the harmful side effects of oxidative radical interactions by supplying the reactive molecule with an electron from its own electron-rich molecular structure.

A critical component of reproduction is the passage of genetic material from the parents to the offspring. Like lipids, oxidative species target DNA molecules and may cause a variety of problems in the genetic material of the cell. Reactive oxidative radicals appear to target the bases and backbone of the DNA molecule and result in a variety of DNA problems, including deletions, chromosomal rearrangements, point mutations, polymorphisms, and single or double stranded DNA breaks [68, 69]. The

spermatozoa have two mechanisms for protecting DNA from damage: the tight packing of the chromatids and the presence of seminal antioxidants which act as a natural repair system. Unlikely the oocyte, spermatozoa have lost most of its redox enzymes containing cytoplasm during the maturation process. While sperm and oocyte's natural repair systems tolerate DNA damage up to a certain degree, an excessive amount of damage can lead to miscarriage, improper embryo development, embryo fragmentation, and gamete apoptosis [68]. While the exact mechanism of oxidative species' damage on sperm DNA is debated, what is known is that infertile men with sperm DNA damage tend to have elevated ROS levels [70]. This must be duly noted because problems with sperm DNA due to oxidation could greatly hinder the effectiveness of assisted reproductive techniques.

## DNA fragmentation

A number of factors can cause sperm DNA damage: advanced paternal age, inadequate dietary intake, drug abuse, environmental pesticide exposure, tobacco use, varicocele, medical diseases, hyperthermia, air pollution, genital inflammation and infectious diseases [3]. DNA damage could occur as a result of protamine sulfate deficiency (with aberrant chromatin remodeling), ROS, abortive apoptosis, and alterations in topoisomerase II activity [71]. DNA damage can also occur due to oxidative stress caused by the generation of ROS from contaminating leukocytes, defective sperm and antioxidant depletion [63]. DNA damage is rare in fertile men whereas abnormal levels of DNA damage are observed in approximately 5% of infertile men with normal semen analysis and in 25% of infertile patients with abnormal semen analysis [72–74]. Damaged DNA may cause errors in DNA replication, transcription, and translation during embryogenesis [75]. Certain cases of childhood leukemia and autism have been suggested to be linked to abnormal DNA fragmentation [71, 76].

Indications for DNA integrity assessment include:

1. Normal semen analysis seen in conventional method in an infertile man.
2. Recurrent pregnancy loss.
3. Determination of the most suitable assisted conception treatment modality.

The clinical utility of assessing sperm DNA integrity relies on three main aspects: (1) identifying the cause of male infertility and (2) choosing the most suitable ART modality, and (3) predicting, to some extent, ART outcomes.

Conventional semen analysis has limited value in detecting DNA damage. Several tests can assess sperm DNA integrity such as the terminal uridine nick-end labeling (TUNEL) assay, comet assay, acridine orange test, sperm chromatin structure assay (SCSA) and sperm chromatin dispersion test (SCD). The TUNEL and SCSA tests detect histone-associated chromatin breaks whereas the Comet assay detects breaks in both protamine- and histone-bound chromatin. The SCD test is based on the principle that sperm with fragmented DNA fails to produce the characteristic halo of dispersed DNA loops that is observed in sperm with non-fragmented DNA, following acid denaturation and removal of nuclear proteins [77]. The sperm DNA fragmentation index (DFI) cutoff value varies from one assay to another. It is approximately 20% in TUNEL and 27–30% in SCSA. These thresholds levels vary in native, unprocessed and processed sperm prepared for ART by density centrifugation [77].

## Counseling of male infertile patients

Infertile men are often anxious, feel guilty regarding their inability to induce a pregnancy, and face depression due to lack of spontaneous conception [78]. Proper counseling of both partners should be one of the top priorities of the treating physician, and in this sense, the gynecologist plays a crucial role. The gynecologist should be updated with the latest information regarding the conditions affecting male infertility, the available treatment options and their results, as well as with the ART available for males with severe infertility. Gynecologist should be able to elicit and record enough information while counseling to help reduce the couple's anxiety, and at the same time, she/he should not give false hopes if the cause is not treatable.

The following list contains practical recommendations for couples with associated male factor infertility who are attempting to conceive:

1. Explain to the couple how to time intercourse with ovulation, possibly using ovulation prediction tests. Also discuss the detrimental effects of lubricants. All commercially available lubricants decrease sperm motility and increase sperm DNA damage. Hydroxylethylcellulose-based lubricant (Pre-Seed) was shown to be relatively less detrimental to sperm than other substances [79]. Saliva and vegetable oil also decrease sperm motility [80, 81].
2. Advise both the male and female partner to stop smoking, limit their use of alcohol to 3–4 U/week and abstain from illicit drug use [4, 82].
3. Prescribe exercise and weight loss for overweight or obese men. A healthy body mass index (BMI) ranges from 22 to 28 [82, 83].
4. Advise the male partner to avoid any situations that can increase scrotal temperature such as wearing tight-fitting underwear, riding a bike for more than 30 min at

a time, sitting in a hot tubs/sauna, and placing portable computers directly on the lap. If occupational exposure to a hot work environment is unavoidable, the patient can take proper precautionary measures to minimize testicular heat exposure [40].

5. Refer men older than 40 and women older than 35 for genetic counseling [3].
6. Abnormal findings in semen analysis require thorough physical examination and further laboratory investigation. This work-up should be discussed with the patient and it should be explained that referral to a clinical andrologist is preferable. In the presence of azoospermia in semen analysis, the couple should not be discouraged and should be informed that treatment modalities are available.
7. In cases of non-obstructive azoospermia, recurrent pregnancy loss, previous history of congenitally anomalous baby, family history of chromosomal disorder, the diagnostic tests such as karyotyping, Y-chromosome microdeletion test, sperm DNA fragmentation test and testicular biopsy if required are to be discussed.
8. In men with abnormal genitalia and/or azoospermia, advise further investigation to rule out genetic conditions such as cystic fibrosis (due to the congenital absence of the vas deferens). Screening of other genetic disorders such as Tay-sachs, sickle cell anemia should be done if required [4].
9. If a palpable varicocele is present, discuss the need for surgical repair along with operative techniques and postsurgical follow-up.
10. Inform the couple about ovulation induction methods and the consequences including multiple pregnancy and preterm labor [84].
11. Be aware that elderly infertile men usually have chronic medical diseases, which should be identified and treated because they can negatively affect fertility.
12. Discuss treatment cost/expenses. In most cases, health insurance does not reimburse for ART, which can range from a couple thousand dollars to $100,000 or more for IVF and ICSI cycles [84].
13. Discuss the success rate of each ART technique. Do not give false hope to couples who are untreatable or have a very poor prognosis.
14. Discuss adoption with couples who have not become pregnant after multiple treatments.

## Prescription of antioxidant therapy

There is a high correlation between ROS and alterations in sperm concentration, viability, motility, and morphology. Antioxidant therapy can possibly prevent or reverse these alternations (see Table 4).

Before prescribing any kind of antioxidants, however, it is important to promote lifestyle changes that can help improve fertility. Unhealthy activities that promote free radical generation, such as smoking, drug use, and excessive alcohol consumption, should be stopped [85]. In addition, exposure to various environmental pollutants and/or radiation should be avoided or reduced. Chemical gonadotoxins, including pesticides found in vegetables and industrial waste, can increase the formation of free radicals due to the unstable chemical compounds found in these products. In addition, radiation exposure, from cell phones and laptop computers, can produce oxidative stress by inducing cellular chemical changes through the electromagnetic waves emitted from the devices.

In some cases, extensive lifestyle and environmental changes may not lower levels of oxidative stress [86]. Patients with conditions that promote continual radical generation (e.g., varicocele or systemic infections) may be ripe candidates for antioxidant treatment. It may also be helpful for patients who may have idiopathic infertility and recurrent pregnancy loss.

Theoretically, antioxidant treatment increases the total antioxidant capacity (TAC) of body fluids including semen, which, in turn, helps control the damage that oxidative radicals create. Several compounds are commonly used for antioxidant therapy, and even more compounds are under vigorous research. These compounds are briefly described below.

Selenium and vitamin E are compounds that have been extensively studied because of their known antioxidant properties. Vitamin E is a lipid soluble molecule that improves cell and mitochondrial bi-layer membrane integrity by hindering oxidation of PUFAs. In a study performed by Moslemi et al. [87], vitamin E was used in combination with selenium as a treatment for idiopathic male infertility. The results showed that the therapy had significant beneficial effects on male fertility when taken for 100 days. The researchers noticed overall

**Table 4** Advantages of antioxidant therapy

| |
|---|
| Improvement in sperm motility and motion kinetics |
| Increase in sperm count in oligozoospermic patients |
| Decrease in abnormal spermatozoa |
| Reduction in PUFA in sperm membrane |
| Suppression of ROS production |
| Reduction in sperm DNA fragmentation |
| Improvement in sperm viability |
| Improvement in oocyte fertilization rate |
| Improvement in pregnancy rate (few studies) |

improvements in seminal parameters such as sperm motility, viability, and morphology in 52.4% of the participants. More importantly, the study indicated that 10.8% of the men who took supplementation of vitamin E were able to initiate a spontaneous pregnancy. The serious drawback in this study, however, was that it lacked a double blind and placebo controlled method. Keskes-Ammar et al. [88] did a similar study with randomization and control groups, and found overall improvements in sperm parameters.

Vitamin C is a water-soluble antioxidant. It is an effective scavenger of oxidative species molecules and helps control endogenous seminal radicals. According to Ross et al. [89], the sole use of vitamin C as a treatment for male infertility has not been satisfactorily explored, and thus its specific effects are unknown. However, several studies have examined the effect of vitamin C used in conjunction with other antioxidants, such as selenium, vitamin A, $ZnSO_4$ and vitamin E and reported improvements in sperm motility, sperm DNA fragmentation, fertilization capacity, and odds of normal sperm count [90–93]. There were several studies, however, that found no significant improvement in sperm parameters [94].

Carotenoids are a class of terpenoid molecules commonly found in red and orange-pigmented vegetables like carrots and tomatoes. Commonly discussed as powerful antioxidant agents, their link to preventing various cancers is well documented. However, carotenoids are under more discussion as a potential aid to reproductive health of males. Lycopene is perhaps the most well-known carotenoid, and its strength as an antioxidant is unmatched by any other carotenoid. Gupta and Kumar [95] reported that lycopene concentrations were lower in the testicles and seminal fluid of infertile men than in those from fertile men. In their study, treatment with 200 μg of lycopene twice a day for 3 months resulted in better sperm concentration and motility. Astaxanthin is another carotenoid commonly mentioned as an antioxidant. In a double-blind randomized control trial, Comhaire et al. [96] demonstrated that astaxanthin therapy increased pregnancy rates by approximately 10%. The observed improvements in pregnancy rate were attributed to the reduction of ROS resulting in enhancement of linear velocity and reduction of sperm DNA damage. However, the authors of the aforementioned studies admitted that larger randomized trials are still needed.

Finally, zinc is an important trace metal antioxidant that has been linked to increased fertilization parameters. Zinc is an important component of superoxide dismutase—a key antioxidant enzyme in the seminal fluid and testicles [97].

**Table 5** Cochrane review on the effect of oral supplementation with antioxidants taken by the male partner of a couple seeking fertility assistance (Adapted from Cochrane Database Syst Rev. 2011 [1]:CD007411 Showell MG, Brown J, Yazdani A, Stankiewicz MT, Hart RJ. Antioxidants for male subfertility)

| Semen parameter | No. of studies No. of patients | Duration of treatment | % Mean difference [95% CI] | Pooled cumulative grade of response | *P* value |
|---|---|---|---|---|---|
| Total motile sperm | 10 trials 514 patients ($T = 302$, $C = 212$) | 3 months or less | 11.72 [6.94–16.49] | Low | $P < 0.00001$ |
| | 7 trials 963 patients ($T = 625$, $C = 338$) | 6 months | 4.19 [3.81–4.56] | Very low | $P < 0.00001$ |
| | 3 trials 332 patients ($T = 181$, $C = 151$) | 9 months | 1.38 [0.81–1.95] | Very low | $P < 0.00001$ |
| Sperm count | 7 trials 320 patients ($T = 162$, $C$=158) | 3 months or less | 6.04 [−5.42–17.50] | No effect | $P = 0.30$ |
| | 6 trials 825 patients ($T = 536$, $C = 289$) | 6 months | 5.25 [4.43–6.08] | Very low | $P < 0.00001$ |
| | 3 trials 332 patients ($T = 181$, $C = 151$) | 9 months | 1.61 [0.61–2.61] | Very low | $P = 0.002$ |
| Sperm DNA fragmentation index | 1 trials 64 patients ($T = 32$, $C = 32$) | 2 months | −13.80 [−17.50 to −10.10] | Low | $P < 0.00001$ |
| Pregnancy rate | 15 trials 964 couples ($T = 515$, $C = 449$) | 4.5 months (follow-up: 3–24 months) | Odds ratio = 4.18 [2.65–6.59] | High | $P < 0.00001$ |
| Live birth per couple | 3 trials 214 couples ($T = 116$, $C = 98$) | 4 months (follow-up: 6–24 months) | Odds ratio = 3.94 [1.14–13.55] | Moderate | $P = 0.03$ |

*T* treated patients, *C* control group, *CI* confidence interval

Despite being similar in the serum, seminal zinc levels are lower in infertile men than in fertile men [98]. Lowered zinc levels in the seminal fluid is correlated with abnormal sperm morphology and reduced sperm motility [99]. Others have indicated that zinc sulfate (200 mg) taken orally twice daily for 3 months either alone or in combination with vitamin E and/or vitamin E were associated with comparable improvement in sperm motility and reduction of sperm oxidative stress markers [90]. Despite the beneficial properties of zinc supplementation, excessive zinc intake can result in damaged sperm DNA and therefore care should be taken exactly as prescribed [100].

A recent Cochrane meta-analysis on the use of oral antioxidants in male infertility found that these agents significantly improved pregnancy rates and live births and decreased sperm DNA damage [101]. An associated statistically significant increase in live birth rate [pooled odds ratio (OR) = 4.85; 95% CI 1.92–12.24; $P = 0.0008$; I (2) = 0%] has been obtained when men taking antioxidants were compared to controls. No studies reported harmful side effects from the antioxidant therapy used. Nevertheless, improvements in semen parameters were not well evident. The evidence suggests that antioxidant supplementation in subfertile males may improve the outcomes of live birth and pregnancy rate for subfertile couples undergoing fertility treatment. However, clinical trials are still necessary to identify the superiority of one antioxidant over the other in different subpopulations of infertile males, as well as other important aspects such as dose and duration of therapy (see Table 5).

## Conclusion

The diagnosis of infertility can have serious psychological consequences not only for the male but to his partner as well. Common patient intuition often leads a couple to the gynecologist who is usually the first to deal with infertility problems. Every gynecologist should consider the possibility of male factor infertility. Thus, it is imperative for them to understand diagnostic tests, be able to interpret the results, and provide referrals for timely urological evaluation and appropriate treatment options. This increased knowledge will help gynecologists better manage and treat infertile couples.

**Conflict of interest** None.

## References


1. WHO (ed) (1984) Report of the meeting on the prevention of infertility at the primary health care level, Geneva
2. Jarow JP, Sharlip ID, Belker AM, Lipshultz LI, Sigman M, Thomas AJ et al (2002) Best practice policies for male infertility. J Urol 167(5):2138–2144
3. Esteves SC, Miyaoka R, Agarwal A (2011) An update on the clinical assessment of the infertile male. Clinics (Sao Paulo) 66(4):691–700
4. Kamel RM (2010) Management of the infertile couple: an evidence-based protocol. Reprod Biol Endocrinol 8:21
5. Shindel AW, Nelson CJ, Naughton CK, Ohebshalom M, Mulhall JP (2008) Sexual function and quality of life in the male partner of infertile couples: prevalence and correlates of dysfunction. J Urol 179(3):1056–1059
6. Singh NP, Muller CH, Berger RE (2003) Effects of age on DNA double-strand breaks and apoptosis in human sperm. Fertil Steril 80(6):1420–1430
7. Sloter E, Nath J, Eskenazi B, Wyrobek AJ (2004) Effects of male age on the frequencies of germinal and heritable chromosomal abnormalities in humans and rodents. Fertil Steril 81(4):925–943
8. Thacker PD (2004) Biological clock ticks for men, too: genetic defects linked to sperm of older fathers. JAMA 291(14):1683–1685
9. Rolf C, Behre HM, Nieschlag E (1996) Reproductive parameters of older compared to younger men of infertile couples. Int J Androl 19(3):135–142
10. Sloter E, Schmid TE, Marchetti F, Eskenazi B, Nath J, Wyrobek AJ (2006) Quantitative effects of male age on sperm motion. Hum Reprod 21(11):2868–2875
11. Kuhnert B, Nieschlag E (2004) Reproductive functions of the ageing male. Hum Reprod Update 10(4):327–339
12. Wyrobek AJ, Aardema M, Eichenlaub-Ritter U, Ferguson L, Marchetti F (1996) Mechanisms and targets involved in maternal and paternal age effects on numerical aneuploidy. Environ Mol Mutagen 28(3):254–264
13. Schlegel PN, Chang TS, Marshall FF (1991) Antibiotics: potential hazards to male fertility. Fertil Steril 55(2):235–242
14. Nudell DM, Monoski MM, Lipshultz LI (2002) Common medications and drugs: how they affect male fertility. Urol Clin North Am 29(4):965–973
15. Corona G, Mannucci E, Schulman C, Petrone L, Mansani R, Cilotti A et al (2006) Psychobiologic correlates of the metabolic syndrome and associated sexual dysfunction. Eur Urol 50(3):595–604 (discussion)
16. Ramlau-Hansen CH, Thulstrup AM, Nohr EA, Bonde JP, Sorensen TI, Olsen J (2007) Subfecundity in overweight and obese couples. Hum Reprod 22(6):1634–1637
17. Jensen TK, Andersson AM, Jorgensen N, Andersen AG, Carlsen E, Petersen JH et al (2004) Body mass index in relation to semen quality and reproductive hormones among 1,558 Danish men. Fertil Steril 82(4):863–870
18. Martini AC, Tissera A, Estofan D, Molina RI, Mangeaud A, de Cuneo MF et al (2010) Overweight and seminal quality: a study of 794 patients. Fertil Steril 94(5):1739–1743
19. Hammoud AO, Wilde N, Gibson M, Parks A, Carrell DT, Meikle AW (2008) Male obesity and alteration in sperm parameters. Fertil Steril 90(6):2222–2225
20. Smit M, Romijn JC, Wildhagen MF, Weber RF, Dohle GR (2010) Sperm chromatin structure is associated with the quality of spermatogenesis in infertile patients. Fertil Steril 94(5):1748–1752
21. Agarwal A, Deepinder F, Sharma RK, Ranga G, Li J (2008) Effect of cell phone usage on semen analysis in men attending infertility clinic: an observational study. Fertil Steril 89(1):124–128
22. Esteves SC, Hamada A, Agarwal A (2012) Evaluation and diagnosis of male infertility. In: Dubey A (ed) infertility:

management and treatment, Jaypee Medical Publisher, New Delhi, pp 2–30
23. Cooper TG, Noonan E, von Eckardstein S, Auger J, Baker HW, Behre HM et al (2010) World Health Organization reference values for human semen characteristics. Hum Reprod Update 16(3):231–245
24. Esteves SC, Zini A, Aziz N, Alvarez JG, Sabanegh ES Jr, Agarwal A (2012) Critical appraisal of World Health Organization's new reference values for human semen characteristics and effect on diagnosis and treatment of subfertile men. Urology 79(1):16–22
25. Moghissi KS, Wallach EE (1983) Unexplained infertility. Fertil Steril 39(1):5–21
26. Foresta C, Moro E, Ferlin A (2001) Y chromosome microdeletions and alterations of spermatogenesis. Endocr Rev 22(2): 226–239
27. Schiff JD, Palermo GD, Veeck LL, Goldstein M, Rosenwaks Z, Schlegel PN (2005) Success of testicular sperm extraction [corrected] and intracytoplasmic sperm injection in men with Klinefelter syndrome. J Clin Endocrinol Metab 90(11):6263–6267
28. Sciurano RB, Luna Hisano CV, Rahn MI, Brugo Olmedo S, Rey Valzacchi G, Coco R et al (2009) Focal spermatogenesis originates in euploid germ cells in classical Klinefelter patients. Hum Reprod 24(9):2353–2360
29. Jequier AM, Ansell ID, Bullimore NJ (1985) Congenital absence of the vasa deferentia presenting with infertility. J Androl 6(1):15–19
30. Tiepolo L, Zuffardi O (1976) Localization of factors controlling spermatogenesis in the nonfluorescent portion of the human Y chromosome long arm. Hum Genet 34(2):119–124
31. Pryor JL, Kent-First M, Muallem A, Van Bergen AH, Nolten WE, Meisner L et al (1997) Microdeletions in the Y chromosome of infertile men. N Engl J Med 336(8):534–539
32. Viswambharan N, Suganthi R, Simon AM, Manonayaki S (2007) Male infertility: polymerase chain reaction-based deletion mapping of genes on the human chromosome. Singap Med J 48(12):1140–1142
33. Stahl PJ, Masson P, Mielnik A, Marean MB, Schlegel PN, Paduch DA (2010) A decade of experience emphasizes that testing for Y microdeletions is essential in American men with azoospermia and severe oligozoospermia. Fertil Steril 94(5):1753–1756
34. Choi JM, Chung P, Veeck L, Mielnik A, Palermo GD, Schlegel PN (2004) AZF microdeletions of the Y chromosome and in vitro fertilization outcome. Fertil Steril 81(2):337–341
35. Hopps CV, Mielnik A, Goldstein M, Palermo GD, Rosenwaks Z, Schlegel PN (2003) Detection of sperm in men with Y chromosome microdeletions of the AZFa, AZFb and AZFc regions. Hum Reprod 18(8):1660–1665
36. Steeno O, Knops J, Declerck L, Adimoelja A, van de Voorde H (1976) Prevention of fertility disorders by detection and treatment of varicocele at school and college age. Andrologia 8(1):47–53
37. Dubin L, Amelar RD (1971) Etiologic factors in 1294 consecutive cases of male infertility. Fertil Steril 22(8):469–474
38. Greenberg SH (1977) Varicocele and male fertility. Fertil Steril 28(7):699–706
39. Gorelick JI, Goldstein M (1993) Loss of fertility in men with varicocele. Fertil Steril 59(3):613–616
40. Jung A, Schuppe HC (2007) Influence of genital heat stress on semen quality in humans. Andrologia 39(6):203–215
41. Twigg J, Fulton N, Gomez E, Irvine DS, Aitken RJ (1998) Analysis of the impact of intracellular reactive oxygen species generation on the structural and functional integrity of human spermatozoa: lipid peroxidation, DNA fragmentation and effectiveness of antioxidants. Hum Reprod 13(6):1429–1436
42. Saleh RA, Agarwal A, Sharma RK, Said TM, Sikka SC, Thomas AJ Jr (2003) Evaluation of nuclear DNA damage in spermatozoa from infertile men with varicocele. Fertil Steril 80(6):1431–1436
43. Nevoux P, Mitchell V, Chevallier D, Rigot JM, Marcelli F (2011) Varicocele repair: does it still have a role in infertility treatment? Curr Opin Obstet Gynecol 23(3):151–157
44. Miyaoka R, Esteves SC (2012) A critical appraisal on the role of varicocele in male infertility. Adv Urol 2012:597495
45. Marmar JL, Agarwal A, Prabakaran S, Agarwal R, Short RA, Benoff S et al (2007) Reassessing the value of varicocelectomy as a treatment for male subfertility with a new meta-analysis. Fertil Steril 88(3):639–648
46. Meng MV, Greene KL, Turek PJ (2005) Surgery or assisted reproduction? A decision analysis of treatment costs in male infertility. J Urol 174(5):1926–1931 (discussion 31)
47. Esteves SC, Glina S (2005) Recovery of spermatogenesis after microsurgical subinguinal varicocele repair in azoospermic men based on testicular histology. Int Braz J Urol 31(6):541–548
48. Inci K, Hascicek M, Kara O, Dikmen AV, Gurgan T, Ergen A (2009) Sperm retrieval and intracytoplasmic sperm injection in men with nonobstructive azoospermia, and treated and untreated varicocele. J Urol 182(4):1500–1505
49. Cayan S, Erdemir F, Ozbey I, Turek PJ, Kadioglu A, Tellaloglu S (2002) Can varicocelectomy significantly change the way couples use assisted reproductive technologies? J Urol 167(4): 1749–1752
50. Esteves SC, Oliveira FV, Bertolla RP (2010) Clinical outcome of intracytoplasmic sperm injection in infertile men with treated and untreated clinical varicocele. J Urol 184(4):1442–1446
51. Report on varicocele and infertility. Fertil Steril 2008 90 (5 Suppl):S247–9
52. EAU (2010) European Association of Urology. Guidelines on male infertility. Available from: http://www.uroweb.org/gls/pdf/Male%20Infertility%202010.pdf. (updated 2010; cited: February 27th, 2011)
53. Sociedade Brasileira de Urologia & Colégio Brasileiro de Radiologia (2011) Projeto Diretrizes da Associação Médica Brasileira: Varicocele. Available from: http://www.projetodiretrizes.org.br/8_volume/40-Varicocele.pdf. (updated 2011; cited 2011 February 27th)
54. Esteves SC, Miyaoka R, Agarwal A (2011) Surgical treatment of male infertility in the era of intracytoplasmic sperm injection—new insights. Clinics (Sao Paulo) 66(8):1463–1478
55. Pisipati S, Pearcy R (2011) The role of urological surgery in male infertility. Hum Fertil (Camb) 13(4):233–241
56. Pavlovich CP, Schlegel PN (1997) Fertility options after vasectomy: a cost-effectiveness analysis. Fertil Steril 67(1):133–141
57. Tournaye H (2010) Update on surgical sperm recovery—the European view. Hum Fertil (Camb) 13(4):242–246
58. Esteves SC, Agarwal A (2011) Novel concepts in male infertility. Int Braz J Urol 37(1):5–15
59. Verza S Jr, Esteves SC (2011) Microsurgical versus conventional single-biopsy testicular sperm extraction in nonobstructive azoospermia: a prospective controlled study. Fertil Steril 96(S3):53
60. Esteves SC, Agarwal A (2011) Sperm retrieval techniques. In: Gardner DKRB, Falcone T (eds) Human assisted reproductive technology: future trends in laboratory and clinical practice. Cambridge University Press, Cambridge, pp 41–53
61. Prudencio C, Seol B, Esteves SC (2010) Reproductive potential of azoospermic men undergoing intracytoplasmic sperm injection is dependent on the type of azoospermia. Fertil Steril 94(S):232–233
62. Verza S Jr, Esteves SC (2008) Sperm defect severity rather than sperm source is associated with lower fertilization rates after intracytoplasmic sperm injection. Int Braz J Urol 34(1):49–56

63. Lewis SE, Boyle PM, McKinney KA, Young IS, Thompson W (1995) Total antioxidant capacity of seminal plasma is different in fertile and infertile men. Fertil Steril 64(4):868–870
64. Sharma RK, Agarwal A (1996) Role of reactive oxygen species in male infertility. Urology 48(6):835–850
65. Aitken RJ (1988) Assessment of sperm function for IVF. Hum Reprod 3(1):89–95
66. Rao B, Soufir JC, Martin M, David G (1989) Lipid peroxidation in human spermatozoa as related to midpiece abnormalities and motility. Gamete Res 24(2):127–134
67. Aitken RJ, Clarkson JS (1987) Cellular basis of defective sperm function and its association with the genesis of reactive oxygen species by human spermatozoa. J Reprod Fertil 81(2):459–469
68. Makker K, Agarwal A, Sharma R (2009) Oxidative stress & male infertility. Indian J Med Res 129(4):357–367
69. Saalu LC (2010) The incriminating role of reactive oxygen species in idiopathic male infertility: an evidence based evaluation. Pak J Biol Sci 13(9):413–422
70. Zini A, San Gabriel M, Baazeem A (2009) Antioxidants and sperm DNA damage: a clinical perspective. J Assist Reprod Genet 26(8):427–432
71. Aitken RJ, De Iuliis GN, McLachlan RI (2009) Biological and clinical significance of DNA damage in the male germ line. Int J Androl 32(1):46–56
72. Kodama H, Yamaguchi R, Fukuda J, Kasai H, Tanaka T (1997) Increased oxidative deoxyribonucleic acid damage in the spermatozoa of infertile male patients. Fertil Steril 68(3):519–524
73. Spano M, Bonde JP, Hjollund HI, Kolstad HA, Cordelli E, Leter G (2000) Sperm chromatin damage impairs human fertility. The Danish first pregnancy planner study team. Fertil Steril 73(1):43–50
74. Zini A, Bielecki R, Phang D, Zenzes MT (2001) Correlations between two markers of sperm DNA integrity, DNA denaturation and DNA fragmentation, in fertile and infertile men. Fertil Steril 75(4):674–677
75. Cooke MS, Evans MD, Dizdaroglu M, Lunec J (2003) Oxidative DNA damage: mechanisms, mutation, and disease. FASEB J 17(10):1195–1214
76. Aitken RJ, De Iuliis GN (2007) Value of DNA integrity assays for fertility evaluation. Soc Reprod Fertil Suppl 65:81–92
77. Lewis SE, Simon L (2010) Clinical implications of sperm DNA damage. Hum Fertil (Camb) 13(4):201–207
78. Zhang LH, Qiu Y, Wang KH, Wang Q, Tao G, Wang LG (2010) Measurement of sperm DNA fragmentation using bright-field microscopy: comparison between sperm chromatin dispersion test and terminal uridine nick-end labeling assay. Fertil Steril 94(3):1027–1032
79. Agarwal A, Deepinder F, Cocuzza M, Short RA, Evenson DP (2008) Effect of vaginal lubricants on sperm motility and chromatin integrity: a prospective comparative study. Fertil Steril 89(2):375–379
80. Tulandi T, Plouffe L Jr, McInnes RA (1982) Effect of saliva on sperm motility and activity. Fertil Steril 38(6):721–723
81. Kutteh WH, Chao CH, Ritter JO, Byrd W (1996) Vaginal lubricants for the infertile couple: effect on sperm activity. Int J Fertil Menopausal Stud 41(4):400–404
82. Anderson K, Nisenblat V, Norman R (2010) Lifestyle factors in people seeking infertility treatment—a review. Aust N Z J Obstet Gynaecol 50(1):8–20
83. Du Plessis SS, Cabler S, McAlister DA, Sabanegh E, Agarwal A (2010) The effect of obesity on sperm disorders and male infertility. Nat Rev Urol 7(3):153–161
84. Brown HL (2009) Preconceptional considerations and counseling for the infertile couple. N C Med J 70(5):463–465
85. Agarwal A, Sekhon LH (2010) The role of antioxidant therapy in the treatment of male infertility. Hum Fertil (Camb) 13(4):217–225
86. Agarwal A, Desai NR, Ruffoli R, Carpi A (2008) Lifestyle and testicular dysfunction: a brief update. Biomed Pharmacother 62(8):550–553
87. Moslemi MK, Tavanbakhsh S (2011) Selenium–vitamin E supplementation in infertile men: effects on semen parameters and pregnancy rate. Int J Gen Med 4:99–104
88. Keskes-Ammar L, Feki-Chakroun N, Rebai T, Sahnoun Z, Ghozzi H, Hammami S et al (2003) Sperm oxidative stress and the effect of an oral vitamin E and selenium supplement on semen quality in infertile men. Arch Androl 49(2):83–94
89. Ross C, Morriss A, Khairy M, Khalaf Y, Braude P, Coomarasamy A et al (2010) A systematic review of the effect of oral antioxidants on male infertility. Reprod Biomed Online 20(6):711–723
90. Omu AE, Al-Azemi MK, Kehinde EO, Anim JT, Oriowo MA, Mathew TC (2008) Indications of the mechanisms involved in improved sperm parameters by zinc therapy. Med Princ Pract 17(2):108–116
91. Scott R, MacPherson A, Yates RW, Hussain B, Dixon J (1998) The effect of oral selenium supplementation on human sperm motility. Br J Urol 82(1):76–80
92. Paradiso Galatioto G, Gravina GL, Angelozzi G, Sacchetti A, Innominato PF, Pace G et al (2008) May antioxidant therapy improve sperm parameters of men with persistent oligospermia after retrograde embolization for varicocele? World J Urol 26(1):97–102
93. Greco E, Iacobelli M, Rienzi L, Ubaldi F, Ferrero S, Tesarik J (2005) Reduction of the incidence of sperm DNA fragmentation by oral antioxidant treatment. J Androl 26(3):349–353
94. Rolf C, Cooper TG, Yeung CH, Nieschlag E (1999) Antioxidant treatment of patients with asthenozoospermia or moderate oligoasthenozoospermia with high-dose vitamin C and vitamin E: a randomized, placebo-controlled, double-blind study. Hum Reprod 14(4):1028–1033
95. Gupta NP, Kumar R (2002) Lycopene therapy in idiopathic male infertility—a preliminary report. Int Urol Nephrol 34(3):369–372
96. Comhaire FH, El Garem Y, Mahmoud A, Eertmans F, Schoonjans F (2005) Combined conventional/antioxidant "Astaxanthin" treatment for male infertility: a double blind, randomized trial. Asian J Androl 7(3):257–262
97. Patel SR, Sigman M (2008) Antioxidant therapy in male infertility. Urol Clin North Am 35(2):319–330 x
98. Chia SE, Ong CN, Chua LH, Ho LM, Tay SK (2000) Comparison of zinc concentrations in blood and seminal plasma and the various sperm parameters between fertile and infertile men. J Androl 21(1):53–57
99. Fuse H, Kazama T, Ohta S, Fujiuchi Y (1999) Relationship between zinc concentrations in seminal plasma and various sperm parameters. Int Urol Nephrol 31(3):401–408
100. Lloyd DR, Carmichael PL, Phillips DH (1998) Comparison of the formation of 8-hydroxy-2'-deoxyguanosine and single- and double-strand breaks in DNA mediated by fenton reactions. Chem Res Toxicol 11(5):420–427
101. Showell MG, Brown J, Yazdani A, Stankiewicz MT, Hart RJ (2011) Antioxidants for male subfertility. Cochrane Database Syst Rev (1):CD007411