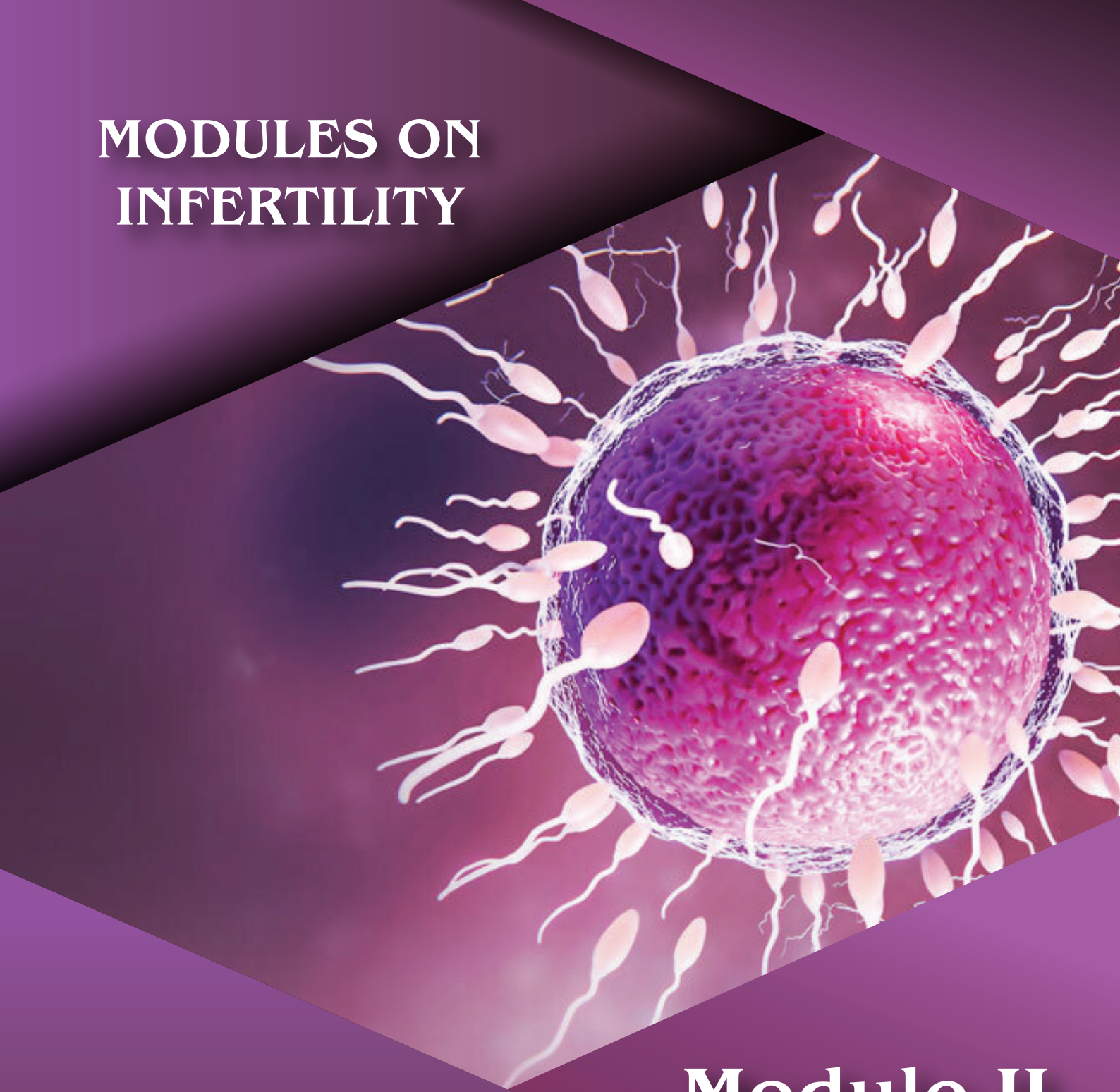
MODULES ON
INFERTILITY



Module II

Male Infertility
&
Female Infertility

CONTENT

Introduction: Male infertility

WHO defines infertility as the inability to conceive after at least 12 months of regular, unprotected sexual intercourse. Infertility is a major health problem world¬ wide and is estimated to affect 8–12% of couples in the reproductive age group. A Global Burden of Disease survey reported that between 1990 and 2017, the age¬ standardised prevalence of infertility increased annually by 0·370% in women and by 0·291% in men.

Infertility causes substantial psychological and social distress, and imposes a considerable economic burden on patients and health¬care systems. Early diagnosis and appropriate management can mitigate these factors. In a prospective study of 384419 Danish men, Glazer and colleagues reported a higher risk of mortality among men with male factor infertility than among men who were fertile. Ventimiglia and colleagues showed that impaired male reproductive health (including poorer semen para¬ meters and lower testosterone levels) was associated with a higher Charlson Comorbidity Index, which is a proxy of decreased general health status. Severe male infertility is also associated with a greater incidence of cancer. Thus, early detection of male subfertility offers the opportunity for identification and correction of medical conditions affecting not only fertility, but also general health and wellbeing.

There is increasing evidence that paternal health at the time of conception can affect the offspring's meta¬bolic health and reproductive potential, through transge¬nerational transmission of epigenetic modifications. Thus, obesity or diabetes might contribute not only to male subfertility, but can also compromise the health of future progeny. A study of 744 men with infertility revealed that 15·4% of men who met the criteria suggestive of prediabetes were at increased risk of hypogonadism, higher sperm DNA fragmentation, and non¬obstructive azoospermia. Men who are oligozoospermic are more likely to have metabolic syndrome than men who are normozoospermic Therefore, it is important to look beyond a semen analysis, and to view male infertility as a condition connected to and promoting a state of impaired metabolism.

The cause of infertility lies solely with the man in 20–30% of cases and a male cause is contributory in a further 20%. In 1992, a large meta-¬analysis by Carlsen and colleagues confirmed that sperm counts had declined by 50% during a 60¬year period. Subsequently, numerous studies have shown similar declines globally, although some studies have disputed this claim. A systematic review by Levine and colleagues reported that sperm counts decreased by 50–60% between 1973 and 2011.

The causes of male subfertility are wide ranging and poorly understood in most cases. Although various diagnostic tests are available, their interpretation is imprecise and often subjective.

Intracytoplasmic sperm injection has made it possible to achieve pregnancy with very poor semen quality—eg, in cases of azoospermia for which surgically retrieved testicular sperm are used. Exciting new therapies using stem cells and in¬vitro sperm maturation are still experimental.

Causes

A multitude of causes and risk factors contribute to the increasing incidence of male infertility which can be stratified as congenital, acquired, and idiopathic (panel 1). The primary known genetic causes of male infertility are congenital bilateral absence of the vas deferens associated with cystic fibrosis gene mutations, Kallmann syndrom chromosomal abnormalities leading to deterioration of testicular function, and Y chromosome microdeletions resulting in isolated spermatogenic defects. Among acquired factors, varicocele is the most common and correctable cause of infertility in men, with a prevalence of 40%. About 30–50% of male infer¬tility cases are idiopathic, with no discernible cause or contributory female infertility. Male oxidative stress infertility involves altered semen characteristics and oxidative stress, and affects about 37 million men with idiopathic male infertility. Environmental or occu¬pational exposure to toxic chemicals38 and various lifestyle factors (eg, smoking, alcohol consumption, recre¬ational drug use, obesity and psychological stress are all potential risk factors for male infertility.

Evaluation and Medical history

Evaluation

Infertility evaluation and treatment is recommended for couples who do not conceive naturally after at least 12 months of regular, unprotected sexual intercourse or after 6 months for couples in which the female partner is older than 35 years. Evaluation and treatment before 12 months might be considered on the basis of medical history and physical examination, and men who have concerns about their future fertility can also be evaluated.

The American Society for Reproductive Medicine (ASRM) and the European Association of Urology (EAU) both recommend an initial evaluation consisting of a reproductive history and at least one semen analysis although the American Urological Association (AUA) insists on two semen analyses. If the initial evaluation shows abnormal results, referral to a reproductive specialist is recommended for a thorough evaluation that includes a physical examination and taking a complete medical history. Depending on the results, further andrological assessments and procedures might be recommended.

Medical History

Successful diagnosis of male infertility can be challenging, because the process of conception involves multiple organs and requires the evaluation of two individuals. The initial step in evaluating infertility is obtaining a thorough history (panel 2). Infertility can be classified as either primary (ie, no previous fertility) or secondary (ie, previously fertile, currently infertile). Although this distinction can narrow differential diagnosis, men classified with primary or secondary infertility should be assessed in the same way. Various childhood conditions (eg, cryptorchidism, post-pubertal mumps orchitis, and testicular torsion or trauma) can result in testicular atrophy or decreased semen quality. Infections of the male urogenital tract (prostatitis, urethritis, epididymitis, and orchitis) can contribute to male infertility.30 The prevalence of male urogenital tract infection was reported to be as high as 35% in a study of more than 4000 men with infertility. A cross¬ sectional study of 1689 men revealed that 20% of men with primary infertility had asymptomatic semen infections, which were associated with impaired sperm concentrations. Prostatitis, a common urogenital disease caused by Escherichia coli, can have detrimental effects on various sperm parameters.56 Among sexually active men younger than 35 years, Chlamydia trachomatis and Neisseria gonorrhoeae are the most common path¬ogens to cause epididymitis. E coli is the predominant pathogen found in men older than 35 years who have infertility. Although semen analysis is not recom¬mended in acute cases of epididymitis or prostatitis, men with chronic epididymitis or prostatitis might present with leukocytospermia (>1×106 white blood cells per mL), which is a sign of inflammation and can be confirmed by peroxidase test in semen.

Lifestyle factors such as smoking, alcohol consumption, recreational drug use (eg, cocaine, opioid narcotics, cannabis, and anabolic steroids), and obesity are also relevant to male infertility. A large meta¬-analysis involving 5865 men from 20 studies showed deterioration of semen quality in moderate and heavy smokers. Similarly, a meta-¬analysis of 15 cross-sectional studies revealed the negative association between alcohol con¬sumption and sperm parameters. Cannabis, the most frequently used recreational drug, negatively effects male fertility by inhibiting the hypothalamic–pituitary– gonadal axis, spermatogenesis, and sperm function. The association between obesity and male infertility has been widely investigated as the global prevalence of obesity continues to rise. Obesity¬ induced endocrine alterations that result in peripheral conversion of testosterone to oestrogen have been linked with reduced sperm con¬ centrations. Among the subsets of obesity, metabolically unhealthy obesity (ie, with metabolic abnormalities such as diabetes, hypertension, dyslipidaemia, insulin resis¬tance) is known to be a risk factor for erectile dysfunction, and the combination of erectile dysfunction and meta-bolically healthy obesity (ie, without evidence of metabolic and cardiovascular disease) in men represents an early marker for future adverse metabolic consequences.

The couple’s sexual practices, including the timing of coitus and erectile and ejaculatory function, should be assessed. Ovulation tracking methods should be used to ensure that couples are timing intercourse effectively. Intercourse is recommended every 48 h around the time of ovulation, to maximise the chance of fertilisation. The most common sexual disorders that affect men with infertility are hypoactive sexual desire and an absence of sexual satisfaction (pleasure, positive feeling, and orgasm). One in six men with infertility has erectile dysfunction, or premature ejaculation, or both. The psychological effects of sexual dysfunction and male infertility can be a substantial barrier to successful fecundity, and should be screened for during clinical evaluation. Also, many couples use vaginal lubricants, but these can be spermicidal. Vegetable oil, raw egg white, and fertility¬ friendly lubricants (eg, Pre¬Seed, ING Fertility, Spokane, WA, USA) have the least spermicidal effects, but couples should still be made aware to use them in moderation.

Semen Analysis

WHO recommends conventional semen analysis as the first step in the evaluation of male fertility potential. The WHO Laboratory Manual for the Examination and Processing of Human Semen and Sperm–Cervical Mucus Interaction has been published since 1980, with the most recent manual released in 2010. The recom¬mended cut off values for semen parameters have evolved dramatically over the years (table), yet nomenclature related to semen quality has remained unchanged (panel 3). The lower reference limits depicted in the latest edition of the WHO manual are derived from the statistical analysis of the semen parameters of 1953 fertile men from around the world. However, these reference limits have been criticised for not considering the female factor, high biological variation among individuals, and the absence of data from representative ethnic groups. Consequently, standard semen analysis has limited accuracy for determining male fertility potential or predicting reproductive success. In fact, interpreting semen analysis using WHO 2010 reference values resulted in samples being considered normal that would have been considered abnormal if using the 1999 manual. Ombelet and colleagues used receiver operating characteristic curve analysis to determine the diagnostic potential and cut off values for single and combined sperm parameters. Their prospective study revealed that single sperm parameters were of little clinical value for differentiating men who were fertile from men with subfertility, and showed it was important to use a combination of sperm parameters to predict a man's fertility status. Another problem with standard semen analysis is that not all laboratories comply strictly with the WHO manual methods. Less than 60% of laboratories in the USA complied with WHO guidelines, and less than 5% in the UK. It is of paramount importance that all laboratories follow the WHO manual guidelines strictly, to provide reliable and comparable results.

Several semi¬automated and fully automated computer¬ assisted sperm analysis systems have been introduced. Despite their shortcomings for evaluating sperm mor¬phology accurately, computer ¬assisted sperm analysis systems are widely used in many andrology and in¬vitro fertilisation clinics that strictly adhere to quality control protocols to quantify semen parameters accurately. Sys¬tems such as the LensHooke (Bonraybio Co, Taichung City, Taiwan) incorporate artificial intelligence to simplify semen analysis. Results of Agarwal and colleagues' prospective study of semen analysis show that this device is a reliable diagnostic tool, providing clinically acceptable results, as defined by WHO 5th edition guidelines.

Home¬based collection of semen samples is another advancement in semen analysis. Technologies that support being able to test sperm at home provide a potential solution for men who feel uncomfortable about providing a semen specimen in an unfamiliar environment. Home¬based sperm testing systems are mainly based on antibody reactions, microfluidics, or smartphone technology. The accuracy of these devices for determining sperm concentration ranges from 95% to 98%, making them a practical and affordable way to do preliminary screening for male infertility.

Physical Examination

Physical examination is a key part of evaluating male infertility, and should include an assessment of body habitus, secondary sexual characteristics, and genitalia. An eunuchoid body habitus, decreased body hair compared with Tanner stage V, obesity, or gynaeco¬mastia might be seen in patients with endocrinopathies (eg, low serum testosterone, Klinefelter syndrome, hyperprolactinaemia).

The genital examination should begin with the phallus, carefully assessing for penile curvature, plaques, epi¬spadias, or hypospadias, all of which can impair semen deposition in the vaginal vault. The testicles should be examined for presence, size, and consistency. Testicular size should be assessed using a Prader orchidometer or callipers (normal volume 20 mL or 4×3 cm). Scrotal ultrasonography can be useful when the patient's body habitus or scrotal anatomy (hydrocele, dilated epididymis, or inguinal testis) might render testicular measurement by Prader orchidometer unreliable. A testicular mass should be ruled out, because men with infertility are at increased risk of testicular neoplasm. The epididymides should be palpated to assess for enlargement that might indicate distal obstruction. A hypoplastic epididymis with either unilateral or bilateral non¬palpable vas deferens is consistent with vasal agenesis and can be associated with genetic or renal abnormalities.

The spermatic cords should be assessed in the supine and standing positions, allowing for the detection of a varicocele. Varicoceles are graded by size: grade 1 is palpable only by Valsalva manoeuvre, grade 2 is palpable without Valsalva manoeuvre, and grade 3 is visible at rest. Although digital rectal examination is not routinely done in young men with subfertility, it is indicated in men with low ejaculate volume. The prostate should be assessed for size and consistency. A midline cyst or prominent seminal vesicles might indicate ejaculatory duct obstruction.

Hormonal Evaluation

Hormonal evaluation is an important tool in the management of male infertility. Many clinicians consider hormonal assessment to be part of the routine inves¬tigation for every male patient with infertility, although international societies recommend limiting use to particular groups of patients, including men with a sperm concentration below 10×106/mL or impaired sexual function, or if endocrinopathy is suspected.

The recommended basic hormonal evaluation should include analysis of follicle ¬stimulating hormone and total testosterone (panel 4). If total testosterone concentration is found to be low, a more thorough endocrine evaluation is recommended. This process includes repetition of total testosterone and addition of luteinising hormone assay to differentiate primary from secondary hypo¬gonadism. Prolactin analysis is also recommended in such cases. The validity of the ASRM guidelines for hormonal evaluation of male infertility has been challenged for predicting hypogonadism. A retro¬spective study by Ventimiglia and colleagues revealed that the guidelines had a low predictive value, with 58% overall accuracy, 75% sensitivity, and 39% specificity. There is no general consensus on the lower cut-off value for testosterone concentrations. The ASRM adopts the value of less than 300 ng/dL as a cut-off for diagnosing hypogonadism, and the EAU recommends 230 ng/dL (8 nmol/L). Measuring total testosterone concentration alone could be insufficient in cases in which sex hormone¬ binding globulin is increased (eg, in men older than 75 years, thyroid disease, or diabetes). In these cases, measurement of free testosterone is recommended. Although reverse equilibrium dialysis is the gold standard for measuring free testosterone, it is expensive and technically chal¬lenging. Using calculated free testosterone can be a more clinically accurate method in assessing men with hypogonadal symptoms.

Although the role of prolactin in female fertility is well established, its role in male infertility is not clear, although mild elevations are not important. Severe hyperprolactinaemia might be associated with lower total testosterone concentrations, thereby affecting spermatogenesis and male sexual function. Hyperpro¬lactinaemia is caused by prolactinomas in 40% of cases. Follicle¬ stimulating hormone is usually negatively associated with spermatogenesis, so increased follicle¬ stimulating hormone would be seen in cases of defective spermatogenesis with absent or diminished spermatogonia. However, in some cases of sper¬matogenic arrest at the level of spermatocyte or sper¬matid, concentrations of follicle¬ stimulating hormone, luteinising hormone, and testosterone might be normal, which limits the predictive value of endocrine evaluation in men with non¬obstructive azoospermia.

Genetic Testing

Genetic abnormalities related to male infertility affect about 15% of men with infertility. A recent systematic review and clinical validity assessment of male infertility genes revealed a total of 78 genes linked to 92 male infer¬tility phenotypes. Several genes and gene mutations related to spermatogenesis have been discovered. Men with genetic abnormalities usually show defective spermatogenesis, resulting in severe oligozoospermia or azoospermia and increased aneuploidy. Genetic muta¬tions in embryos might lead to repeated intracytoplasmic sperm injection failure, recurrent miscarriage, or vertical transmission of paternal genetic defects. Therefore, identifying genetic defects is crucial for diagnostic purposes and proper counselling before intracytoplasmic sperm injection. Vertical transmission of genetic defects can be prevented through preimplantation genetic testing and transfer of genetically healthy embryos.109 Genetic testing is also important for predicting the success of sperm retrieval.

Karyotyping (also known as chromosomal analysis) detects numerical chromosomal defects, or structural defects. Karyotype anomalies are the most common type of genetic defect, with a prevalence of 12–15% in azoospermia, 5% in severe oligozoospermia, and less than 1% in normal semen. The most common karyotype defect is Klinefelter syndrome (also known as 47,XXY), followed by translocations, inversions, and deletions. Different professional societies agree on recommending karyotype analysis for men with azoospermia or severe oligozoospermia (sperm count <5×106/mL). However, the EAU extended their guideline recommendations to include men with a sperm count of less than 10×106/mL. The EAU also recommends obtaining karyotype if there is a family history of recurrent spontaneous abortions, malfor¬mations, or intellectual disability regardless of the sperm concentration. This recommendation was retrospectively validated in a cohort study of 1168 men, which found that the suggested threshold had moderate sensitivity (80%), but low specificity (37%) and discrimi-nation (59%).Therefore, use of the EAU guidelines primarily on the basis of sperm count might lead to unnecessary use of karyotype analysis, which is an expensive and laborious test.

Y chromosome microdeletion analysis is indicated for patients with azoospermia or oligozoospermia and a sperm count of less than 5×106/mL. A meta-¬analysis by Kohn and colleagues showed that the majority of Y chromosome microdeletions occur in men with sperm counts of less than 1×106/mL. The latest EAU guide¬ lines recommend Y chromosome microdeletion testing if sperm concentrations are less than 5×106/mL, and make such testing mandatory for sperm concentrations of less than 1 × 106/mL. Y chromosome microdeletion affects azoospermia factor a, b, or c in the long arm of the Y chromosome. Although sperm can be retrieved from the testes of men with azoospermia factor c deletions, azoospermia factor a or b deletions carry a very poor prognosis and sperm retrieval is not advised in such cases.

Importantly, Y chromosome microdeletions can be transmitted to male offspring, so counselling couples is recommended before intracytoplasmic sperm injection.

Most patients with cystic fibrosis have congenital bilateral absence of the vas deferens and about two thirds of men with this condition have *CFTR* mutations without any other cystic fibrosis manifestations. For men with structural abnormalities of the vas deferens, it is recommended that both partners be tested for *CFTR* mutations containing a minimal panel of common point mutations and the 5T allele.

Imaging

Full evaluation of a man with infertility can involve imaging in some circumstances. Scrotal ultrasonography is a preferred imaging modality because of its non¬ invasive nature, safety, and low cost. It provides details about testicular size and volume, testicular echogenicity and blood flow, varicocele presence, and epididymal anatomy. Because scrotal ultrasonography is not indi¬cated for the diagnosis of subclinical varicocele, ultrasonography can be avoided in men with a normal physical examination result. Patients in whom proximal genital tract obstruction is suspected (on the basis of history, physical examination, and semen analysis) need to have transrectal ultrasound to evaluate for seminal vesicle dilation, midline prostatic cyst, and ejaculatory duct dilation. Transrectal ultrasound can be used in combination with seminal vesicle aspiration to more accurately diagnose ejaculatory duct obstruction. If more detailed imaging of the genitourinary tract is required, MRI can be done. In men with infertility, hypogonadism, and elevated prolactin, cranial MRI can diagnose a pituitary pathology (most commonly pro¬lactinoma) as an underlying cause of hyperprolactinaemia and hypogonadism. Vasography is an invasive imaging modality to confirm patency or delineate an obstruction of the vas deferens or ejaculatory duct, and is usually done only as part of definitive reconstructive surgery. In many cases, physical examination alone allows a specialist in male infertility to make a diagnosis, but the aforementioned imaging methods can be used for inconclusive cases, or intraoperatively during recon¬structive microsurgery.

Specialised Tests

Conventional semen parameters do not detect defects associated with functional aspects of spermatozoa, so sperm function tests have been developed to augment semen analysis (figure 1). The clinical importance of the sperm function tests came to light after the emergence of in-vitro fertilisation and intracytoplasmic sperm injection. In conventional in vitro fertilisation, defec¬tive sperm–zona interaction is the main reason for fertilisation failure. However, in the current era of intracytoplasmic sperm injection, hemizona or acrosome function assays are no longer used in clinical practice, because the penetrating capability of sperm is bypassed by intracytoplasmic sperm injection. Therefore, greater emphasis is placed on the assessment of sperm chromatin quality using sperm DNA fragmentation testing.

Sperm DNA fragmentation assays potentially provide a more comprehensive assessment of the overall fertility status than conventional semen parameters.

Currently, terminal deoxynucleotidyl transferase mediated dUTP nick end labelling, sperm chromatin structure assay, and sperm chromatin dispersion are among the most commonly used sperm DNA fragmentation assays.

Although test protocols and cutoff values have substan¬tially improved precision and decreased variations for the sperm DNA fragmentation test, the absence of strict standardisation and clear threshold values deter its wider application. Hence, although emerging evidence supports the role of sperm DNA fragmentation in reproductive outcomes (whether natural or via assisted reproductive techniques),routine use of sperm DNA fragmentation testing is not recommended by the AUA or ASRM. In 2017, a publication on clinical practice guidelines consolidated the available data on sperm DNA fragmentation testing and provided recommendations in four specific clinical scenarios(panel 5). The EAU guidelines recommend sperm DNA fragmentation testing in couples with recurrent pregnancy loss, or in men with unexplained infertility. A DNA fragmentation index of more than 30% by sperm chromatin structure assay is associated with a lower incidence of pregnancy via natural conception or intrauterine insemination.

Measuring seminal oxidative stress could be another means of sperm functional assessment, considering the close and potentially causal relationship between sperm DNA fragmentation and reactive oxygen species. Excessive amounts of reactive oxygen species, if not counterbalanced by antioxidants, lead to oxidative stress and result in protein, lipid, and DNA damage. Direct measurement of reactive oxygen species in semen using chemiluminescent or fluorescent techniques can have prognostic value in the evaluation of the male fertility potential, with a cutoff value of less than 102·2 RLU/s/106 sperm per mL to distinguish between men who are fertile and men with infertility. Seminal oxidation¬ reduction potential is a novel concept intro duced to measure global oxidative stress in semen samples using the Male Infertility Oxidative System, which is a quick and simple test. The potential clinical value of the oxidation¬ reduction potential assay was reported in a multicentre study that established a cutoff value of 1·34 mV/106 sperm per mL to differentiate men with normal and abnormal semen parameters. Although seminal oxidative stress can be determined by various assays, the EAU guidelines recommend that routine testing of reactive oxygen species should remain experimental until these tests are validated in randomised controlled trials (RCTs).

Figures & Tables

***Panel 1*: Causes and risk factors of male infertility**

Congenital factors

- Anorchia
- Congenital absence of vas deferens
- Cryptorchidism
- Y chromosome microdeletions
- Chromosomal or genetic abnormalities
  - Klinefelter syndrome and its variants (47,XXY; 46,XY/47,XXY mosaicism)
  - Kallmann syndrome
  - Robertsonian translocation
  - Mild androgen insensitivity syndrome
- Genetic endocrinopathy
- Congenital obstruction

Acquired factors

- Varicocele
- Testicular trauma
- Testicular torsion
- Germ cell tumours
- Acquired hypogonadotrophic hypogonadism
- Recurrent urogenital infections (prostatitis, prostatovesciculitis)
- Postinflammatory conditions (epididymitis, mumps orchitis)
- Urogenital tract obstruction
- Exogenous factors (eg, chemotherapy, medications, radiation, heat)
- Systemic diseases (live cirrhosis, renal failure)
- Anti-sperm antibodies
- Surgeries that can comprise vascularisation of the testis
- Sexual dysfunction (erectile or ejaculatory dysfunction)

Idiopathic risk factors

- Smoking
- Alcohol
- Recreational drugs
- Obesity
- Psychological stress
- Advanced paternal age
- Dietary factors
- Environmental or occupational exposure to toxins

***Panel 2*: Important attributes of history taking in the evaluation of men with infertility**

Infertility history

- Duration of infertility
- Previous pregnancies and outcomes (primary *vs* secondary infertility)
- Partner's fertility history
- Previous fertility investigation and treatment

Sexual history

- Libido
- Erectile dysfunction
- Ejaculatory dysfunction
- Type of lubricants
- Frequency and timing of coitus
- Sexually transmitted disease

Medical history

- Cryptorchidism
- Timing of puberty
- Anosmia
- History of testicular torsion
- History of testicular trauma
- Diabetes
- Neurological conditions (spinal cord injury, multiple sclerosis)
- Infections (urinary infections, epididymitis or prostatitis, tuberculosis, mumps orchitis, recent febrile illness)
- Renal disease
- Cancer

Surgical history

- Orchidopexy
- Retroperitoneal or pelvic surgery
- Herniorrhaphy
- Vasectomy
- Bladder neck or prostatic surgery

Gonadotoxin exposures

- Medications (endocrine modulators, antihypertensives, antibiotics, antipsychotics)
- Environmental (pesticides, heavy metals)
- Chemotherapy or radiotherapy
- Lifestyle (obesity, tobacco, vaping, recreational drugs, anabolic steroids)

Family history

- Infertility
- Cystic fibrosis
- Androgen receptor deficiency

	WHO manual 1st edn (1980)[69]	WHO manual 2nd edn (1987)[70]	WHO manual 3rd edn (1992)[71]	WHO manual 4th edn (1999)[72]	WHO manual 5th edn (2010)[57]
Volume	ND	≥2·0 mL	≥2·0 mL	≥2·0 mL	≥1·5 mL
Sperm concentration	20–200 × 10^6/mL*	≥20 × 10^6/mL	≥20 × 10^6/mL	≥20 × 10^6/mL	≥15 × 10^6/mL
Total sperm count	≥40 × 10^6/mL	≥40 × 10^6/mL	≥40 × 10^6/mL	≥40 × 10^6/mL	≥39 × 10^6/mL
Sperm motility (% progressive)	≥60%	≥50%	≥50%	≥50%	≥32%
Sperm vitality (%)	ND	≥50%	≥75%	≥75%	≥58%
Sperm morphology (% normal)	≥80·5%†	≥50%	≥30%‡	≥15%§	≥4%

Data extracted from the WHO manuals. ND=not defined. *Probably based on MacLeod's work.[73] †Mean of fertile population. ‡Arbitrary value. §Value not defined but strict criteria and in-vitro fertilisation data suggest a 14% cutoff value.

Table: **The evolution of normal values for semen parameters from 1980 to 2010 across the first five editions of the *WHO Laboratory Manual for the Examination and Processing of Human Semen and Sperm–Cervical Mucus Interaction***

Panel 3: Nomenclature related to semen quality

Aspermia
No semen (no ejaculation or retrograde ejaculation)

Asthenozoospermia
Percentage of progressively motile spermatozoa below the lower reference limit

Asthenoteratozoospermia
Percentages of both progressively motile and morphologically normal spermatozoa below the lower reference limits

Azoospermia
No spermatozoa in the ejaculate (given as the limit of quantification for the assessment method used)

Cryptozoospermia
Spermatozoa absent from fresh preparations but seen in a centrifuged pellet

Haemospermia (haematospermia)
Presence of erythrocytes in the ejaculate

Leukospermia (leukocytospermia, pyospermia)
Presence of leucocytes in the ejaculate greater than the threshold value

Necrozoospermia
Low percentage of live, and high percentage of immotile, spermatozoa in the ejaculate

Normozoospermia
Total number (or concentration, depending on outcome reported)* of spermatozoa, and percentages of progressively motile and morphologically normal spermatozoa, equal to or greater than the lower reference limits

Oligoasthenozoospermia
Total number (or concentration, depending on outcome reported)* of spermatozoa, and percentage of progressively motile spermatozoa, less than the lower reference limits

Oligozoospermia
Total number (or concentration, depending on outcome reported)* of spermatozoa less than the lower reference limit

Teratozoospermia
Percentage of morphologically normal spermatozoa less than the lower reference limit

The suffix spermia refers to the ejaculate and zoospermia refers to the spermatozoa. Therefore, the following terms should not be used: asthenospermia, asthenoteratospermia, cryptospermia, oligoasthenospermia, oligoteratospermia, oligospermia, teratospermia.

Adapted from WHO 5th edn, 2010.[57] *Preference should always be given to the total number, as this parameter takes precedence over concentration.

Panel 4: Clinical interpretations of hormonal assessments in men with infertility

Hypogonadotrophic hypogonadism
Decreased concentrations of follicle-stimulating hormone, luteinising hormone, and testosterone

Testicular failure (oligoasthenoteratozoospermia or non-obstructive azoospermia)
Increased concentrations of follicle-stimulating hormone and luteinising hormone, and decreased or normal concentrations of testosterone

Inconclusive: normal spermatogenesis or defective spermatogenesis
Normal concentrations of follicle-stimulating hormone, luteinising hormone, and testosterone

Hyperprolactinaemia
Increased concentrations of prolactin, and normal or decreased concentrations of testosterone

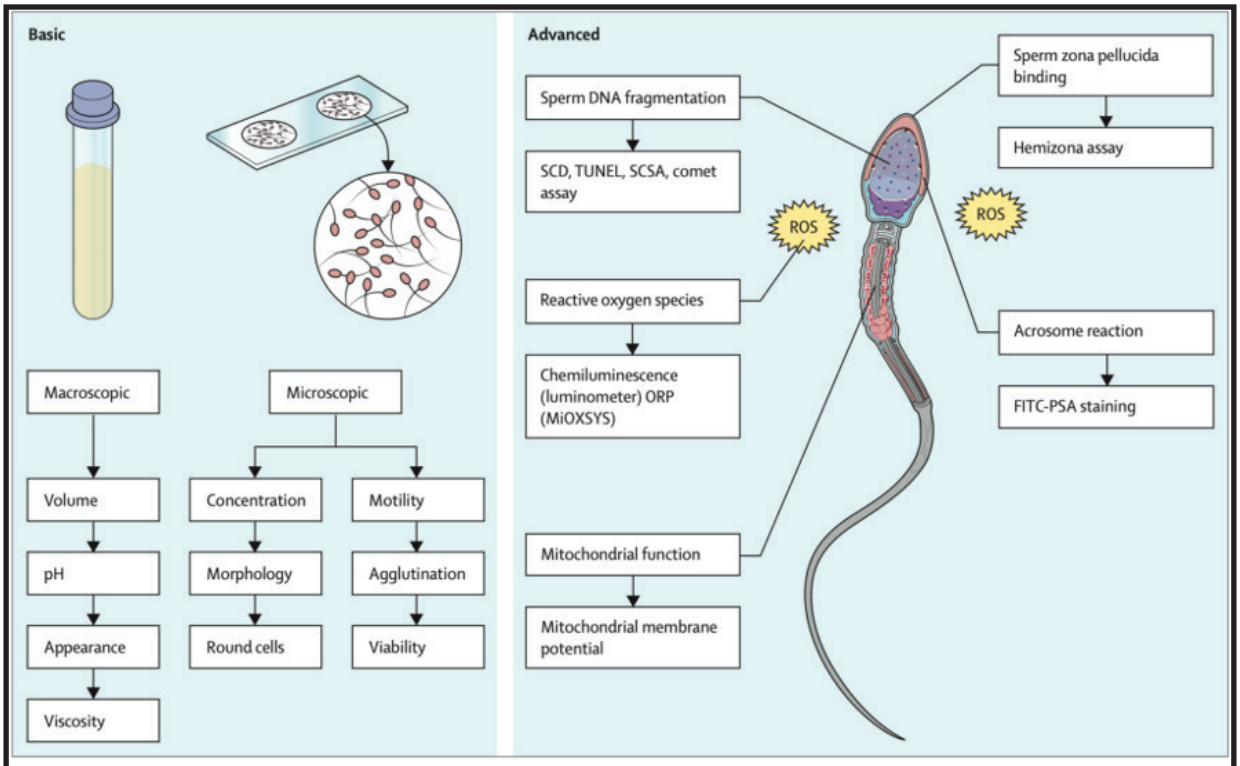


Figure 1: **Laboratory evaluation for male infertility**
Standard semen analysis comprises the analysis of macroscopic and microscopic parameters. An advanced sperm function test comprises the determination of ROS, sperm DNA fragmentation, acrosome reaction, and MMP using different techniques. FITC-PSA=fluorescein isothiocyanate-labelled *Pisum sativum* agglutinin. MiOXSYS=male infertility oxidative system. MMP=mitochondrial membrane potential. ORP=oxidation-reduction potential. ROS=reactive oxygen species. SCD=sperm chromatin dispersion test. SCSA=sperm chromatin structure assay. TUNEL=terminal deoxynucleotidyl transferase-mediated dUTP nick-end labelling. Adapted from Agarwal and colleagues,[128] by permission of the Korean Society for Sexual Medicine and Andrology.

Panel 5: Clinical indications for sperm DNA fragmentation testing

Clinical varicocele

- Sperm DNA fragmentation testing is recommended in patients with grade 2 or 3 varicocele with normal conventional semen parameters
- Sperm DNA fragmentation testing is recommended in patients with grade 1 varicocele with borderline or abnormal conventional semen parameters

Unexplained infertility or intrauterine insemination failure or recurrent pregnancy loss

- Sperm DNA fragmentation testing should be offered to couples with infertility and recurrent pregnancy loss, or before intrauterine insemination
- Early in vitro fertilisation or intracytoplasmic sperm injection might be an alternative treatment for couples with infertility and recurrent pregnancy loss or failed intrauterine insemination

In vitro fertilisation failure, or intracytoplasmic sperm injection failure, or both

- Sperm DNA fragmentation testing is indicated in patients with recurrent failure of assisted reproduction
- The use of testicular sperm rather than ejaculated sperm might be beneficial in men with oligozoospermia, high sperm DNA fragmentation, and recurrent in vitro fertilisation failure

Borderline abnormal (or normal) semen parameters with risk factor

- Sperm DNA fragmentation testing should be offered to patients who have a modifiable lifestyle-risk factor for male infertility

Female Infertility

Emotional problems and philosophical questions affect clinical practice and are of profound importance in the provision of services. They include damage to patients' self-esteem and sense of well-being, their grieving for children that never were, usually in secret, and frustrations owing to service limitations by budgetary constraints. The medical profession and society must decide whether everyone has a right to have a child and therefore access to treatment. The welfare of any offspring who may result from treatment should be of overriding concern. The risk to offspring caused by treatment should be minimized; the main risk in current practice is from multiple pregnancy. Treatments employing donor gametes involve specific emotional risks not only for the recipient parents and for their children but also for the donors, and counselling must be provided accordingly.

In clinical practice it is worth appreciating that patients' objectives are often quite different from those of physicians. The goal of physicians is to reach a diagnosis and administer treatments whose effectiveness can be measured in terms of pregnancy and live birth rates. In contrast, patients desire a baby and see themselves as sustaining emotional and interrelation problems requiring understanding, counselling, and education.

Essentials of underlying physiology

Any of several distinct functional elements of conception can fail, leading to infertility. All of these factors need to be considered in practice, including ovulation, tubal transport of the oocyte, timed coital delivery of sperm, cervical mucus receptivity, sperm production and motility, fertilization, and uterine/ endometrial receptivity for implantation. Some functions such as cervical mucus receptivity are cyclical and linked to follicular development and ovulation. Others such as sperm production or function are independently controlled or can be damaged in isolation. The most common defined causes of infertility are listed in Table 1. Best understood are the control of ovulation and the causes and treatment of ovulation failure. Although a detailed analysis of the physiology and pathophysiology of the causes of infertility is beyond the scope of this article, some of the key points of practical relevance are summarized in the following sections. A clinically oriented reviewz1and basic reference textl2 on reproductive physiology can be found elsewhere.

Ovulation (Oocyte Release and Follicular Maturation)

Although follicular growth and rupture can be observed by serial ultrasonography, follicle size correlates only weakly with functional maturity, which is best reflected by hormone production. The functional capacity of the corpus luteum reflects preovulatory follicular maturation, and midluteal serum progesterone measurement is an accurate index of ovulation with the potential for conception. Variation between cycles requires repeated measurement. Assessment of multiple ovulation induced by stimulation therapy must take into account the progesterone contribution of the mature and subsidiary follicles.

Follicular maturation determines not only ovulation but also oocyte maturation and fertilizing ability. Oocyte quality is independent of those functions and although it cannot be recognized clinically, it determines embryo quality and ultimate viability. Declining oocyte quality is the main cause for declining fecundability as women age and is associated with declining follicle numbers in the ovaries. Diminishing inhibin levels and consequently rising follicle-stimulating hormone (FSH) levels during the early follicular phase are clinically important indices of potential fertility.

Tuba1 Transport of the Oocyte

Oocyte uptake and transport along the tube depend on healthy, unrestricted fimbriae and ciliary action of the endotubal mucosa. Assessment of the fimbriae and mucosa is essential in clinical practice, in addition to a determination of patency and restrictive adhesions.

Timed Coital Delivery of Sperm

Optimal timing of coitus to achieve conception is 1 to 2 days before ovulation, that is, the day of or day before the onset of the luteinizing hormone (LH) surge. That event is linked to peak receptivity of cervical mucus, at which time spermatozoa should be present and available to the oocyte as soon as ovulation occurs. Spermatozoa are stored in the female reproductive tract and normally retain fertilizing ability for at least 48 hours, effectively bridging intervals of 2 days between coitus. More frequent intercourse is more effective (not less as sometime believed), whereas infrequent intercourse reduces sperm quality.

Cervical Mucus Secretion and Receptivity

Cervical mucus is a tissue and not a fluid, containing a mesh like mucoprotein matrix that acts as a challenging selector of sperm having favourable motility. Full development and receptivity coincide with the peak estradiol level, normally lasting 2 to 3 days, and are quickly interrupted by the antioestrogen effects of progesterone secreted in response to the LH surge, even before ovulation.

Sperm Production and Motility

Spermatozoa are normally produced in vastly excessive numbers; the most important factor is their functional ability. Sperm motility must be forwardly progressive with a fast tail beat and only slight lateral head movement to enable rapid progression through cervical mucus and onward to reach the oocyte. The pattern changes dramatically to slow lashing of the tail and marked lateral head movement (”activated motility,” a feature of capacitation) to enable penetration of the outer layers of the oocyte (granulosa cumulus cells and zona pellucida). Even when precisely quantitated using computerized imaging, sperm motility in semen correlates only weakly with mucous penetration, fertilization, and the chance of natural conception.

Fertilization

Before fertilization can occur, the oocyte must mature in response to the LH surge by resuming meiosis to reach metaphase I1 (extrusion of the first polar body), and the spermatozoa must develop activated motility and undergo the acrosome reaction (shedding of the acrosomal cap) to release the proteolytic enzymes required to assist penetration of the zona pellucida and attachment to the oocyte membrane (vitelline).Entry of the oocyte by the (haploid) spermato- zoon induces the second meiotic division of the oocyte (reducing it from diploid to haploid) and expulsion of the second polar body. Fertilization is defined by formation of the pronuclei of the oocyte and spermatozoon. Syngamy soon follows, and the first cleavage division of the embryo occurs about 24 hours after the meeting of oocyte and spermatozoa.

Uterine/Endometrial Receptivity for Implantation

Endometrial proliferation in response to estrogen and subsequent secretory differentiation in response to progesterone generally occur given appropriately timed hormonal signals. Full secretory differentiation, that is, decidualization, is induced locally by paracrine signals from the blastocyst. There is virtually no clinical value to be gained from endometrial histology. Implantation success or failure is determined largely by embryo quality, which, in turn, reflects original gamete quality. The age-related decline in female fecundability is primarily the result of a decline in oocyte quality, thereby reducing implanting ability (without affecting ovulation and fertilization).

INFERTILITY CAUSES AND OUTCOME

The causes of infertility and their relative frequencies are listed in Table 1. The frequencies add up to more than 100% because some couples have more than one cause. In primary infertility, endometriosis and sperm disorders are relatively more frequent (usually present from the start), whereas in secondary infertility, tuba1 infective damage is more frequent (sometimes resulting from complications of childbirth, miscarriage, or termination of pregnancy).

Although several other conditions have been described as causes of infertility, controlled studies have not shown these entities to reduce fertility nor does corrective treatment improve the outlook. These conditions include intermittent luteal deficiency, hyperprolactinemia in ovulating women, endometrial and endotubal polyps, minor endometrial adhesions, and most fibroids (if not impinging on the uterine cavity).

Outcome Measures

Outcome related to diagnostic and therapeutic procedures is best described by pregnancy or preferably birth rates related to specific time frames. Most results have been reported as pregnancy rates, although in vitro fertilization (IVF) results are more commonly presented as birth rates. The difference is particularly important in women with a high risk of miscarriage, such as that associated with age or polycystic ovarian disease (PCO).

The cumulative chance of success for a couple depends on the duration of exposure to the chance of natural conception or to the number of cycles of active treatment. Rates given per cycle may be misleading if limited to the first one or two cycles of treatment because the rate tends to fall in subsequent cycles. For these reasons, cumulative time-specific or cycle-specific rates are presented herein whenever possible.

Cumulative rates are calculated using the life-table method, which takes into account those couples who are not followed up or actively treated as long as others. Because biases can occur leading to calculation of exaggerated success rates owing to selective reporting by successful patients or selective discontinuation of cyclical treatment, all patients are actively followed up, and the life-table method is applied to specifically defined groups of patients categorized by diagnosis, age, and duration of infertility who are treated consistently. Patients selectively withdrawn from treatment must remain in the life-table calculation as continuing failures.

For the use or a Registered Medical Practitioner or a Hospital or a Laboratory only

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Weston Medical Education Foundation of India

Office No:- 99, 9th Floor, Kalpataru Avenue, Opp. ESIC Hospital,
Kandivali (East), Mumbai - 400101. M: 9322615653 | W: www.wmefi.co.in