Male fertility and infertility

Allan A Pacey

Abstract
In 30–50% of sub-fertile couples the male partner has poor semen quality, either because of low sperm count, poorly motile sperm or sperm with abnormal size and shape (morphology). This may result from inherent genetic factors, poor pre-natal testicular development or adult exposure to gonadotoxic agents. There are few proven therapies to improve semen quality. Therefore, the number and quality of sperm that can be recovered from the ejaculate, or obtained by surgical sperm recovery, determines the available options for assisted conception. In recent years, the reliance upon donor sperm has reduced markedly as intracytoplasmic sperm injection can be attempted. Reproductive technologies are considered relatively safe, with healthy babies born; however, there are some concerns about potential reproductive problems in males conceived in this way. These may be related to the techniques used, or the underlying infertility of the parents, and are currently the subject of follow-up studies.

Keywords donor insemination; intracytoplasmic sperm injection; male fertility; semen analysis; spermatozoa

Introduction
In most males the onset of sperm production (spermatogenesis) begins at puberty, typically between the ages of 13 and 15 years old. Once spermatogenesis has started, evidence suggests that in most males it continues almost constantly until death and the current consensus is that there is no significant reduction in the number, or concentration, of sperm ejaculated with age. Typically, the ejaculates of fertile men can contain up to several hundred million sperm per ml, although this is highly variable both within and between individuals.

The production of sperm takes approximately 3 months and is a consequence of both mitotic and meiotic activity leading to cell proliferation and a reduction in the number of chromosomes from diploid to haploid. An appreciation of the timeline of sperm production is important to recognise when attempting to advise patients over the potential benefit of lifestyle changes in improving semen quality.

Allan A Pacey BSc PhD is a Senior Lecturer in Andrology at the University of Sheffield, Academic Unit of Reproductive and Developmental Medicine, School of Medicine and Biomedical Sciences, The University of Sheffield, Sheffield, UK.

Studies have attempted to characterise the sperm that are able to ascend the female reproductive tract, and bind to the unfertilised oocyte, and these provided the opportunity for scientists to establish the optimum size and shape of sperm. This information is of value in clinical practice as part of diagnostic procedures. Current evidence suggests that ideal human sperm are at least 50 μm in length with an oval head that should be between 4.0 and 5.0 μm in length, and 2.5 to 3.5 μm in width, when observed on a dried fixed smear stained with Papanicolaou. A recent study of the semen quality of 889 men prior to vasectomy found that the mean percentage (± standard deviation) of normal sperm morphology by this criterion was 17.57 ± 9.50.

Incidence of male infertility
Population estimates of male infertility are difficult to establish reliably. General census data of semen quality is always biased towards those with experience of infertility and few cross-sectional studies of the general (fertile) population are available. However, it is known that there is a male factor problem contributing to the infertility in 30–50% of all couples undergoing in vitro fertilisation (IVF). Given that there are over 1000 cycles of IVF performed per million European inhabitants per year, male infertility is experienced by a substantial number of men.

Causes of male infertility
Male infertility may be due to a number of different reasons including

- diseases of the hypothalamus and pituitary gland that affect the endocrine signals to the testes or prevent adequate testicular development at puberty
- disorders at the testicular level that may affect the rate or the quality of sperm production
- dysfunction of the seminal ducts that prevents or inhibits sufficient numbers of sperm being ejaculated
- disorders of sexual function and/or ejaculation that interfere with intromission, such as spinal cord injury.

Each of these largely results in ejaculates containing either too few sperm (oligozoospermia), insufficiently motile sperm (asthenozoospermia) or insufficient proportions of morphologically normal sperm (teratozoospermia) to allow any reasonable chance of unassisted conception occurring within 1 year. Less than 1% of men are truly sterile and do not produce any spermatozoa (i.e. are consistently azoospermic).

In recent years, concern has been growing over whether or not male infertility is becoming more common. This has been highlighted by studies and meta-analyses that have suggested in many parts of the world there has been an apparent drop in semen quality alongside an increase in the incidence of developmental disorders of the male reproductive tract seen in newborns (e.g. cryptorchidism and hypospadias) and an increase in the incidence of germ cell tumours among young men. These pathologies are now thought to be linked by a common mechanism, termed testicular dysgenesis syndrome (TDS). This is thought to occur as a result of a disruption of gonadal development during critical neonatal stages (Figure 1).

Whether or not the apparent reduction in semen quality and associated increase in male reproductive problems is genetically
and clinical manifestations of testicular dysgenesis syndrome (TDS) and how direct effects on the male foetus in utero can lead to the appearance of a suite of phenotypes such as reduced semen quality, testicular cancer, maldescent or hypospadias. Redrawn from Skakkaæbaek et al. 2001 and reproduced with kind permission of Oxford University Press.

or environmentally driven, some authors have suggested that a general decline in male fertility may explain why there has been a significant fall in the birth rate in many developing and industrialised countries. In some countries as many as 20% of young men may have sperm concentrations below the World Health Organization (WHO) reference ranges (see Table 1) and therefore, in theory, may be more likely to require assisted conception to be able to conceive with their partner. However, the fact that there is now a greater use of contraceptives among couples, as well as the active decision to wait until the female partner is older (and therefore less fertile herself) before they have children, makes it difficult to separate biology from social factors as an explanation for the apparent reduction in birth rate.

**Diagnosis**

The diagnosis of male infertility involves an exploration of the patient’s medical history and physical examination followed by laboratory tests such as semen analysis (see below) and, if indicated, genetic tests such as karyotype and tests for cystic fibrosis carrier status. The WHO, the Royal College of Obstetricians and Gynaecologists (RCOG) and the National Institute for Health and Clinical Excellence (NICE) have each produced guidelines for the evaluation of the infertile male. However, physical examination is always recommended to preclude the possibility of testicular cancer and also symptoms of sexually transmitted infection. As a typical rule of thumb, when an ejaculate contains less than 5 million sperm, then tests for cystic fibrosis carrier status and a karyotype should be performed.

**Semen analysis**

The laboratory techniques of semen analysis are defined by the WHO. However, in spite of these, significant variation can exist in the data generated between laboratories that analyse aliquots of the same sample circulated for quality assurance purposes. It has been shown that the most precise measurements are made when semen analysis is performed in specialist andrology laboratories rather than general laboratories available in many hospitals (including embryology laboratories). The danger is that without adequate precision, the results may guide a doctor away from some of the supplementary tests outlined in Figure 2. Clinicians should, therefore, be aware of such variability and make efforts to understand the robustness of the measures generated in the laboratories they use to undertake semen analysis on their patients.

In spite of the problems in performing semen analysis, follow-up studies of couples attempting to conceive show that where semen analysis is performed robustly, there are good relationships between the individual measures of semen quality obtained (concentration, motility and morphology) and the probability of conception. A study in Denmark following the conception attempts of 430 Danish couples (in which the men were aged 20–35 years) found that over a 6-month period there was an increasing probability of conception as the concentration of sperm rose to about 50 million per ml, above which there is no additional benefit of having greater numbers of sperm in the ejaculate. What was impressive about this study was that results of semen analysis alone to guide their decisions. However, physical examination is always recommended to preclude the possibility of testicular cancer and also symptoms of sexually transmitted infection.

**Reference values for minimum semen quality compatible with normal fertility according to WHO (1999)**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Value</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume</td>
<td>≥2.0</td>
<td>ml</td>
</tr>
<tr>
<td>pH</td>
<td>&gt;7.2</td>
<td>pH units</td>
</tr>
<tr>
<td>Concentration</td>
<td>&gt;20</td>
<td>×10^6 per ml</td>
</tr>
<tr>
<td>Total number</td>
<td>&gt;40</td>
<td>×10^6 per ejaculate</td>
</tr>
<tr>
<td>Motility</td>
<td>≥50</td>
<td>% grades a + b</td>
</tr>
<tr>
<td>Morphology</td>
<td>n/a</td>
<td>% normal forms</td>
</tr>
<tr>
<td>Vitality</td>
<td>≥75</td>
<td>% alive</td>
</tr>
<tr>
<td>White blood cells</td>
<td>≤1.0</td>
<td>×10^6 per ml</td>
</tr>
<tr>
<td>Sperm antibodies</td>
<td>≤50</td>
<td>% of coated sperm</td>
</tr>
</tbody>
</table>

*a The editorial committee for the WHO (1999) did not provide a reference value for the percent of normal morphology compatible with normal fertility and instead provided as a footnote the following text: ‘Multi-centre population-based studies utilising the methods of morphology assessment in this manual are now in progress. Data from assisted reproductive technology suggest that, as sperm morphology falls below 15% normal forms using the methods described in this manual, the fertilisation rate in vitro decreases.’

**Table 1**

The theoretical pathogenetic links between the components and clinical manifestations of testicular dysgenesis syndrome (TDS)
this relationship existed in spite of the apparent day-to-day variation in ejaculate quality, the fact that semen analyses were not performed in the conception cycle, and that variation in female physiology introduces confounding factors that are difficult to control for. However, it did suggest that the 20 million sperm per ml reference value provided by the WHO and widely used by doctors to guide clinical decisions (see Table 1) may require revision. This view was further supported by a study in the United States that found that in order for a man to be definitively classified as fertile, his sperm concentration had to exceed 48 million per ml. Yet, he could be classified as infertile only if his sperm concentration fell below 13.5 million per ml. Clearly, therefore, there is an extensive ‘grey area’ over which it is difficult to use sperm concentration to make reasonable predictions of fertility. Similar ‘grey areas’ are evident with measures of sperm motility and morphology, although in this study, while males whose ejaculates contained a minimum of 12% morphologically normal sperm could be classified as fertile, those with less than 9% could be classified as infertile. Therefore, it would seem that sperm morphology might be a better parameter obtained at semen analysis to distinguish fertile from infertile men, because the ‘grey area’ is smaller.

Other laboratory tests
In an attempt to improve the accuracy of laboratory tests, many researchers have developed more sophisticated assays designed to investigate specific aspects of sperm biology or to mimic in some way aspects of the journey sperm make to the site of fertilisation. These are termed sperm function tests and 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.

Although for each of these there is a reasonable body of evidence to suggest correlations with outcome of IVF or unassisted conception, none of these tests has, to date, been universally incorporated into clinical practice.

Perhaps the tests with most current promise are those that examine the integrity of sperm DNA. For example, the sperm chromatin structure assay has been used to investigate the DNA integrity of men entering a 2-year follow-up study with their partners, and who were attempting to conceive for the first time. It was shown that poor quality chromatin structure detected by this technique was highly predictive of male infertility regardless of the number, motility or morphology of spermatozoa. No pregnancies were reported when more than 30–40% of spermatozoa were seen to contain damaged DNA and it is, therefore, perhaps not unsurprising to find that men attending infertility clinics have higher levels of DNA damage in their sperm. During IVF, embryo quality has shown to be positively related to sperm DNA quality (as measured in the Comet assay) in both fresh ($r = 0.698$) and prepared ($r = 0.718$) semen. Using the terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate (dUTP) in situ DNA nick end labelling (TUNEL) technique found a significant negative correlation ($r = -0.4$) between blastocyst development and the percent of TUNEL positivity in the sperm sample prepared for oocyte insemination. Interestingly, in spite of such research, the American Society for Reproductive Medicine recently reviewed the evidence base for sperm DNA testing and have concluded for the time being at least that there

Figure 2: Idealised pathway for diagnosis of male fertility using semen analysis data to guide the choice of further investigations, depending on the number of sperm in the ejaculate.
is insufficient evidence for such tests to be offered on a routine basis. As such, patients should only be offered the option to have their DNA quality tested in the context of an appropriately designed clinical trial.

Options to improve semen quality

There are few proven medical therapies to improve semen quality in men who are found at semen analysis to have an abnormal profile. Only in those rare cases of specific endocrine disorders, where the induction of puberty or the stimulation of sperm production could be achieved by adding back the missing endocrine factors, have merit. Clearly, many men with abnormal semen profiles are eager to know if there is anything that they can change in their lifestyle to improve their semen quality and thereby increase their chances of conceiving either naturally or through cycles of assisted conception. There are many epidemiological studies identifying possible risk factors that may lead to poor semen quality and these include exposure to:

- tobacco smoke
- solvents such as glycol ethers (e.g. painters and decorators)
- heat (either in the workplace or through the wearing of tight underwear)
- lead (through exposure to vehicle exhaust emissions).

However, in spite of these data there are no authoritative studies to show that behavioural change (i.e. removal of exposure) can improve semen quality significantly. Perhaps the most convincing are those that show that, in a randomised trial, the oral intake of some dietary supplements (e.g. vitamin E and C or folic acid and zinc) can marginally improve semen quality and/or the DNA integrity of sperm in the treatment arm of the trial. However, none have successfully demonstrated that such changes can result in increased pregnancy rates. Therefore, while patients may gain psychological benefit from making lifestyle changes, and there would seem to be an underlying logic that such changes may improve their outcome, they should be cautioned that there is currently no convincing evidence to support this.

Treatment options for sub-fertile men

Techniques to assist sub-fertile men become fathers include: donor insemination; intra-uterine insemination (IUI); and IVF and intracytoplasmic sperm injection (ICSI).

Donor insemination

For many years, this was the only effective treatment option for men who were azoospermic or severely oligozoospermic. Such treatments are now not without controversy and in 2006 the number of donor insemination treatment cycles carried out in the UK was at the lowest level since records began in 1991. While this may reflect a shift in the medical management of couples toward ICSI with their own sperm, it seems more likely that this is largely due to the lack of availability of donor sperm following the change in legislation that abolished the right of sperm donors to remain anonymous. It remains to be seen if over time the number of donors recruited will be sufficient to meet the UK needs or whether donor sperm will continue to be a limited resource.

IUI

For 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. Clearly, this is limited to those couples where the female partner has a patent reproductive tract and the sperm can migrate unhindered to the site of fertilisation in the Fallopian (uterine) tubes following insemination. However, a recent study has shown that in cases of unexplained infertility, unstimulated IUI does not result in significantly higher birth rates compared with expectant management. Therefore, although IUI can be performed on women in the natural cycle, it is now almost always combined with some element of ovarian stimulation.

IVF and ICSI

Although IVF was first developed mainly as a treatment option for women with blocked or absent Fallopian tubes, the ability to culture human embryos in vitro following the micro-manipulation of human sperm has revolutionised the treatment options for infertile men. While a number of different micro-manipulation techniques were attempted in the early days, it was the direct injection of a single human sperm into the cytoplasm that proved the most successful in facilitating fertilisation. Retrospective analyses have indicated that both fertilisation and pregnancy outcome are unrelated to the three basic parameters of semen analysis (i.e. count, motility and morphology). Data from the European Society of Human Reproduction and Embryology show that the clinical pregnancy rate using ICSI in 2003 was 26.5% per egg collection and 28.7% per embryo transfer, respectively.

With the widespread introduction of ICSI, developments in surgical sperm retrieval techniques have also opened up opportunities for men who are classically azoospermic at semen analysis but in whom small numbers of viable sperm can be recovered from the epididymis (PESE) or from pieces of testicular tissue taken by biopsy (TESE). This includes both obstructive and non-obstructive cases. The latter is particularly important in the context of men who are azoospermic following chemotherapy treatment for cancer, and who do not have adequate supplies of banked sperm (or did not have the opportunity). In such men, it has been shown that between 40 and 60% of them can have sufficient sperm recovered by testicular biopsy to enable successful ICSI to take place.

Cautionary notes for assisted conception

Although there is widespread acceptance that current assisted reproduction technology (ART) procedures are generally safe, the evidence for this, particularly in terms of long-term safety, is relatively weak when compared with other similarly established clinical techniques. Therefore, many researchers are currently conducting long-term follow-up studies to determine if those individuals born through assisted conception are as healthy as those conceived naturally. With regard to the future fertility of any sons born there are some specific cautionary notes.

Multiple births

In general terms, babies born as twins or triplets are less healthy than their singleton counterparts and low birth weight can affect many aspects of future health. With regard to future fertility,
there is concern that impaired foetal growth in the uterus may be associated with increased risk of hypospadias, cryptorchidism and testicular cancer. This is consistent with the theory of TDS. However, it remains to be seen if low birth weight following twin or triplet births as a consequence of IVF will lead to lower adult fertility and a greater reliance on ART in those individuals to have their own families.

Ovarian stimulation
There is a theoretical concern about the effect that ovarian stimulatory drugs, given to the woman receiving treatment, may have on the testicular development of her sons if she became pregnant. Again, this might be predicted from TDS theory (see above) if the drug administered is still able to exert some endocrine disruptive effect during the critical time of testicular development of any sons in utero. A single study, to date, has found that after controlling for confounding factors, the men whose mothers had received fertility treatment had smaller testicles as well as a lower median sperm concentration and total sperm count, fewer motile sperm and a lower number of morphologically normal sperm in their ejaculates when compared with men whose mothers had conceived them naturally.

Recovery of sperm for ART
Concerns have been raised about whether sperm obtained by surgical means are appropriate to be used in ART procedures because they might not yet have completed spermatogenesis or epididymal maturation. In this context it is interesting that, in children born through IVF and ICSI there is a small but significant increase in rare clinical disorders known to result from imprinting disorders, such as Beckwith–Wiedemann syndrome and Angelman syndrome. Whilst it is unclear whether this is specifically related to the sperm used in ART (it could also be influenced by the embryo culture techniques or genetic instability because of the underlying infertility of the couple), it is a matter of concern.

Sperm injection directly into the oocyte (ICSI)
Following the development of ICSI technique, its safety has been the subject of ongoing debate given that the direct injection of a single sperm into the egg cytoplasm would seem to bypass all of the natural barriers and selection processes ordinarily imposed on sperm during natural conception in vivo. There are two main risks. First, with sperm injection directly into the oocyte, there is the potential for vertical transmission of genetically related infertility from the father to offspring that would not normally occur. For example, men with micro-deletions of the Y chromosome generally have poor sperm quality and following ICSI the same deletion can be found in their sons suggesting that when they become adults they may also be sub-fertile and require ICSI to become fathers themselves. Second, are the risks associated with the sperm-injection procedure itself since animal models have shown that there are a number of subtle differences in the cytoskeletal events that occur during ICSI fertilisation when compared with IVF. It has been suggested that these may explain why there is a slight increase in chromosomal abnormalities in children conceived by ICSI.

Male age
Several studies have shown that increasing paternal age (> 40 years) is linked with a failure to father children either through infertility or increasing risk of miscarriage. Men above the age of 40 years are thought to be about half as fertile as men under the age of 25 (with no detectable change in semen quality) and moreover, that the risk of miscarriage in women over the age of 35 was 3.38 times higher (95% CI 1.76–6.47) if the man was aged 25–39 years and 6.73 times increased (95% CI 3.50–12.95) if he was older than 40 years. There is now considerable evidence that increased age of paternity is associated with an increased prevalence of offspring born with various pathological conditions including: achondroplasia; dyskinetic cerebral palsy; Apert syndrome; retinoblastoma schizophrenia; multiple sclerosis; cleft palate; and autism spectrum disorders. This is almost certainly due to new de novo gene mutations that arise as a consequence of the number of mitotic cell divisions of the germ line that occurs throughout a man’s life. There is little evidence that sperm counts decrease with age.

Underlying genetic issues of the parents
Because ART procedures are generally performed to overcome an infertility problem in either parent it is perhaps unsurprising that many studies have noted that there is an increased incidence of genetic abnormality in the offspring born. However, little research has been devoted to attempting to separate the effect of ART from that of infertility itself. Current data suggests that aspects of the ART procedure may be related to the occurrence of malformations of genital organs, but the reported increased prevalence of congenital malformations seen in singletons born after ART is partly due to the underlying infertility or its determinants. This could be explained, at least in part, by the high proportion of chromosomally abnormal sperm that has been observed in infertile men. However, since it is not possible to do any kind of genetic analysis of the sperm that is injected into the oocyte an absolute link cannot be determined.

FURTHER READING
Skakkebaek NE, Rajpert-De Meyts E, Main KM. Testicular dysgenesis syndrome: an increasingly common developmental disorder with environmental aspects. Hum Reprod 2001; 16: 972–978.


**Practice points**

- Poor semen quality contributes to the sub-fertility of 30–50% of couples undergoing IVF and needs to be carefully assessed
- Semen analysis remains the main diagnostic tool and the results used to guide further tests (e.g. karyotype, screen for cystic fibrosis genes in cases of severe oligozoospermia or azoospermia) as well as the appropriate treatment plan
- Semen analysis should be performed in an appropriately skilled laboratory that participates in an external quality assurance programme and in which the staff are suitably trained and resourced
- There are no proven strategies to improve semen quality and increase the probability of pregnancy, although exposure to cigarette smoke, heat and some chemical agents are correlated with poor semen quality
- The development of ICSI has reduced the reliance upon using donor sperm to overcome male sub-fertility
- The long-term safety of ICSI remains to be evaluated fully and is the focus of ongoing research efforts