Male fertility and infertility

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Abstract
Male factor infertility accounts for 30–50% of infertility cases. Over the past two decades, there have been several papers published describing declining numbers and quality of sperms. An increasing incidence of urogenital abnormalities in the new born and rising incidences of testicular cancer from many different countries have stimulated public interest and concern about male infertility. In this review different aetiological factors in male infertility are considered with an attempt to provide an up-to-date account on the investigations and management of male infertility.

Keywords male infertility; semen analysis; spermatozoa; testicular factors; intracytoplasmic sperm injection; donor insemination

Introduction
The true incidence of male infertility is unknown due to great variability in the prevalence of infertility. However in approximately 30–50% of sub-fertile couples the male partner has suboptimal semen quality, either because of low sperm count, poorly motile sperm or sperm with abnormal size and shape (morphology). In more than 50% of male infertility cases, the aetiology remains unknown and the infertility is classified as idiopathic. Male infertility evaluation must go far beyond a simple semen analysis which has to be complemented with a comprehensive history and physical examination as well as relevant endocrine, genetic and other investigations.

The testis comprises of two distinct components, the seminiferous tubules (the site of spermatogenesis) and the Leydig cells (the source of testosterone). The process of spermatogenesis is directed by genes located on the Y chromosome and takes approximately 70 days to complete from the spermatocyte stage. Another 12–21 days are required for the transport of sperm from the testis through the epididymis to the ejaculatory duct. During passage through the epididymis, sperm mature further to develop the capacity for sustained motility. The long time required for sperm development and transit implies that the results of a semen analysis reflect conditions existing many weeks earlier. Semen includes secretions contributed by the prostate, the seminal vesicles, and the distal vas deferens (Figure 1).

Causes of male infertility
This can be classified as shown in Table 1.

Evaluation of male infertility
The diagnosis of male infertility involves taking a thorough medical history, physical examination followed by laboratory tests such as semen analysis.

The medical history should include the following:
- Developmental history — testicular descent, age of puberty, change in the shaving
- Frequency and loss of body hair
- Infection history — Mumps, sexually transmitted diseases, prostatitis
- Surgical repair — Hernia repair, vasectomy
- Drugs/Environmental — Smoking, alcohol, anabolic steroids, chemotherapy, toxic chemicals and radiation
- Sexual history — Libido, frequency of intercourse and previous fertility assessment
- Chronic medical illness

Physical examination (Table 2): examination of the urogenital system is always recommended to preclude the possibility of testicular cancer and also to look for symptoms of sexually transmitted infection.

Testicular ultrasound (Figure 2)
Testicular ultrasound is frequently utilised in order to assess the scrotal contents for testicular volume and morphology. This non-invasive test substantially detects more pathological conditions compared to clinical examination. Additionally, this may give indirect evidence of the presence of possible reversible pathology in the form of obstructive azoospermia. Further imaging in the form of transrectal ultrasound and Magnetic Resonance Imaging (MRI) is then often able to categorise the level of obstruction and facilitate treatment planning.

Semen analysis: The semen analysis should be performed according to the World Health Organization (WHO, 2010) laboratory manual for examination and processing of human semen (Tables 3 and 4). The sample should be collected after 2–7 days of sexual abstinence, preferably at the fertility clinic by masturbation. A study in 2006 by Castilla et al. showed a large biological variability in semen quality when five healthy young volunteers assessed by WHO recommended methods over a one and a half year period. If first analysis is abnormal, biologically, the optimal time for the second sample is at least three months after the initial sample because the cycle of spermatozoa formation takes about three months to complete. However, this delay may cause anxiety and the timing of the second sample and should take into consideration the preferences of the man. If azoospermia or severe oligozoospermia is reported in the initial semen analysis, a repeat test should be undertaken within two to four weeks.

Less than 1% of men are truly sterile and do not produce any spermatozoa (i.e. are consistently azoospermic).

Hormone analysis: if repeat semen analysis demonstrate severe oligozoospermia (<5 million spermatozoa/ml) or azoospermia, then basal serum follicle-stimulating hormone (FSH), luteinising
hormone (LH), and testosterone will be valuable. If serum concentrations of FSH, LH, and testosterone are normal and the man has azoospermia, a post-ejaculatory urine sample will provide evidence about retrograde ejaculation if sperm are seen in the urine. If spermatozoa are not present in the post-ejaculatory urine, the man has obstructive azoospermia or impaired spermatogenesis. Low serum FSH, LH and testosterone warrants gonadotrophin treatment (secondary hypogonadism). High serum FSH, LH and low testosterone indicate primary hypogonadism (Testicular failure). Men with low sperm counts and low LH (and FSH) who are well-androgenised should be suspected of anabolic steroid abuse (exogenous testosterone suppresses intratesticular testosterone production, which is an absolute prerequisite for normal spermatogenesis.) The serum testosterone can be low, normal, or high depending upon the specific substance taken. Sperm production recovers in most men when they stop using anabolic steroids, however, this process can take months to years. Prolactin should be measured in men who complain of reduced libido and have low serum testosterone. Low serum inhibin B may be a more sensitive indicator of primary testicular dysfunction than high FSH.

Genetic testing: if an ejaculate contains less than 5 million sperm/ml, then tests for cystic fibrosis carrier status, karyotype and a Y chromosome micro deletion is recommended. The blood tests for screening for cystic fibrosis analyses the most common mutation seen in a selected population group. The values vary depending on the ethnic origin of the patient. In patients with Congenital bilateral absence of Vas Deferens (CBAVD), non classical cystic fibrosis mutation might be present that is not detected by routine screening. Therefore it is prudent to offer both partner screening to establish the carrier status. Cytogenetic abnormalities have been observed in 10–15% of azoospermic men and in ~5% of men with oligospermia. Additionally, inversions and translocations of autosomes are observed at a higher frequency among infertile men than in the general population. Y microdeletions have a frequency of ~2–10% or higher among infertile men, depending on the population studied. Three regions of Yq (AZFa, AZFb and AZFc) have been shown to be deleted. Deletions of AZFc are the most common (~60%).

Sperm function test
There are specific sperm function tests and these include measuring the ability of sperm to:

- enter and make progression in mid-cycle cervical mucus (sperm mucus penetration tests)
- hyperactivate following capacitation
- bind to the zona pellucida
- undergo the acrosome reaction
- penetrate zona-free hamster eggs.

DNA fragmentation and fluorescent in situ hybridization testing are replacing some of the previously used evaluations of sperm function. However, both the American Society for Obstetrics, Gynaecology and Reproductive Medicine 24:11

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Reproductive Medicine (ASRM) and the European Society for Human Reproduction and Embryology (ESHRE) have recently reviewed the evidence base for sperm DNA testing and have concluded, for the time being at least, that there is insufficient evidence for such tests to be offered on a routine basis.

### Treatment options for male infertility

#### Lifestyle changes
- Men who have a BMI of 30 or over should be informed that they are likely to have reduced fertility and should be encouraged to lose weight.
- There is an association between smoking and reduced semen quality (although the impact of this on male fertility is uncertain) and that stopping smoking will improve their general health.
- Excessive alcohol intake is detrimental to semen quality, alcohol consumption within the Department of Health’s recommendations of 3–4 units per day for men is unlikely to affect their semen quality.
- There is an association between elevated scrotal temperature and reduced semen quality but it is uncertain whether wearing loose-fitting underwear improves fertility.
- There is no consistent evidence of an association between consumption of caffeinated beverages (tea, coffee and cola) and fertility problems.

### Medical treatment
Men with hypogonadotrophic hypogonadism should be offered gonadotrophins because these are effective in improving fertility. If the patient is on testosterone replacement, testosterone treatment should be stopped. HCG pre-treatment is

### Pathophysiological factors

#### Pathophysiological classification of male infertility

**Hypothalamic pituitary disease**
- **Congenital**
  - Congenital GnRH deficiency (Kallmann syndrome)
  - Genetic disorders that affect multiple organs (e.g. Prader–Willi and Laurence–Moon–Biedl syndromes)
  - Haemochromatosis
- **Acquired**
  - Pituitary and hypothalamic tumours
  - Trauma (surgery, irradiation)
  - Vascular
  - Acromegaly, cushings, hyperprolactinemia
  - Infiltrative disorders (sarcoidosis, tuberculosis)
  - Hormonal (↑prolactin, ↑testosterone, ↑oestrogen)
  - Systemic disorders (chronic illness, malnutrition, morbid obesity)

**Testicular disease**
- **Congenital**
  - Klinefelter's syndrome (47XXY)
  - Cryptorchidism (undescended testis)
  - Y chromosome microdeletions
  - Defective androgen synthesis or response (5α-reductase deficiency, androgen insensitivity)
- **Acquired**
  - Varicocele
  - Infection (e.g: mumps orchitis, chlamydia, gonococci)
  - Drugs (e.g: cimetidine, sulfasalazine, steroids, spironolactone, nitrofurantoin)
  - Environmental toxins
  - Smoking, alcohol
  - Hyperthermia
  - Antisperm antibodies
  - Testicular torsion and trauma
  - Testicular cancer, chemotherapy and radiotherapy
  - Systematic disorders

**Sperm transport problems**
- Obstruction in the epididymis, vas deferens, seminal vesicles and prostate
- Defective ejaculation
- Vasectomy

**Unexplained**
- Unexplained (idiopathic)
commenced to normalise testosterone and this can take up to 3–6 months. Once testosterone is normalised, spermatogenesis is augmented by using a combination of FSH and LH. Men with idiopathic semen abnormalities should not be offered anti-oestrogens (e.g. clomiphene citrate, tamoxifen), gonadotrophins, androgens, bromocriptine or kinin-enhancing drugs because they have not been shown to be effective. Oxidative stress (OS) has been identified as one of the many mediators of male infertility by causing sperm dysfunction. This explores the benefits of using antioxidants (such as Vitamin C, E and glutathione) in a clinical setting. There is currently insufficient evidence about the timing, duration and dosage of antioxidants that is most beneficial. Clearly this is an area for further study.

__Surgical treatment__

**Obstructive azoospermia:** obstructive azoospermia is suggested by the combination of azoospermia, normal size testes, normal serum FSH, LH and testosterone. Both surgery and assisted reproductive treatment (ART) may be options for such patients. Obstruction of the epididymis can be treated by surgical correction. The results are variable and depend on the site of reanastomosis, the skill of the operator and the duration of obstruction. Vasectomy reversal has been found to be more successful and cost-effective than microsurgical epididymal sperm aspiration followed by In vitro fertilisation (IVF) or Intra-cytoplasmic sperm injection (ICSI). Vasectomy reversal is a low risk procedure and reversal may be performed with a high degree of success, particularly with a short obstructive interval (97% patency if performed <3 years following vasectomy). Longer obstructive interval is associated with lower patency and pregnancy rate and ART using surgical sperm recovery (SSR) followed by ICSI is recommended. CBAVD is a consistent feature of men affected with cystic fibrosis. Men with CBAVD and their partners should be offered genetic screening and counselling.

**Surgical sperm recovery (SSR):** SSR is offered for men with non obstructive azoospermia, some cases of obstructive azoospermia, idiopathic azoospermia, failed reversal of vasectomy, and congenital bilateral absence of vas deferens. The aim of this procedure is to obtain sperm for ICSI. This can be done as a diagnostic procedure with storage of sperms for a future use or as treatment procedure.

- Percutaneous epididymal sperm aspiration (PESA): in PESA sperms are aspirated through a 21G butterfly needle that is placed into the caudal portion of the epididymis on the selected side at different angles allowing for suction of secretion (Figure 3).
- Testicular sperm extraction (TESE) is where the seminiferous tubules are extracted using a 19G butterfly needle directly from the testes.
WHO (2010) reference values The fertile ranges are a sperm concentration of ≥15 million/ml, vitality of 58%, progressive motility of ≥32% and morphological normal forms of ≥24%

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Lower reference limit</th>
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<tbody>
<tr>
<td>Semen volume (ml)</td>
<td>1.5 (1.4–1.7)</td>
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<tr>
<td>Total sperm number (10⁹ per ejaculate)</td>
<td>39 (33–46)</td>
</tr>
<tr>
<td>Sperm concentration (10⁶ per ml)</td>
<td>15 (12–16)</td>
</tr>
<tr>
<td>Total motility (PR + NP, %)</td>
<td>40 (38–42)</td>
</tr>
<tr>
<td>Progressive motility (PR, %)</td>
<td>32 (31–34)</td>
</tr>
<tr>
<td>Vitality (live spermatozoa, %)</td>
<td>58 (55–63)</td>
</tr>
<tr>
<td>Sperm morphology (normal forms, %)</td>
<td>4 (3.0–4.0)</td>
</tr>
</tbody>
</table>

Table 3

- Micro TESE is where an operating microscope is used to identify dilated seminiferous tubules and sperm extracted from them.
- Scrotal exploration and testicular biopsy is used when all the above have failed to obtain sperm. The risks associated with testicular biopsy include pain, infection, hae-matoma, atrophy of the testis and fibrosis at the aspiration site.

Intrauterine insemination (IUI)/Donor insemination (DI): men who ejaculate reasonable numbers of sperm and from which 5 million sperm per ml or more can be isolated using sperm washing techniques, IUI has been a common treatment option previously. Clearly, patent fallopian tubes in a female partner are a pre requisite for fertility. A NICE clinical guideline for people with fertility problems (2013) recommends that IUI has no role in mild male factor infertility and should not be routinely offered.

Donor insemination is the alternative treatment for many couples with male infertility, including those who failed to conceive with ART with severe male factor infertility, single woman parent and female same-sex couples. Sperm donors are rigorously screened for sexually transmitted diseases and genetic conditions. The semen sample is then quarantined for 6 months storage before using for any treatment. From April 2005 children conceived using donor sperm are able to trace their biological parent. The pregnancy rate per treatment cycle is around 15% using insemination. This alternative, together with adoption and childlessness, should be offered in appropriate couples with male factor infertility.

Retrograde ejaculation can be treated with IUI using the male partner’s spermatozoa collected after alkalisation of the urine and washing of the sperm. Alternatively, the spermatozoa can be used for IVF or ICSI.

IVF/ICSI: IVF is a reproductive technology in which stimulation of the ovaries with gonadotrophins and the aspiration of oocytes from the ovarian follicles. These oocytes are then fertilised in vitro. ICSI involves the direct injection of a single spermatozoon into the cytoplasm of an oocyte (Figure 4). It has revolutionised the treatment of men with very severe oligoasthenoteratozoospermia, obstructive and nonobstructive azoospermia. When there are no sperm in the ejaculate, ICSI can be performed with spermatozoa isolated from testicular fine needle aspiration or testicular biopsy. Successful pregnancy has been reported even with injection of immature sperm cells, such as elongated spermatids. Certain genetic and developmental defects in a very small number of children born using ICSI treatment have been reported. It is still not known whether the risks associated with ICSI are related to the procedure or to inherent sperm abnormalities. More boys conceived by ICSI were found to have undescended testis and required urogenital surgery. The risk of hypospadiasis which had previously been associated with ICSI was no longer significantly different from the risk in the general population. A recent study (2013) of 106,013 children born after assisted conception found no increased risk of cancer in ART children. Several studies found no significant differences in the long-term developmental outcome of ICSI offspring. In men with Klinefelter’s syndrome and those with Y micro-deletions, their offspring might carry the same gene or have an increased risk of sex chromosome aneuploidy. If ICSI is deemed the most suitable method in the therapeutic armamentarium, then the associated risks of birth defects must be conveyed to the couple. It is imperative that the children being born through ICSI and other ART continue to be monitored.

Intracytoplasmic morphologically selected sperm injection (IMSI): IMSI is a real time method where sperms are selected before the microinjection into the oocytes. This is a variation of ICSI that uses an inverted microscope that is able to provide a much greater magnifying power (around 6000 times) than those that are normally used in reproductive laboratories (400 times) to carry out ICSI. With this microscope, embryologists can see the

Nomenclature related to semen quality

<table>
<thead>
<tr>
<th>Oligozoospermia</th>
<th>Total number (or concentration) of spermatozoa below the lower reference limit</th>
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<tbody>
<tr>
<td>Asthenozoospermia</td>
<td>Percentage of progressively motile (PR) spermatozoa below the lower reference limit</td>
</tr>
<tr>
<td>Teratozoospermia</td>
<td>Percentage of morphologically normal spermatozoa below the lower reference limit</td>
</tr>
<tr>
<td>Azoospermia</td>
<td>No spermatozoa in the ejaculate</td>
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Table 4
internal morphology of sperm and discard those with abnormalities. Being able to select the sperm without morphological alterations is believed to increase the chances of successful implantation of embryo and decrease the likelihood of miscarriage. This technique still needs well designed studies to demonstrate its efficacy and validity to be used routinely in the laboratory of assisted reproduction and to be recommended with sufficient scientific evidence.

Physiological intracytoplasmic sperm injection (PICSI): this is yet another new technique using hyaluronan, a protein that naturally occurs in human cells as well as in the membrane surrounding the egg. The sperm is added to the culture dish with hyaluronan. The mature and structurally sound sperm will bind to hyaluronan. Research shows that sperm that binds to hyaluronan has a lower probability of chromosomal abnormalities and higher DNA integrity. Large multicentre trials are ongoing in the United Kingdom (UK) to find the efficacy of this particular technique.

Controversies in male infertility

Genital infections: the presence of increased leucocytes in the semen may decrease the functional capacity of the sperm by the release of reactive oxygen species. These men are sometimes labelled as having chronic prostatitis, but specific organisms are rarely identified at culture. The poor results of any antibiotic treatment make it difficult to demonstrate a causal relationship between genital infections and male infertility. However, infections can lead to obstruction of the epididymis and vas deferens and therefore antibiotics treatment is warranted.

Sperm autoantibody: Antisperm antibodies (ASAB) are produced after trauma, operations such as vasectomy and infection of the genital tract. Autoimmunity to spermatozoa occurs due to a breach in the blood–testicular barrier. Diagnosis of ASAB is by mixed antiglobulin reaction (MAR) test or the immunobead test (IBT). There are three types of ASAB. IgG and IgM are mainly found in the serum whereas IgA is found locally in the genital tract. They impair sperm motility, reduce sperm penetration in the cervical mucous and affect the capacitation and acrosomal reaction interfering with sperm oocyte interaction. Detection of ASAB is not diagnostic of infertility. Infertility from ASAB is possible if more than 50% of sperm are bound to the sperm antibodies. Immunosuppressant therapy (i.e. corticosteroids) is very controversial for a number of reasons. In placebo/double blind crossover trials there is no agreement with the results.
(pregnancy rates), dosage of corticosteroids and the duration of treatment. There were significant side effects to show improvement in ASAB suppression (Cushingoid features with decreased bone mineral density occurred in 60% of patients). ICSI is the treatment of choice at present. NICE clinical guidelines (2013) do not recommend any treatment for ASAB.

**Varicocele:** varicoceles are abnormally dilated testicular veins (pampiniform plexus) in the scrotum, which is normally secondary to internal spermatic vein reflux. Varicocele is found in approximately 15% of the general population. While there is a higher rate of infertility in men with a varicocele compared to those without, most men with varicoceles are not infertile. The causal relationship between varicocele and male infertility has been ascribed to increased testicular temperature. The NICE clinical guideline (2013) recommends that men should not be offered surgery for varicoceles as a form of fertility treatment because it does not improve pregnancy rates.

**Undescended testis:** the incidence of undescended testes (cryptorchidism) is 2–5% in new born baby boys and is more common in preterm boys. The incidence decreases to 1–2% spontaneously at 3 months of age. Aetiology is multifactorial. The maldescent may occur in the transabdominal or inguinal phase and may be due to endocrine disruption, defective anti-mullerian regulation of abdominal descent and gene deletion expressed on Leydig cells affecting the androgen production. The most important long-term complications of undescended testes include infertility and testicular cancer. Orchidopexy performed at the early age between 6 and 12 months can result in a catch up of growth (versus orchidopexy at 3 years of age) and may possibly improve spermatogenesis. Orchidopexy also reduces incidence of malignancy. Testicular atrophy due to vascular damage is a serious complication of orchidopexy. Although hormonal treatment with human chorionic gonadotropin (hCG) helps in testicular descent in 15–20% cases, one-fifth of these re-ascend later on. Also, treatment with hCG may be harmful to future spermatogenesis through increased apoptosis of germ cells. Hormone treatment therefore has no place in the treatment of maldescended testes.

**Sperm cryopreservation:** there is an increase in referrals for fertility preservation for men for medical, social and work reasons. Sperm cryopreservation should be offered before starting chemotherapy, radiotherapy or before any gonadotoxic therapy that is likely to affect their fertility. Most of the laboratories in the UK use freezing in liquid nitrogen vapour as the preferred cryopreservation technique for sperm. Cryopreservation of sperm was initially offered for a period of 10 years and then continued storage beyond 10 years (up to a maximum of 55 years) to men where there is a risk of significant infertility.

**Emotional impact:** for most men, the initial appointment at the fertility clinic is the first time they have had to answer personal questions and to be examined intimately. It is to be noted that most men will be unable to share their thoughts with anyone else. The psychological and social issues associated with the diagnosis and treatment of male infertility cannot be underestimated. It is absolutely vital to provide appropriate support and counselling with locally available resources.

**Future perspective:** during the past few years a considerable progress in the derivation of male germ cells from embryonic pluripotent stem cells has been made. Stem cells are considered as potentially new therapeutic agents for the treatment of male infertility. In vitro derived sperms provide the possibility of having genetically related children for people who currently cannot produce sperm on their own. Scientists are concerned that the very complex process needed to generate them has great potential for chromosome abnormalities and other severe genetic problems.

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**Practice points**

- Poor semen quality contributes to the sub-fertility in 30–50% of couples undergoing IVF.
- A male infertility evaluation must go far beyond a simple semen analysis, as it has to be complemented by a comprehensive history taking, physical examination, and relevant endocrine, genetic, and other investigations.
- Men with hypogonadotropic hypogonadism should be offered gonadotrophins drugs which are effective in improving fertility.
- No role for IUI with partner’s sperm for male factor infertility except in couples with physical, ethical or moral objection to IVF/ICSI.
- Surgical sperm recovery in the form of PESA, TESE and open testicular biopsy are performed to obtain sperm for ICSI.
- ICSI has revolutionised the treatment of male infertility with newer advancements like IMSI and PICSI.
- Sperm cryopreservation is commonly used for fertility preservation in men before gonadotoxic therapy.
- Embryonic stem cells are considered as potentially new therapeutic agents for the treatment of male infertility.