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Male infertility: Advances in diagnosis, therapeutic strategies and assisted reproductive technologies

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Abstract
Male infertility is a complex, global issue caused by genetic, hormonal, structural, and environmental factors. Because standard semen analysis often misses subcellular deficits, advanced diagnostics like sperm DNA fragmentation assays and super-resolution Ultrasound Localisation Microscopy are essential for identifying chromatin damage and microvascular changes. Management requires a stratified strategy using targeted pharmacotherapies (SERMs, aromatase inhibitors, gonadotropins) to restore HPG axis balance, alongside surgical interventions (microsurgical varicocelectomy, micro-TESE) for physical defects and non-obstructive azoospermia. When natural conception fails, Assisted Reproductive Technologies—especially Intracytoplasmic Sperm Injection—offer reliable fertilization. Ultimately, combining advanced diagnostics, tailored medical and surgical care, lifestyle changes, and ART optimizes treatment and improves reproductive success.

Keywords: Male infertility, sperm DNA fragmentation, aromatase inhibitors, varicocelectomy, micro-Tese, ICSI

Introduction

According to the World Health Organization (WHO), infertility is defined as the inability of a couple to conceive after one year of regular, unprotected sexual intercourse. It affects approximately 15% of couples worldwide. Among infertile couples, nearly 50% of cases are attributed solely to female-related factors, around 20% are associated exclusively with male factors, while the remaining 30% result from a combination of both male and female factors [1]. Infertility falls into two types: primary and secondary. According to the WHO, it has emerged as a worldwide health issue that impacts millions of men and women of childbearing age, with roughly 15% of couples experiencing this problem [2]. Primary male infertility describes men who have never been able to father a pregnancy with their partner, even though they have had regular unprotected intercourse. Secondary male infertility describes men who have fathered at least one pregnancy before but are now unable to do so [3]. Male infertility is defined as a man's inability to cause a pregnancy in a female partner after 12 months of regular unprotected intercourse. It is a multifactorial condition with many possible causes, which may be congenital or acquired. These include congenital and genetic abnormalities, hormonal (endocrine) disorders, blockages in the reproductive tract, ejaculation problems, exposure to substances that damage the testes, chronic illnesses, lifestyle habits, advancing age, environmental toxins, and impaired sperm production, among other factors [4]. Male infertility can result from a wide range of pathophysiological factors. These include varicocele-associated testicular overheating and oxidative stress, abnormalities of the urogenital system, genetic disorders such as chromosomal aneuploidies and Y-chromosome microdeletions, hormonal or endocrine disorders, cryptorchidism leading to impaired sperm production, obstructive azoospermia, exposure to environmental factors, unexplained (idiopathic) causes, and lifestyle-related risk factors [5]. The causes of male infertility can broadly be categorized into coital infertility and azoospermia. Coital infertility may arise from structural abnormalities, such as penile deformities, as well as erectile dysfunction, premature ejaculation, retrograde ejaculation, and absence of ejaculation (anejaculation). Azoospermia, on the other hand, is broadly classified into two types: obstructive and non-obstructive [6].

Epidemiology

Worldwide, male infertility-related DALYs grew by 17.79% (EAPC 0.51%), while its prevalence went up by 16.90% (EAPC 0.50%). The burden was heavier in South Asia, where DALYs climbed by 45.66% (EAPC 1.40%) and prevalence by 47.19% (EAPC 1.50%). India recorded the largest increase, with DALYs and prevalence growing by 55.87% and 58.82%, respectively. Men aged 25-29 years were the most affected group [7].

Etiology

- **Spermatogenesis**

Spermatogenesis is a complex and continuously regulated process that depends on various factors, particularly genetic and hormonal mechanisms. Therefore, any disruption or dysfunction in these regulatory factors can interfere with sperm production and may lead to male infertility [8]. Spermatogenesis depends on maintaining appropriate levels of several hormones, with testosterone being the primary hormone involved. The production and regulation of these hormones occur through interactions within the hypothalamic–pituitary–gonadal (HPG) axis, which includes gonadotropin-releasing hormone (GnRH) released by the hypothalamus, follicle-stimulating hormone (FSH) and luteinizing hormone (LH) secreted by the anterior pituitary gland, and sex hormones produced by the gonads [9].

- **Recombination deficiencies during spermatogenesis**

Crossing-over, which produces recombination during meiosis, can introduce errors through nuclease-generated DNA breaks. Because sperm chromatin is so tightly compacted, it has a strong tendency toward DNA-DNA and DNA-protein cross-linking, which makes further condensation difficult. Examples include oestrogen found covalently bound to DNA in human spermatozoa, as well as highly cross-linked chromatin in sperm [10].

- **Abnormal Spermatid Maturation**

Histone hyper-acetylation and DNA breaks are both important for chromatin packaging, and Topoisomerase II both creates and ligates these breaks. To relieve torsional stress, which helps protamines replace histones, the DNA undergoes nicking [10]. Chromatin packaging and DNA repair must be completed before sperm are transferred to the epididymis. However, DNA fragmentation can occur when temporary breaks are not fully sealed, and the presence of unsealed DNA nicks in spermatozoa indicates defective chromatin remodelling during spermatogenesis [11].

- **Protamine I and II Ratio**

This replacement of histones with protamines takes place in the late phase of spermatogenesis and affects 85-95% of histone proteins. It proceeds in a stepwise sequence: hyperacetylation first, then replacement by transition proteins, and finally substitution of the remaining histones with protamines 1 and 2 (P1 and P2). For accurate chromatin packaging and proper gene expression, P1 and P2 need to be present in a 1:1 ratio [10]. Changes in the protamines that replace histones can also lead to infertility. A P1/P2 ratio that deviates from the normal value, either higher or lower, has been observed in men with high levels of sperm DNA fragmentation (SDF) [12].

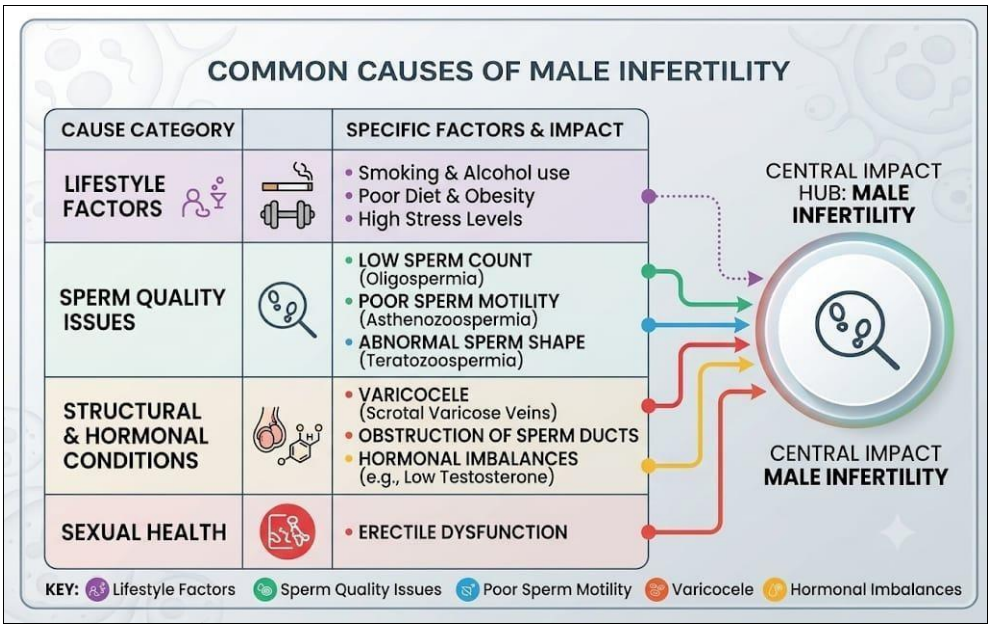
- **Cryptorchidism**

Cryptorchidism, or undescended testis, is associated with reduced sperm concentration and impaired sperm quality. Men with this condition may have a lower sperm count and decreased sperm quality.

Germ cell loss: Undescended testes are exposed to higher-than-normal temperatures, which can cause progressive loss of germ cells and may eventually result in infertility.

Sertoli and Leydig cell dysfunction: Cryptorchidism may impair testosterone production and cause disturbances in the normal hormonal environment required for spermatogenesis.

Increased risk of malignancy: Men with cryptorchidism have approximately a 4–6-fold higher risk of developing testicular cancer, and this increased risk may remain even after surgical correction by orchiopexy [13].



- **Oxidative Stress**

Male subfertility can be associated with oxidative stress (OS) caused by excessive levels of reactive oxygen species (ROS). Normally, ROS production in semen is regulated by naturally occurring antioxidant systems. Oxidative stress occurs when there is an imbalance between ROS generation and antioxidant defenses. Both experimental and clinical evidence suggests that excessive ROS can contribute to male infertility and impaired sperm function.

At controlled, low concentrations, ROS play an important role in sperm–zona pellucida interaction, while superoxide anion radicals can promote capacitation and the acrosome reaction. However, increased oxidative stress and reduced antioxidant capacity of spermatozoa are associated with elevated ROS levels and may adversely affect sperm function [14].

- **Varicocele**

Varicocele is a common condition, occurring in approximately 15% of the general male population and in about 40% of men experiencing infertility. Men with varicocele may exhibit abnormalities in sperm DNA and immature chromatin, which can contribute to impaired fertility. These abnormalities have been observed when men with varicocele were compared with fertile men having normal semen parameters (normozoospermia) [15]. Scrotal hyperthermia, or increased heat exposure to the testes, may be one of the factors responsible for reduced semen quality and quantity, as well as increased DNA fragmentation index

(DFI), in men with varicocele. This elevated temperature can promote oxidative stress, which may negatively affect sperm function [16].

- **Genital Tract Infections**

Reproductive tract infection is a common cause of male infertility. Organisms such as Ureaplasma urealyticum, Chlamydia trachomatis, Mycoplasma, Staphylococcus aureus, and Pseudomonas aeruginosa can cause urogenital tract infections that may raise SDF by disrupting the structure of sperm DNA and chromatin [17]. These infections can also alter the protamine ratio in men, as the P1 and P2 ratio has been found to be abnormal in affected males who also have high SDF levels [18].

- **Age**

Advancing age is considered an important factor that can negatively influence semen parameters. As men age, increased mitochondrial oxidative stress can lead to higher production of reactive oxygen species (ROS) and increased lipid peroxidation, which may contribute to age-related deterioration in sperm quality [17]. The relationship between age and sperm DNA fragmentation (SDF) levels remains uncertain because studies have reported conflicting findings. However, one study involving normozoospermic men found that older men aged 40 years or above had greater sperm DNA damage, with a DNA fragmentation index (DFI) exceeding 30%, compared with men younger than 40 years [19].

- **Obesity and Metabolic Syndrome**

Several mechanisms may contribute to male reproductive dysfunction in obesity. Increased aromatase activity can elevate oestradiol levels, which may suppress the hypothalamic–pituitary–gonadal axis and disrupt hormonal regulation. In addition, oxidative stress and excessive reactive oxygen species (ROS) can cause damage to sperm membranes and DNA. Increased scrotal fat may also contribute to scrotal hyperthermia, which can interfere with the temperature-sensitive process of spermatogenesis. Furthermore, vascular and neurogenic changes associated with obesity may contribute to erectile dysfunction. Studies have shown that weight reduction can improve semen quality and restore hormonal balance in obese men [20].

- **Tobacco and substance use**

Cigarette smoking is linked to lower sperm counts and reduced sperm motility, and the effect is stronger in moderate to heavy smokers. Smoking also harms sperm DNA integrity and thereby increases sperm DNA fragmentation, though these effects may be reversible once a person quits. Abuse of anabolic-androgenic steroids is a severe but preventable cause of hypogonadism in men. Misuse of anabolic steroids, sometimes associated with bodybuilding, can cause severe hypogonadotropic hypogonadism and markedly impair spermatogenesis [21].

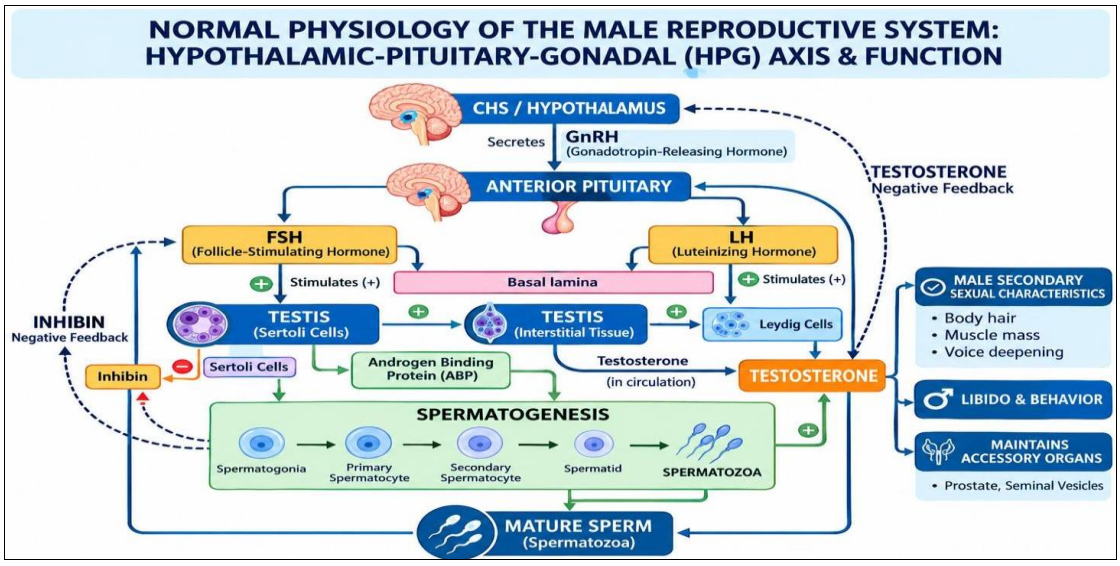
- **Persistent organic pollutants (POPs)**

Environmental contaminants such as organochlorine pesticides, including DDT, polychlorinated biphenyls (PCBs), and dioxins, can accumulate in reproductive tissues. Their presence may interfere with normal sperm production and contribute to inflammatory processes and oxidative stress, potentially impairing male reproductive function [22]. Heavy metals such as arsenic (As), lead (Pb), cadmium (Cd), and mercury (Hg) can contribute to male reproductive dysfunction by inducing oxidative stress and disturbing hormonal regulation through the inhibition of steroidogenic enzymes. They may also alter the protamine-to-histone ratio, cause epigenetic changes, and interfere with normal spermatogenesis. These effects can result in decreased sperm count and motility, abnormal sperm morphology, and increased sperm DNA fragmentation [23].

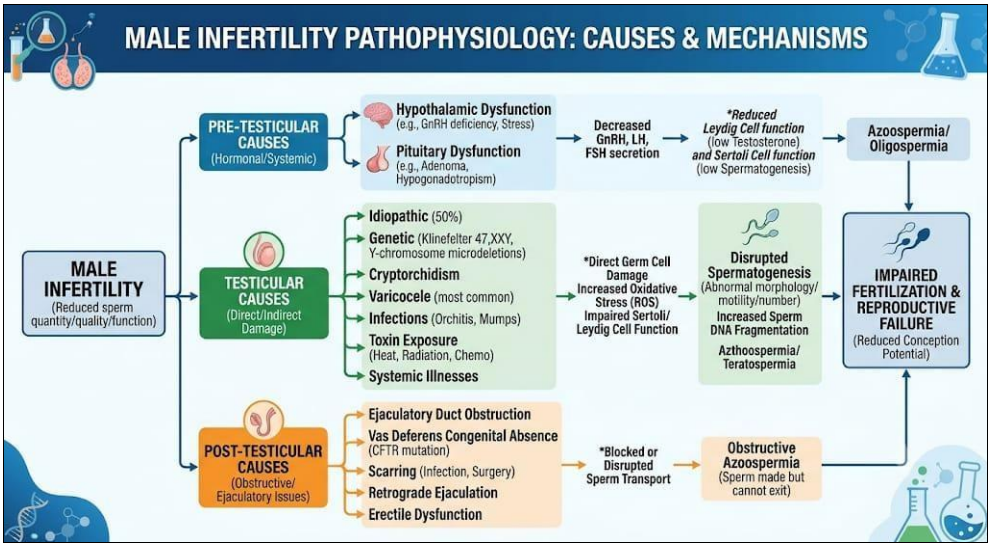
- **Other Factors**

Heat stress, the cytotoxic effects of radiotherapy and chemotherapy, the position of the testis, and antidepressant drugs can all lead to abnormal SDF. Various environmental and chemical factors also affect sperm DNA fragmentation. Tobacco use, for example, leads to the production of H2O2 through the Fenton process mentioned earlier, resulting in DNA damage. Similarly, cadmium and lead, which are present in cigarette smoke, can cause DNA strand breaks. These metals have also been detected in seminal fluid and serve as indicators of oxidative stress. Tobacco also contains nicotine, which has been shown to induce double-stranded DNA breaks in sperm *in vitro*, and its main primary metabolite, cotinine, has been detected in the seminal vesicles of men who smoke [24].

Normal Physiology of Male Reproductive System

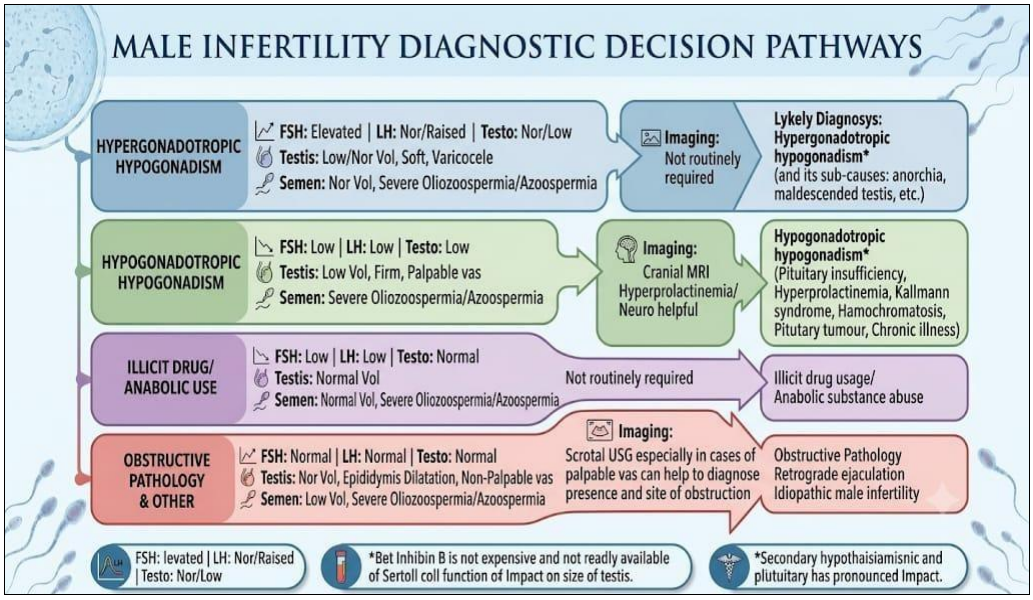


Pathophysiology for Male Infertility



Diagnosis

A thorough evaluation using a multidimensional approach is essential for diagnosing and effectively managing male infertility because of its complex and multifactorial nature. Standard semen analysis assesses sperm concentration and count, motility, morphology, semen volume, and pH. Further evaluation of male infertility includes hormonal investigations, genetic testing, assessment of anti-sperm antibodies, analysis of sperm nuclear DNA, transrectal ultrasonography, and other relevant imaging techniques.[25] Years of fertility research have led to the development of a wide range of treatment options, including pharmacological therapy, surgical interventions, and assisted reproductive technologies (ART) such as intracytoplasmic sperm injection (ICSI), *in vitro* fertilization (IVF), and intrauterine insemination (IUI). These are among the most commonly used ART methods for clinically addressing fertility problems [26].



Since the introduction of intracytoplasmic sperm injection (ICSI) in the early 1990s, men with severe oligozoospermia or azoospermia have had the opportunity to achieve fatherhood through the direct injection of a single sperm into the cytoplasm of an oocyte [27].

Certain causes of male infertility can be detected and successfully treated or improved through appropriate medications or surgical procedures, whereas some causes can be identified but cannot be reversed. The main objectives of evaluating male infertility are to

1. Identify and treat reversible causes of male infertility, with the aim of enabling natural conception through sexual intercourse or using the least advanced reproductive technology possible;
2. Recognize irreversible conditions that may be managed with assisted reproductive technologies (ART) using sperm obtained from the male partner;
3. Identify irreversible conditions in which the male partner's sperm cannot be obtained, allowing the couple to consider alternatives such as donor sperm or adoption;
4. Detect underlying medical conditions that may contribute to infertility and require appropriate treatment; and

5. Determine specific genetic causes of infertility that could potentially be passed on to the offspring.

In certain cases, a testicular biopsy can serve both diagnostic and therapeutic purposes. If mature spermatozoa are identified in the biopsy tissue, the recovered sperm may be cryopreserved for future use in assisted reproductive procedures. When non-obstructive azoospermia (NOA) is suspected or the clinical findings are inconclusive, a diagnostic testicular biopsy or fine-needle aspiration (FNA) may be performed to assess the presence of mature sperm and determine the likelihood of successful sperm retrieval from the testes. Various histopathological patterns may be observed, and each pattern is associated with a different probability of obtaining viable spermatozoa [28].

Sperm DNA fragmentation

Conventional semen analysis is widely regarded as a key diagnostic method for male infertility worldwide. However, the WHO parameters cannot fully explain infertility, since their reference values only account for poor semen quality. The usefulness of conventional [17] semen analysis remains limited in infertile men, as about 40% of them have semen parameters that fall within the WHO normal reference range. Therefore, conventional semen analysis alone cannot assess the many molecular and subcellular factors associated with male infertility. Sperm DNA fragmentation has been linked to male infertility and reproductive failure [29]. Because oxidative stress plays a role in male infertility, tests that directly measure SDF (e.g., TUNEL, SCSA, SCD, and Comet) may be preferable to tests that assess chromatin compaction [30]. Each assay may have its own clinical threshold, since they detect DNA damage at different sites and differ in their technical aspects [31].

Sperm Chromatin Structure Assay (SCSA)

This assay relies on acid denaturation of DNA at sites where single- or double-strand breaks already exist. Acridine orange (AO) is the stain used: it enters the sperm chromatin and intercalates into double-stranded (intact) DNA, which emits green fluorescence under blue laser light. When AO binds to single-stranded DNA, however, it forms a complex that shifts the emission (metachromatic shift) to red fluorescence. This red signal therefore marks DNA strands arising from single- or double-strand break sites. A flow cytometer records the fluorescence patterns of the spermatozoa, and the proportion of red fluorescence relative to total fluorescence (green + red) is used to work out the percentage of sperm with fragmented DNA, known as the DNA fragmentation index (DFI).

Terminal deoxynucleotidyl transferase mediated DUTP biotin nick end labelling (TUNEL)

This assay depends on the enzyme terminal deoxynucleotidyl transferase (TdT) to directly label the free 3′ ends of DNA. Break sites can then be detected using fluorescence microscopy or flow cytometry. The TUNEL test essentially assesses the linker DNA between protamine toroids, because the relatively large TdT molecule may not be able to penetrate the highly compacted toroids themselves. To improve its sensitivity, modified protocols have been introduced, including DNA decompaction with dithiothreitol (DTT).

Sperm Chromatin Dispersion (SCD)

The assay is based on the principle that spermatozoa with fragmented DNA do not form the characteristic halo of dispersed DNA loops that is seen in sperm cells with intact DNA after acid-induced denaturation and removal of nuclear proteins. In this method, sperm suspensions are embedded in agarose gel on microscope slides and subsequently treated with an acidic denaturation solution, such as hydrochloric acid (HCl), to produce restricted DNA structures. The process generates single-stranded DNA regions at the sites of pre-existing single- or double-strand DNA breaks. The denaturation step is then terminated, and the spermatozoa are treated with a lysis solution containing dithiothreitol (DTT), sodium dodecyl sulfate (SDS), and sodium chloride (NaCl) to remove the sperm membrane and nuclear proteins. The slides are subsequently stained using DAPI (4′,6-diamidino-2-phenylindole) or Diff-Quik. Spermatozoa showing either dispersed or non-dispersed chromatin loops are then identified using fluorescence or bright-field microscopy, respectively, allowing the percentage of sperm cells with DNA fragmentation to be determined. The presence of halos represents relaxed DNA loops attached to the remaining nuclear structure, which is typically observed in spermatozoa with low or absent sperm DNA fragmentation (SDF). In contrast, spermatozoa showing very small halos or no halos are associated with SDF. This finding can be confirmed using DNA breakage detection–fluorescence in situ hybridization (DBD-FISH), a technique that detects and quantifies the restricted single-stranded DNA sequences produced at sites of DNA breaks.

Single-cell gel electrophoresis assay (Comet)

This assay relies on DNA decompaction and protein removal, followed by single-cell electrophoresis in an agarose microgel. Stripping away protamines and histones leaves a nucleoid-like structure made up of supercoiled DNA loops. Under alkaline or neutral pH conditions, the double-stranded DNA unwinds, and during electrophoresis the single- and double-stranded DNA fragments move toward the anode, forming a comet tail that can be viewed under fluorescence microscopy. The amount of fluorescence in the tail relative to the head reflects the level of SDF, so sperm with brighter comet tails have greater chromatin damage. Additional quantitative parameters, such as nucleus diameter, olive tail moment, and comet length, can be used to improve the precision of the test. The alkaline Comet assay detects global SDF, meaning both single- and double-stranded DNA breaks as well as alkali-labile sites, whereas the neutral Comet assay detects only double-stranded DNA breaks. Alternatively, the two-tailed Comet assay can be used to identify and distinguish the type of DNA break within the same spermatozoon. It first applies neutral lysis and electrophoresis to detect double-strand breaks, then the slide is rotated 90º and subjected to alkaline lysis and electrophoresis to detect single-strand breaks. The Comet assay yields three parameters: ACS: the average SDF across 100 individual spermatozoa HCS: the proportion of spermatozoa with high DNA damage LCS: the proportion of spermatozoa [32].

Treatment

Testosterone Replacement Therapy

Testosterone replacement therapy (TRT) has become increasingly popular as more clinical evidence shows its

benefits for men with hypogonadism. Low testosterone levels can compromise cardiovascular health, metabolism, sexual function, bone and muscle mass, and mental well-being, whereas TRT has been found to improve these areas [33]. Experts who advise against testosterone replacement therapy for idiopathic male infertility are chiefly worried that supplemental androgen triggers negative feedback on the hypothalamic-pituitary-gonadal axis.1 This suppresses serum gonadotropin levels and intratesticular testosterone, which in turn can hinder sperm production [34]. Anti-estrogens like clomiphene and tamoxifen promote and sustain sperm production by raising the body's own levels of follicle-stimulating hormone, luteinizing hormone, and testosterone [35]. Combining tamoxifen with TU to treat idiopathic oligozoospermia can effectively improve sperm parameters, and it has proven more effective than tamoxifen alone. Aside from tamoxifen and clomiphene, no other medications have been approved or recommended for treating male infertility [36]. Testosterone administration has a suppressive effect on both baseline and gonadotropin-releasing hormone-stimulated secretion of pituitary gonadotropins, as well as on baseline and human chorionic gonadotropin-stimulated Leydig cell function.

Hormonal Therapy

Hormonal therapies are intended to boost sperm production. Selective estrogen receptor modulators (SERMs), including clomiphene citrate and tamoxifen, along with aromatase inhibitors, have been used to adjust the body's natural hormone levels. That said, the evidence that they improve semen parameters is mixed, so they are typically used only under certain conditions and decided on an individual basis [3]. However, long-term use of externally supplied testosterone is associated with suppression of the hypothalamic–pituitary–gonadal (HPG) axis, which reduces the body's own testosterone and sperm production and can ultimately lead to male infertility [37]. When empiric or targeted treatment improves sperm quantity or quality only modestly, falling short of what's needed for natural conception, the gain may still be enough to permit less intensive assisted reproduction, such as intrauterine insemination (IUI) or *in vitro* fertilization (IVF), and to avoid intracytoplasmic sperm injection (ICSI). This is clearly a benefit that often goes unrecognized. There is not enough evidence to confirm that supplements such as vitamins, antioxidants, and nutritional formulas improve male fertility. Current data indicate these agents are generally safe, but their clinical benefit remains unclear [3].

Gonadotropins

Gonadotropins are the only class of medications the FDA has approved for the medical management of male factor infertility. This class includes GnRH, LH, and FSH, which are used to treat hypogonadism and impaired sperm production (hypospermatogenesis) Hypogonadism is a reduction in the function of Sertoli and/or Leydig cells, which lowers sperm and/or testosterone (T) production. It can stem from testicular hypofunction (primary failure) or from insufficient stimulation by the hypothalamic-pituitary axis (secondary failure), also known as hypogonadotropic hypogonadism (HH). In secondary hypogonadism, both LH and FSH levels are low, leading to reduced testosterone and impaired sperm production [38].

Clomiphene citrate (CC)

CC is a nonsteroidal compound with both estrogen agonist and antagonist activity, and it exists as a racemic mixture of two isomers (enclomiphene and zuclomiphene) [39]. This selective estrogen receptor modulator blocks estrogen's negative feedback at the hypothalamic and pituitary level, which indirectly boosts the release of LH and FSH from the anterior pituitary. The increased LH and FSH stimulation of the testes should raise testosterone production and spermatogenesis, respectively. Recommended doses range from 12.5 to 400 mg/day, and one study reported that 100 mg three times a week was safe for up to 15 months [40].

Aromatase Inhibitors

Anastrozole and letrozole are prescription drugs in the aromatase inhibitor (AI) class. AIs are divided into steroidal and nonsteroidal types. Steroidal inhibitors, such as testolactone, formestane, and exemestane, mimic androstenedione and competitively inhibit aromatase, which causes irreversible inactivation of the enzyme. Letrozole and anastrozole are nonsteroidal inhibitors, and their inhibition of the enzyme is reversible Although anastrozole and letrozole suppress estrogen by nearly 100% in women, men do not show such a marked reduction, probably because of their high plasma testosterone levels. Even so, letrozole is a more potent AI than anastrozole, and both are used off label to treat oligospermia and azoospermia [41].

Androgens

Androgens are essential for the development of the male reproductive organs (penis, prostate, seminal vesicles, vas deferens, and epididymis), as well as for later puberty, sexual function, and fertility. Androgen receptors (ARs) are present in all male reproductive organs and are activated by testosterone and its more potent derivative, dihydrotestosterone (DHT) [42].

Pentoxifylline and Trental

Pentoxifylline (PF) is a methylxanthine derivative that affects cyclic adenosine monophosphate (cAMP) metabolism by blocking phosphodiesterase, which raises cAMP levels. Higher cAMP levels have been associated with better acrosome reactions and improved sperm motility [43]. PF administration has been shown to improve microcirculation by making red blood cells more deformable and by lowering both blood viscosity and platelet aggregation. Better microcirculation may help counter the link between idiopathic male factor infertility and reduced testicular perfusion [44].

Surgical Therapy

Surgical procedures such as varicocelectomy are generally recommended for patients with a palpable varicocele and low sperm counts, with the aim of improving semen parameters and raising pregnancy rates. Patients who have both a varicocele and azoospermia do not benefit from the procedure. In cases of azoospermia, surgical sperm retrieval methods such as testicular sperm extraction (TESE) and microdissection TESE (micro-TESE) are used. These procedures are especially helpful for men with non-obstructive azoospermia or for those whose hormonal therapy has been unsuccessful. Spermatogenesis depends on Leydig cells producing and maintaining a high concentration of testosterone within the testicles, which acts

mainly on the spermatogenic epithelium to promote sperm generation. Low testosterone levels can lead to spermatogenesis dysfunction. Testosterone in human testicular veins can reach 50-1200 ng/ml, about 250 times the level in surrounding venous blood, which indicates that spermatogenic cells need far higher testosterone concentrations in the testis than in the serum to develop and mature.

Varicocelectomy

Elevated sperm DNA fragmentation (SDF) is also seen in men with varicocele, and varicocele repair can lower it to as little as 3.37%, making it one of the more promising methods for reducing SDF in these men [45]. Varicocelectomy has also proven useful for reducing SDF. In a post-varicocelectomy study, SDF levels dropped from 35.2% to 30.2%, after which 37% of patients conceived naturally and another 24% conceived through assisted reproductive technology (ART) [46].

Robot-assisted microsurgical varicocelectomy uses robotic systems (such as the da Vinci) that provide high-definition 3D visualization and greater microsurgical precision. Its advantages include superior 3D, high-definition visualization, improved dexterity and accuracy, reduced tremor, and the potential for better preservation of vessels. The outcomes are as follows: Semen parameter improvements are comparable to, or better than, those achieved with conventional microsurgery.

The complication rate is low. Limitations include higher cost, the need for specialized equipment and training, and limited availability [47].

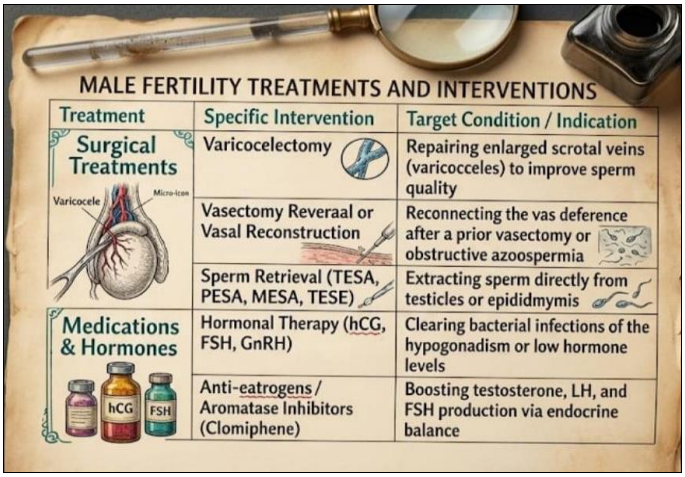
Assisted Reproductive Technologies (ARTs)

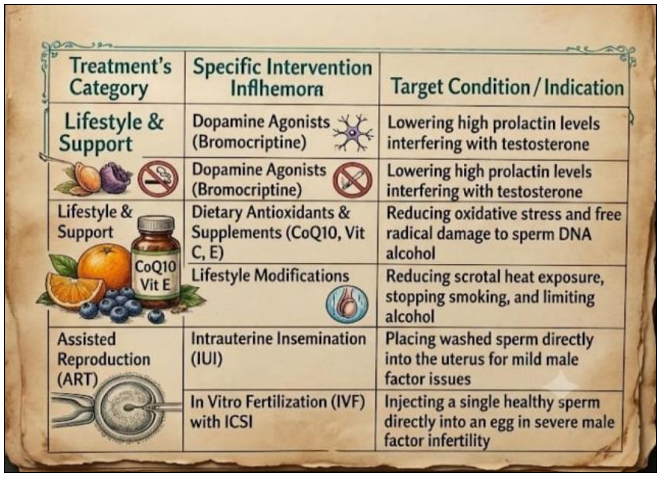
Assisted reproductive techniques such as intrauterine insemination (IUI), *in vitro* fertilization (IVF), and intracytoplasmic sperm injection (ICSI) have transformed the treatment of male infertility [48]. The goal of ARTs is to isolate functional spermatozoa that are capable of fertilizing oocytes. Indications based on total motile sperm count and clinical context are as follows:

Intrauterine insemination (IUI): suitable when the total motile sperm count is above 5 million and the post-wash sperm count exceeds 1 million; as either partner's age increases, pregnancy outcomes tend to worsen [49].

***In vitro* fertilization (IVF):** indicated when the total motile sperm count is 0.2-1 million, after a previous IUI failure, or in cases of moderate-to-severe male infertility [50].

Intracytoplasmic sperm injection (ICSI): The introduction of ICSI has changed the way male infertility is treated and has improved reproductive outcomes. IVF and related techniques were used for male infertility in the past, but most ART centers now rely on ICSI as the primary approach. Researchers have proposed various criteria for choosing suitable ICSI candidates. In general, these include severe oligospermia, progressive motility below 5%, normal morphology below 4%, use of cryopreserved sperm, the presence of anti-sperm antibodies, and previous failed IVF attempts. For patients with non-obstructive azoospermia, microdissection testicular sperm extraction (micro-TESE) achieves higher sperm retrieval rates and better ICSI outcomes than conventional approaches [51].





Life Style Modification

- For overweight men, high Body Mass Index (BMI) combined with metabolic syndrome (MetS) specifically impacts sperm DNA fragmentation (SDF) levels. Consequently, males presenting with both an elevated BMI and MetS should be screened for SDF testing [52].
- Obesity leads to hormonal alterations, with increased levels of LH, FSH, and estradiol, along with decreased testosterone levels. Bariatric surgery may be beneficial in such cases, as it can help restore the balance of reproductive hormones and improve SDF levels [53].
- Nutraceuticals containing myo-inositol, alpha-lipoic acid, coenzyme Q10, selenium, zinc, and vitamin B may benefit men with idiopathic infertility by reducing SDF levels, while also improving semen parameters and sperm vitality [54].
- Quitting smoking and alcohol consumption, along with regular exercise and effective weight management, may help improve SDF levels [55].
- Stress, which is one of the contributing factors to increased SDF levels, can be managed through cognitive behavioral therapy, mind–body stress management techniques, meditation, and behavioral therapy, which may help reduce overall stress [56].

Discussion

In a 2026 randomized clinical trial, Yifan Sun and colleagues evaluated the efficacy of daily 2.5 mg letrozole

combined with vitamins C and E versus vitamins C and E alone over a 3-month course in men suffering from severe male factor infertility. The study noted that inhibiting aromatase prevents testosterone from converting into estradiol, which triggers the hypothalamic-pituitary-gonadal axis to raise intratesticular hormone levels and potentially support sperm production. Results showed that the letrozole group achieved a significantly higher rate of improvement in sperm concentration categories than the control group (14.3% vs. 5.4%), along with increased levels of total testosterone, FSH, and LH, alongside reduced estradiol. Although letrozole was linked to a higher frequency of reduced libido, the overall findings suggest it can reduce the severity of spermatogenic failure, enabling couples to consider less invasive assisted reproductive procedures [57].

Nikoleta Papanikolaou and co-authors evaluated the diagnostic and monitoring potential of Ultrasound Localisation Microscopy (ULM) for male reproductive health in their 2026 study, "Ultrasound localisation microscopy tracks testicular microvascular adaptations to endocrine function in male infertility." The researchers compared testicular microvascular structure in healthy men against patients with hypogonadotrophic hypogonadism (HH), examined variations among treatment groups (testosterone replacement, gonadotrophin therapy, and untreated controls), tracked 12-month longitudinal microvascular shifts during fertility treatment, and evaluated a juvenile rodent model with induced pubertal blockade. They described ULM as a non-invasive, super-resolution technique that visualizes microvascular blood flow far below standard diffraction limits, demonstrating that metrics including vessel density, diameter, area, and tortuosity correlate directly with essential clinical, hormonal, and spermatogenic parameters. Ultimately, the study established that ULM reliably differentiates HH patients from healthy controls, reveals that gonadotrophin therapy promotes greater microvascular recovery than testosterone replacement, and identifies testicular reactivation just three months into fertility treatment substantially earlier than traditional clinical indicators like testicular volume or serum inhibin B levels [58].

In their 2014 research chapter, "Treatment of Male Infertility," Palermo. DG, et.al evaluate traditional diagnostic and therapeutic options including medication, varicocelectomy, intrauterine insemination (IUI), and standard *in vitro* fertilization (IVF) against advanced micromanipulation techniques such as intracytoplasmic sperm injection (ICSI). The authors also evaluate clinical outcomes across different sperm sources (ejaculated compared to surgically retrieved epididymal or testicular sperm) and preservation states (fresh versus cryopreserved). Additionally, they outline how genetic factors (including Y-chromosome microdeletions and chromosomal abnormalities), epigenetic mechanisms (such as DNA methylation and protamine packaging), and environmental influences impact both spermatogenesis and sperm function. The authors trace the evolution of assisted fertilization techniques, starting with early methods designed to penetrate the zona pellucida—such as zona drilling, partial zona dissection, and sub-zonal insemination—which ultimately paved the way for intracytoplasmic sperm injection (ICSI). They demonstrate that ICSI achieves robust fertilization and pregnancy outcomes even when using severely impaired sperm or surgically harvested samples (including MESA and TESE). However, MESA-retrieved sperm and fresh tissue consistently yield higher clinical pregnancy rates than TESE-retrieved or frozen samples. Additionally, while prolonged searches for viable sperm in non-obstructive azoospermia (NOA) cases correlate with time-dependent drops in fertilization and pregnancy success, the effort remains clinically justified given its potential to enable infertile men to father children. Finally, the authors stress that because ICSI circumvents male factor infertility without addressing its root causes, ongoing investigation into the long-term genetic and epigenetic health risks for offspring remains essential [59].

Conclusion

Male infertility is a complex, widespread condition stemming from genetics, hormonal disruptions, structural issues like varicoceles, oxidative stress, and environmental or lifestyle factors. Because standard semen analysis often misses molecular and subcellular defects, advanced diagnostics including sperm DNA fragmentation (SDF) tests and cutting-edge imaging like Ultrasound Localisation Microscopy (ULM) have become essential for detecting microvascular changes and assessing chromatin integrity. Managing the condition effectively calls for a tailored, step-by-step strategy. Medical therapies such as selective estrogen receptor modulators (SERMs), aromatase inhibitors like letrozole, and gonadotropins help re-establish hormone balance and boost sperm production, reducing infertility severity to make less invasive conception possible. When structural issues or azoospermia are present, surgical procedures like microsurgical varicocelectomy and advanced sperm retrieval techniques (such as micro-TESE) offer direct solutions. When natural conception isn't possible, assisted reproductive technologies like Intracytoplasmic Sperm Injection (ICSI) offer dependable fertilization success, even in cases of severe male-factor infertility. Ultimately, integrating targeted medication, surgical interventions, lifestyle modifications, and modern ART optimizes patient outcomes while highlighting the need to monitor long-term genetic and epigenetic safety for future generations.

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