Male infertility

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It is estimated that infertility affects 8–12% of couples globally, with a male factor being a primary or contributing cause in approximately 50% of couples. Causes of male subfertility vary highly, but can be related to congenital, acquired, or idiopathic factors that impair spermatogenesis. Many health conditions can affect male fertility, which underscores the need for a thorough evaluation of patients to identify treatable or reversible lifestyle factors or medical conditions. Although semen analysis remains the cornerstone for evaluating male infertility, advanced diagnostic tests to investigate sperm quality and function have been developed to improve diagnosis and management. The use of assisted reproductive techniques has also substantially improved the ability of couples with infertility to have biological children. This Seminar aims to provide a comprehensive overview of the assessment and management of men with infertility, along with current controversies and future endeavours.

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Introduction

WHO defines infertility as the inability to conceive after at least 12 months of regular, unprotected sexual intercourse.[1] Infertility is a major health problem worldwide and is estimated to affect 8–12% of couples in the reproductive age group.[2] 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.[3]

Infertility causes substantial psychological and social distress,[4,5] and imposes a considerable economic burden on patients and health-care systems.[6] Early diagnosis and appropriate management can mitigate these factors. In a prospective study of 384 419 Danish men, Glazer and colleagues[7] reported a higher risk of mortality among men with male factor infertility than among men who were fertile. Ventimiglia and colleagues[8] showed that impaired male reproductive health (including poorer semen parameters and lower testosterone levels) was associated with a higher Charlson Comorbidity Index, which is a proxy of decreased general health status.[9] Severe male infertility is also associated with a greater incidence of cancer.[10] 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.[11]

There is increasing evidence that paternal health at the time of conception can affect the offspring's metabolic health and reproductive potential, through transgenerational transmission of epigenetic modifications.[12] Thus, obesity or diabetes[13] 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.[14] Men who are oligozoospermic are more likely to have metabolic syndrome than men who are normozoospermic.[15] 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%.[16,17] In 1992, a large meta-analysis by Carlsen and colleagues confirmed that sperm counts had declined by 50% during a 60-year period.[18] Subsequently, numerous studies have shown similar declines globally,[19,20] although some studies have disputed this claim.[21,22] A systematic review by Levine and colleagues[23] 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.[24–26] Although various diagnostic tests are available, their interpretation is imprecise and often subjective.[27] 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.[28] Exciting new therapies using stem cells and in-vitro sperm maturation are still experimental. This Seminar aims to review our current understanding of these issues

Search strategy and selection criteria

We searched Scopus and PubMed for relevant articles on male infertility using the search term "male infertility" in combination with the search terms "epidemiology", "etiology", "pathophysiology", "investigations", "azoospermia", "oligoasthenoteratozoospermia", "asthenozoospermia", "varicocele", "genetic abnormalities", "cryptorchidism", "testicular cancer", "obstruction", "hypogonadism", "ejaculatory dysfunction", "idiopathic", "risk factors", "diagnosis", "clinical evaluation", "sperm DNA fragmentation index", "reactive oxygen species", "genetic testing", "imaging", "management", "treatment", "antioxidant therapy", "varicocelectomy", "ART", or "omics". We selected articles mostly published in the past 5 years and highly cited older publications. We also reviewed the reference list of the retrieved articles and selected articles that discussed male infertility, were published within the last 5 years, and were not retrieved in the initial search. Highly referenced reviews and book chapters are cited to provide readers with more information and references than this Seminar can accommodate.

and provide practice guidelines for individualising and optimising the management of men with subfertility.

Causes

A multitude of causes and risk factors contribute to the increasing incidence of male infertility,[29,30] 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 syndrome,[31] 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%.[30,32–34] About 30–50% of male infertility cases are idiopathic, with no discernible cause or contributory female infertility.[35,36] Male oxidative stress infertility involves altered semen characteristics and oxidative stress, and affects about 37 million men with idiopathic male infertility.[37] Environmental or occupational exposure to toxic chemicals[38] and various lifestyle factors (eg, smoking,[39,40] alcohol consumption,[41] recreational drug use,[42–44] obesity,[45,46] and psychological stress[47]) are all potential risk factors for male infertility.[48]

***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

Evaluation

Infertility evaluation and treatment is recommended for couples who do not conceive naturally after at least 12 months of regular, unprotected sexual intercourse,[49,50] 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,[30,49] although the American Urological Association (AUA) insists on two semen analyses.[50] 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).[1] Although this distinction can narrow differential diagnosis, men classified with primary or secondary infertility should be assessed in the same way.[50]

Various childhood conditions (eg, cryptorchidism, postpubertal mumps orchitis, and testicular torsion or trauma) can result in testicular atrophy or decreased semen quality.[51–53] 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.[54] 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.[55] 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 pathogens to cause epididymitis. *E coli* is the predominant pathogen found in men older than 35 years who have infertility. Although semen analysis is not recommended in acute cases of epididymitis or prostatitis, men with chronic epididymitis or prostatitis might present with leukocytospermia (>1×10^6 white blood cells per mL), which is a sign of inflammation[30] and can be confirmed by peroxidase test in semen.[57]

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.[40–42] A large meta-analysis involving 5865 men from 20 studies showed deterioration of semen quality in moderate and heavy smokers.[40] Similarly, a meta-analysis of 15 cross-sectional studies revealed the negative association between alcohol consumption and sperm parameters.[41] Cannabis, the most frequently used recreational drug, negatively effects male fertility by inhibiting the hypothalamic–pituitary–gonadal axis, spermatogenesis, and sperm function.[58] The association between obesity and male infertility has been widely investigated as the global prevalence of obesity continues to rise.[12] Obesity-induced endocrine alterations that result in peripheral conversion of testosterone to oestrogen have been linked with reduced sperm concentrations.[59] Among the subsets of obesity, metabolically unhealthy obesity (ie, with metabolic abnormalities such as diabetes, hypertension, dyslipidaemia, insulin resistance) is known to be a risk factor for erectile dysfunction, and the combination of erectile dysfunction and metabolically healthy obesity (ie, without evidence of metabolic and cardiovascular disease) in men represents an early marker for future adverse metabolic consequences.[60]

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.[61] 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).[62] One in six men with infertility has erectile dysfunction, or premature ejaculation, or both.[63] 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.[64,65] 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.[66–68]

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,[69–72] with the

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***

most recent manual released in 2010.[57] The recommended cutoff 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[57] are derived from the statistical analysis of the semen parameters of 1953 fertile men from around the world.[74] 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.[75–77] 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.[78] Ombelet and colleagues used receiver operating characteristic curve analysis to determine the diagnostic potential and cutoff values for single and combined sperm parameters.[79] 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.[79] 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.[80,81] 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 morphology accurately,[82,83] 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.[84] Systems such as the LensHooke (Bonraybio Co, Taichung City, Taiwan) incorporate artificial intelligence to simplify semen analysis. Results of Agarwal and colleagues' prospective study[85] 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.[86] 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.[87,88] 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.[89]

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 gynaecomastia might be seen in patients with endocrinopathies (eg, low serum testosterone, Klinefelter syndrome, hyperprolactinaemia).[90,91]

The genital examination should begin with the phallus, carefully assessing for penile curvature, plaques, epispadias, 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).[92] 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.[93] A testicular mass should be ruled out, because men with infertility are at increased risk of testicular neoplasm.[94] 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.[95] 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.[92]

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 investigation for every male patient with infertility,[96] although international societies recommend limiting use to particular groups of patients, including men with a sperm concentration below 10×10^6/mL or impaired sexual function, or if endocrinopathy is suspected.[49,50]

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 hypogonadism. Prolactin analysis is also recommended in such cases.[49,50] The validity of the ASRM guidelines for hormonal evaluation of male infertility has been challenged for predicting hypogonadism.[97] A retrospective study by Ventimiglia and colleagues[97] 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 cutoff value for testosterone concentrations. The ASRM adopts the value of less than 300 ng/dL as a cutoff for diagnosing hypogonadism, and the EAU recommends 230 ng/dL (8 nmol/L).[98,99]

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 challenging. Using calculated free testosterone can be a more clinically accurate method in assessing men with hypogonadal symptoms.[99,100]

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

***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

Oligoasthenoteratozoospermia
Total number (or concentration, depending on outcome reported)* of spermatozoa, and percentages of both progressively motile and morphologically normal spermatozoa, less than the lower reference limits

Oligoteratozoospermia
Total number (or concentration, depending on outcome reported)* of spermatozoa, and percentage of morphologically normal 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

spermatogenesis and male sexual function.[101] Hyperprolactinaemia is caused by prolactinomas in 40% of cases.[102]

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.[103,104] However, in some cases of spermatogenic arrest at the level of spermatocyte or spermatid, 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.[105] A recent systematic review and clinical validity assessment of male infertility genes revealed a total of 78 genes linked to 92 male infertility phenotypes.[106] Several genes and gene mutations related to spermatogenesis have been discovered.[26,107] Men with genetic abnormalities usually show defective spermatogenesis, resulting in severe oligozoospermia or azoospermia and increased aneuploidy.[108] Genetic mutations 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.[109]

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.[110–112] 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 $<5\times10^6$/mL).[113–115] However, the EAU extended their guideline recommendations to include men with a sperm count of less than 10×10^6/mL.[30,35] The EAU also recommends obtaining karyotype if there is a family history of recurrent spontaneous abortions, malformations, or intellectual disability,[30,35] regardless of the sperm concentration.[30] This recommendation[35] 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 discrimination (59%).[116] 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 5×10^6/mL.[117] A meta-analysis by Kohn and colleagues showed that the majority of Y chromosome microdeletions occur in men with sperm counts of less than 1×10^6/mL.[118] The latest EAU guidelines recommend Y chromosome microdeletion testing if sperm concentrations are less than 5×10^6/mL, and make such testing mandatory for sperm concentrations of less than 1×10^6/mL.[30] 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.[119,120]

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.[121,122] 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.[30]

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 indicated for the diagnosis of subclinical varicocele,[123] 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.[93,124] Transrectal ultrasound can be used in combination with seminal vesicle aspiration to more accurately diagnose ejaculatory duct obstruction.[124] 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 prolactinoma) as an underlying cause of hyperprolactinaemia and hypogonadism.[125] Vasography is an invasive imaging modality to confirm patency or delineate an obstruction of the vas deferens or ejaculatory duct,[126] 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 reconstructive microsurgery.[92]

Specialised tests

Conventional semen parameters do not detect defects associated with functional aspects of spermatozoa,[127] 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.[129–131] In conventional in vitro fertilisation, defective 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.[132–134]

Sperm DNA fragmentation assays potentially provide a more comprehensive assessment of the overall fertility status than conventional semen parameters.[135] 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.[136] Although test protocols and cutoff values have substantially improved precision and decreased variations for the sperm DNA fragmentation test, the absence of strict

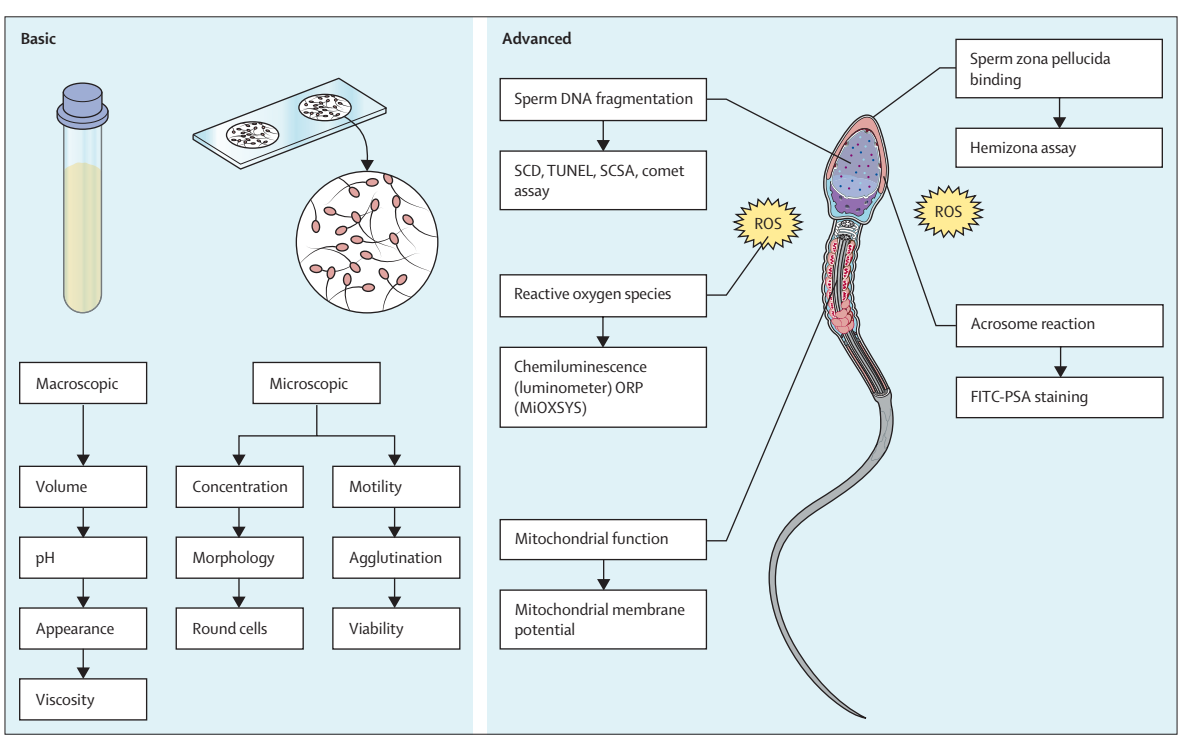

Figure 1: **Laboratory evaluation for male infertility**
Standard semen analysis comprises the analysis of macroscopic and microscopic parameters. An advanced sperm function test comprises the determination of ROS, sperm DNA fragmentation, acrosome reaction, and MMP using different techniques. FITC-PSA=fluorescein 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

standardisation and clear threshold values deter its wider application.[137] Hence, although emerging evidence supports the role of sperm DNA fragmentation in reproductive outcomes (whether natural or via assisted reproductive techniques),[49] routine use of sperm DNA fragmentation testing is not recommended by the AUA or ASRM.[30,49,50] 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[138] (panel 5). The EAU guidelines recommend sperm DNA fragmentation testing in couples with recurrent pregnancy loss, or in men with unexplained infertility.[30] 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.[138]

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.[139,140] 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,[141–143] with a cutoff value of less than 102·2 RLU/s/10^6 sperm per mL to distinguish between men who are fertile and men with infertility.[144] Seminal oxidation-reduction potential is a novel concept introduced to measure global oxidative stress in semen samples using the Male Infertility Oxidative System, which is a quick and simple test.[145] 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/10^6 sperm per mL to differentiate men with normal and abnormal semen parameters.[146] 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).[30]

Management

Azoospermia

The causes of azoospermia can be classified as pretesticular, testicular, or post-testicular. Pretesticular causes of azoospermia include endocrine abnormalities involving the hypothalamic–pituitary–gonadal axis. Although congenital and acquired hypogonadotropic hypogonadism is rare, it is one of the few medically treatable causes of male infertility. Common notable causes of hypogonadotropic hypogonadism include Kallmann syndrome and exogenous androgen excess. Hypogonadotropic hypogonadism is characterised by low concentrations of serum follicle-stimulating hormone and testosterone during the hormonal evaluation of men who are azoospermic. The combination of human chorionic gonadotropin and human menopausal gonadotropin is commonly used in clinical practice as a substitute for luteinising hormone and follicle-stimulating hormone respectively, to induce fertility in patients with hypogonadotropic hypogonadism. Successful pregnancies were reported for female partners of 16–57% of men with congenital hypogonadotropic hypogonadism after treament.[147]

Once a pretesticular cause has been ruled out, azoospermic men are categorised as having either obstructive azoospermia or non-obstructive azoospermia (figure 2). Testicular biopsy is no longer recommended to make a diagnosis. Generally, a cutoff value of 7·6 mIU/mL for follicle-stimulating hormone and a testicular long axis of 4·6 cm are used to differentiate obstructive azoospermia from non-obstructive azoospermia.[148] Patients with obstructive azoospermia have several options, including epididymal or testicular sperm retrieval for intracytoplasmic sperm injection or surgical reconstruction.[149]

Impaired spermatogenesis as a result of primary testicular failure is the most common cause of non-obstructive azoospermia. Although successful testicular sperm retrieval from men with obstructive azoospermia is highly likely, success rates in men with non-obstructive azoospermia are substantially lower.[150–152] Although sperm production in non-obstructive azoospermia is often inadequate to reach the ejaculate, the finding of heterogeneous patchy spermatogenesis on testicular biopsy, and demonstrable sperm within the testes in 60% of men with

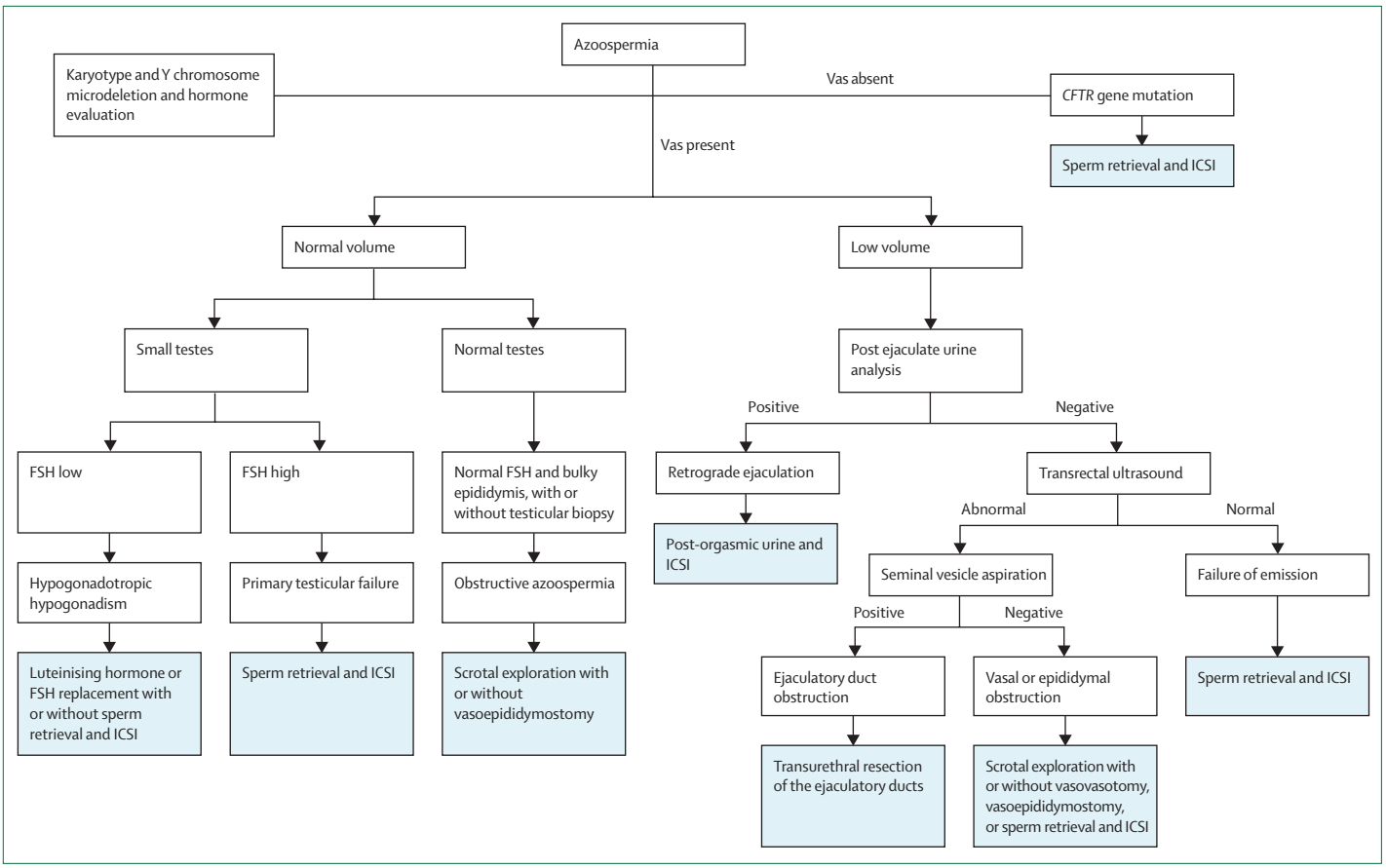

Figure 2: **Classification of azoospermia**
FSH=follicle-stimulating hormone. ICSI=intracytoplasmic sperm injection. Adapted from Agarwal and colleagues,[128] by permission of the Korean Society for Sexual Medicine and Andrology.

non-obstructive azoospermia, provide the rationale for sperm retrieval in the management of non-obstructive azoospermia.[150,151] Although testicular sperm aspiration can be done percutaneously using local anaesthetic, low sperm retrieval renders the procedure uncommon, except when used in conjunction with testicular mapping.[153] Microdissection testicular sperm extraction might be more efficient than conventional testicular sperm extraction (surgical sperm retrieval 52% *vs* 35%), on the basis of a meta-analysis of data from 15 case-controlled studies.[154] Importantly, microdissection testicular sperm extraction gets a larger quantity of sperm with less testicular tissue removed and has the lowest complication rates.[151,154] However, a subsequent meta-analysis showed no difference in sperm retrieval or livebirth outcomes between microdissection and conventional testicular sperm extraction in men with non-obstructive azoospermia.[152] Similar findings were noted in patients with Klinefelter syndrome, for whom surgical sperm retrieval and livebirth outcomes were compared after either conventional or microdissection testicular sperm extraction.[155] Further well designed RCTs are needed to clarify which technique is more efficient. Several variables (eg, surgical skill, testicular histology, cost, and risk of complications) should be considered before counselling patients about a particular sperm retrieval technique, as there is no clear recommendation about which technique to use.[30] There has been considerable debate about the role of varicocele repair in patients with non-obstructive azoospermia, because surgical sperm retrieval rates and outcomes for intracytoplasmic sperm injection have yet to be defined.[156] Despite advances in reproductive medicine, sperm retrieval is not successful in about 50% of men with non-obstructive azoospermia, leaving these men with the option of donor sperm insemination or adoption.

Varicocele

Varicoceles are dilations of the veins of the pampiniform plexus that drain blood from the testicles, and are present in 15% of healthy men and 25% of men with abnormal semen analysis.[30] The mechanism by which varicoceles affect testicular function is likely to be multifactorial, but the most commonly accepted theory includes a relative stasis of venous blood in the pampiniform plexus, which increases testicular temperature and results in elevated reactive oxygen species.[157]

The indications and surgical approach for varicocele repair have been a matter of controversy. In men with

clinical varicoceles and abnormal semen analysis, varicocele repair can significantly improve semen parameters.[30,33,158] Current guidelines do not recommend varicocelectomy in men with infertility who have a normal semen analysis, or in men with a subclinical varicocele. However, varicocele repair is recommended for men with infertility who have clinical varicocele, abnormal semen parameters, and unexplained infertility with a female partner who has healthy hormone levels indicating good egg counts.[30] A systematic review and meta-analysis concluded that varicocele repair improved livebirth outcomes following assisted reproductive technology procedures, even if semen parameters did not improve.[159]

Surgical repair is the primary treatment approach for varicocele, and radiological percutaneous embolisation is a viable alternative.[160] Varicocelectomy can be done through retroperitoneal, laparoscopic, or robot-assisted laparoscopic, microsurgical inguinal, or subinguinal approaches.[161] There is no substantial difference in the success rates between the different surgical approaches, but microsurgical subinguinal varicocelectomy has been considered the gold standard on the basis of a lower risk of varicocele recurrence (0·4%) or postoperative hydrocele formation (0·44%) than other approaches.[30,32] Varicocelectomy can improve semen parameters and reduce oxidative stress, potentially sparing couples from costly assisted reproductive technology procedures.[32]

Idiopathic male infertility

In men with idiopathic infertility, despite completing diagnostic investigations, the cause of altered semen parameters cannot be identified.[35] Current treatment of idiopathic male infertility consists of assisted reproductive technology or empirical medical therapy, which includes lifestyle improvement and hormonal or non-hormonal therapy. Lifestyle modifications (eg, weight loss, physical activity, and cessation of smoking) are important non-invasive measures,[30] and have been linked to improved sperm parameters.[162–165]

The mainstays of hormonal empirical medical therapy are selective oestrogen receptor modulators and aromatase inhibitors. Selective oestrogen receptor modulators (specifically clomiphene citrate) have been used off-label to improve semen parameters, but there are too few high-quality RCTs to prove its efficacy conclusively.[166,167] Exogenous testosterone should not be used for male infertility treatment because it inhibits spermatogenesis.[168]

The role of oxidative stress as a cause of male infertility is supported by elevated seminal oxidation-reduction potential in 80% of men with infertility.[37] Because oxidative stress is potentially reversible, this provides an opportunity for treatment. As a result, oral antioxidants are the most commonly adopted empirical medical therapy. Although there is heterogeneity across studies in the literature, a systematic review showed the efficacy of antioxidant therapy in improving semen parameters and reducing oxidative stress in men with infertility.[169] A 2019 Cochrane review that meta-analysed 61 RCTs in 6264 men with subfertility treated with a combination of antioxidants, reported low-quality evidence suggesting improvement in the proportion of clinical pregnancies and livebirths with antioxidant supplementation.[170] The review recognised important limitations, including low-quality RCTs with serious risk of bias owing to poor reporting of randomisation methods, failure to report on clinical outcomes (eg, livebirths, clinical pregnancies), high attrition rates, and imprecision owing to an often low number of events and small overall sample sizes.[170] Further large-scale RCTs reporting clinically relevant outcomes are therefore necessary before an optimal antioxidant regimen can be recommended.

Role of assisted reproductive technology

The use of assisted reproductive technology has substantially improved the ability of couples with infertility to have biological children. For intrauterine insemination, progressively motile sperm are separated from the semen and inseminated directly into the uterine cavity during the time of ovulation. In cases of more severe male factor infertility, conventional in vitro fertilisation or intracytoplasmic sperm injection can be used. Despite the success of these techniques, some couples still have poor outcomes, which might result from the poor quality of the egg, or sperm, or both. Lee and colleagues showed that intracytoplasmic sperm injection cycles using sperm from men with severe oligoasthenoteratozoospermia and non-obstructive azoospermia had worse outcomes in terms of embryo implantation and clinical pregnancy than for men who were normozoospermic.[171] This finding shows the importance of the paternal contribution, and the need to select the best sperm before intracytoplasmic sperm injection. Optimal management of couples with infertility should involve correction of sperm defects, even for couples destined for assisted reproductive technology. The use of testicular derived sperm is of growing importance, because testicular sperm can have lower amounts of sperm DNA fragmentation compared with ejaculated sperm.[172] As such, testicular sperm extraction–intracytoplasmic sperm injection can be used in men who are not azoospermic but have elevated sperm DNA fragmentation and have had previous failed intracytoplasmic sperm injection cycles. However, additional evidence is needed to support this practice in the routine clinical setting.

Future therapies and challenges

Advancements in the current era of omics technologies facilitate the diagnosis and management of male infertility at genetic, molecular, and cellular levels. Next-generation sequencing technologies, such as disease-targeted sequencing, whole exome and genome sequencing, and epigenetic analysis of sperm, are promising techniques in genetic testing.[173] Next-generation

sequencing technologies have enabled identification of novel candidate genes associated with male infertility conditions such as azoospermia,[174] oligozoospermia,[175] and idiopathic male infertility.[107] Discoveries on the role of small RNAs and microRNAs in epigenetic regulations,[176] and their involvement in spermatogenesis and epididymal sperm maturation, have expanded current understanding of these processes.[177–180] Metabolic fingerprinting of seminal plasma is another promising area of research, especially in cases of idiopathic male infertility.[181] A study published in 2019 found that reactive oxygen species-induced epigenetic alterations of sperm DNA and seminal metabolic profile were correlated with semen quality in men with infertility who were normozoospermic.[182]

The paradigm shift to proteomic research of male reproduction has revealed several proteins as biomarkers that are associated with various causes of male infertility, such as oxidative stress-mediated sperm dysfunction,[183,184] varicocele,[176,185,186] asthenozoospermia,[187,188] globozoospermia,[189,190] and testicular cancer.[191,192] A major problem is the identification of a unique biomarker associated with a specific condition. Strategically, correct diagnosis can be achieved by developing a protein biomarker panel with high specificity for the diagnosis of a particular male infertility condition. Also, before the clinical implementation of omics findings, it is vital to identify the applicability of suitable omics data or their combination with proper clinical validation.[193]

The future diagnostics and management of male infertility are moving towards the fusion of andrology with artificial intelligence, using intensive machine learning. Algorithms are being developed to predict which men are azoospermic and might require genetic investigation, sperm detection, and selection for assisted reproductive technology and embryo selection for in vitro fertilisation.[194] The use of artificial intelligence in andrology and assisted reproductive technology is still in its early phase and comes with ethical issues, hence further comprehensive and extensive research is warranted.[195,196]

In the past decade, research in male reproduction has seen substantial advancements in next-generation therapeutics using stem cells. Different in-vitro methods and organ models using embryonic stem cells, induced pluripotent stem cells, and glioblastoma stem cells were developed for successful production of spermatozoa.[197] Fang and colleagues highlighted the possible use of human induced pluripotent stem cells in the therapeutics of male infertility.[198] Human induced pluripotent stem cells can potentially be used to rebuild spermatogenesis, and in the CRISPR-Cas9 gene editing technique to correct genetic disorders. Furthermore, human induced pluripotent stem cell-derived exosomes might hold therapeutic implications in regaining spermatogenic function in patients who have had chemotherapy or radiotherapy.[198] Similar regenerative and self-renewal characteristics possessed by spermatogonial stem cells have opened up new perspectives in the therapeutics of male infertility.[199,200] Autografting cryobanked spermatogonial tissue was proposed as a new strategy of fertility preservation for paediatric patients who have undergone gonadotoxic therapy.[201] However, several barriers, including ethical issues and the risk of transmitting genetic insults to the offspring during in vitro culture of stem cells, must be overcome before stem cell therapy can be used for the management and treatment of male infertility.

Contributors

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Declaration of interests

We declare no competing interests.

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