Chapter 7

Male infertility

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INTRODUCTION

Infertility is defined by the World Health Organization (WHO) [1] as a failure to conceive a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse, and is based on observing that 84% of couples will conceive after 1 year, 92% after 2 years and 93% after 3 years [2]. One third of infertility is reported as being due to the male partner, but the true extent of male infertility is likely to be underestimated. In 2014, on average 25.4% of patients at UK fertility clinics were undergoing treatment for male factor infertility and 11.5% for mixed male and female factor infertility [3]. The probability of natural conception is related to the total number of motile sperm in the ejaculate. Men with a sperm concentration in the bottom 5% of the population (defined as < 15 million sperm per mL by the WHO) [4], are oligospermic. Men with less than 40% sperm motility in the ejaculate are asthenospermic. Men with low levels of androgen have hypogonadism. This chapter will discuss the major aetiological factors in male infertility and hypogonadism, and then discuss approaches to managing affected patients.

PHYSIOLOGY OF MALE FERTILITY

Male fertility is orchestrated by the hypothalamic-pituitary-gonadal axis [5] (**Figure 7.1**). The median eminence of the hypothalamus releases gonadotrophin-releasing hormone (GnRH) to the portal circulation in a pulsatile manner, which stimulates the anterior pituitary to secrete pulses of the gonadotrophin hormones, luteinising hormone (LH) and follicle stimulating hormone (FSH). LH stimulates the testicular Leydig cells to synthesise testosterone, and FSH stimulates Sertoli cells in the seminiferous tubules to support spermatogenesis. LH and FSH levels are negatively regulated by testosterone and inhibin, respectively. An entire cycle of spermatogenesis lasts on average 64 days[6].

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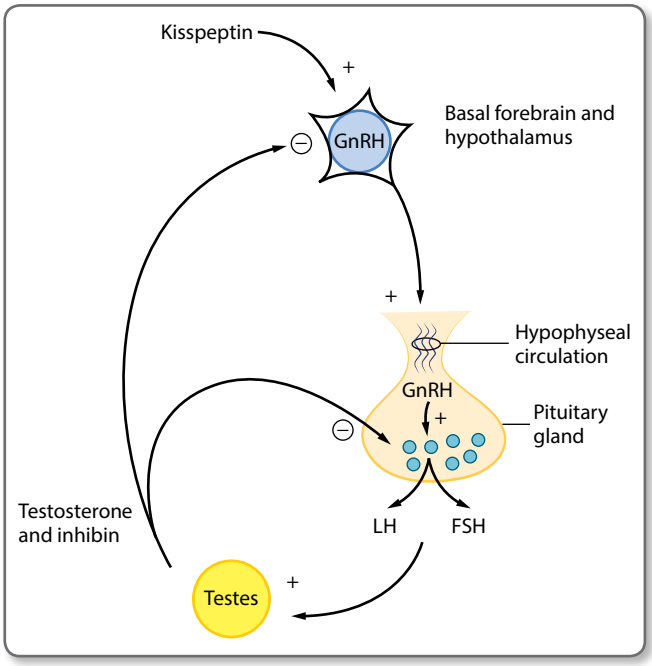

Figure 7.1 The male hypothalamo-pituitary-gonadal axis.

AETIOLOGY OF MALE INFERTILITY

For the purposes of this chapter, the causes of male infertility [7-11] have been broadly sub-divided into testicular failure, obstructive male infertility (more commonly termed obstructive azoospermia) and hypothalamo-pituitary disease. However, male factor infertility may be multifactorial and both male and female factor components to infertility may be present.

TESTICULAR FAILURE

Primary testicular failure classically results in low testosterone, high LH and FSH secretion and oligoasthenospermia or azoospermia associated with deficient sperm production. Azoospermia may arise from complete testicular failure associated with genetic factors, cryptorchidism, mumps orchitis, testicular cancer, radiotherapy and chemotherapy agents such as cyclophosphamide. Milder testicular failure associated with oligoasthenozoospermia may be caused by lifestyle factors or drugs [7,12,13] such as allopurinol, colchicine, sulfasalazine, cyclosporine, methotrexate, cimetidine, amiodarone, nitrofurantoin, erythromycin, carbamazepine, sodium valproate, phenytoin, calcium channel blockers and antiretroviral agents.

Lifestyle factors causing testicular failure

Obesity contributes to infertility in several ways, and is associated with a decreased total sperm count, concentration and motility. Adipocytes express the aromatase cytochrome p450 enzyme which converts androgens into oestrogen, and may suppress the pituitary secretion of FSH leading to a decrease in spermatogenesis [14]. Obesity is also linked with

hyperinsulinaemia and decreased levels of sex hormone binding globulin (SHBG), which may further increase the proportion of oestrogen which is free and therefore biologically active. Obese men are also more likely to suffer sleep apnoea which disrupts the normal circadian pattern of testosterone secretion [15]. These patients may present with low levels of free and total testosterone and a decreased ratio of testosterone to oestrogen. Obesity is also associated with increased reactive oxygen species (ROS) and oxidative stress which can cause sperm DNA damage and an increased DNA fragmentation index [16]. An increase in scrotal fat can also cause an increase in scrotal temperature and the local accumulation of fat-soluble environmental toxins.

Chronic alcohol abuse impairs male fertility by decreasing semen volume, sperm concentration and motility and may be linked with reduced libido and erectile function [17]. Acute alcohol intoxication has also been associated with abnormal spermatogenesis and sperm function however it has not been proven that a moderate alcohol intake impairs fertility [13]. Smoking may decrease sperm concentration by, on average, 22% when compared with non-smokers [18]. Reduced motility and abnormal morphology have also been reported and it is proposed that these abnormal parameters are caused by ROS present in cigarette smoke.

Recreational opioid use inhibits endogenous GnRH secretion, and the sex steroid properties of cannabis may disrupt spermatogenesis. Furthermore, exogenous androgen administration is increasingly employed by amateur body builders, but profoundly impairs spermatogenesis.

Genetics of testicular failure

Genetic abnormalities [19] account for 10–15% of male factor infertility and can be broadly classified into chromosomal abnormalities, Y-chromosome microdeletions, X-linked gene mutations, autosomal gene mutations, polymorphisms and epigenetic errors.

Chromosomal abnormalities

The overall prevalence of chromosomal abnormalities is estimated to be around 5% in infertile men, but over 10% in azoospermic men [20]. The most common chromosome abnormality associated with male infertility is Klinefelter's syndrome caused by the 47,XXY karyotype (or 47,XXY/46,XY mosaics). Klinefelter's causes primary testicular failure during early adulthood, with increased gonadotrophins and low testosterone. It had previously been assumed that these patients had complete azoospermia, it has now been reported that approximately 25% may have evidence of spermatozoa on semen analysis although spontaneous conception is rare [21]. Recent reports suggest that microsurgical testicular sperm extraction (mTESE) followed by intracytoplasmic sperm injection (ICSI) could be used to successfully achieve conception in patients with Klinefelter's syndrome [22]; however it should be emphasised to patients that the chance of sperm retrieval is low and patients should be counselled regarding the increased risk of chromosome aneuploidy and the possibility of performing preimplantation genetic diagnosis (PGD). Another aneuploidy which can occur is 47,XYY syndrome which occurs in 1 in 1,000 live male births [23]. There is a higher prevalence in infertile men although many with this condition may be fertile. Patients may have a normal phenotype and testosterone level however tall stature, behavioural problems, delayed language skills and mild learning disability may feature. In contrast to Klinefelter's there does not appear to be an increased risk of aneuploidy in the offspring of patients with XYY. Men with azoospermia may rarely have a 46,XX karyotype [24]; a fragment of the Y-chromosome containing the *SRY* gene causes male sexual differentiation, but the absence of other genes

on the Y-chromosome is incompatible with spermatogenesis. Chromosome translocations may also be associated with male infertility. Robertsonian translocations occur when two acrocentric chromosomes (13, 14, 15, 21, 22) fuse together. It has been reported that 0.8% of infertile men are carriers of a Robertsonian translocation which is up to nine times higher than the general population [25]. Reciprocal translocations involve an exchange of genetic material between two nonhomologous chromosomes. The incidence of reciprocal translocations has been reported to be seven times higher in infertile men than couples who have had a live birth [21]. Carriers of balanced translocations may have a normal phenotype however as there is a risk that children may inherit an unbalanced translocation these patients should also be offered genetic counselling. It is also possible to have X-autosomal and Y-autosomal translocations, the resulting impact on fertility is determined by the location of the breakpoints.

Y-chromosome microdeletions

The Y-chromosome contains genes which are crucial for male gonadal development and spermatogenesis. Microdeletions in the Yq11 region on the long arm of the Y-chromosome known as the azoospermic factor (AZF) region are prevalent in 10–15% of cases of non-obstructive azoospermia and 5–10% of severe oligozoospermia [26]. A microdeletion may span several genes. These are commonly subdivided into three regions. Deletions of the AZFa region have the worst prognosis for any Y-microdeletion, since they are largely incompatible with spermatogenesis. Deletions in the AZFb region also have a poor prognosis, with incomplete spermatogenesis and azoospermia. However, deletions of the AZFc may be compatible with a degree of spermatogenesis in some patients. AZFc microdeletions (including the *DAZ* gene family) are the most common type of Y microdeletion. It is important to note that affected patients with successful sperm retrieval should be given genetic counselling since the Y microdeletion will be inherited by male offspring.

Gene mutations

Approximately 2% of infertile men have mutations in the androgen receptor gene located on the long arm of the X chromosome [20]. The androgen receptor gene plays an important role in spermatogenesis and patients may be oligo or azoospermic. Infertility may be the only presenting symptom however mutations can also lead to androgen insensitivity syndrome and the neurodegenerative disorder Kennedy's syndrome. The ubiquitin specific protease 26 (*USP26*) gene and the transcription regulator factor gene *TAF7L* gene, which are located on the X-chromosome, are recognised for their role in spermatogenesis. Two small cohort case control studies have shown a significant correlation between mutations in these genes in men with non-obstructive azoospermia [28,29]. Approximately 5% of men with cryptorchidism have mutations in the *INSL3* gene [30] (insulin-like 3 on chromosome 19) and its receptor *LGR8* (relaxin/insulin-like family peptide receptor 2 on chromosome 13). *INSL3* is a peptide produced by Leydig cells which has a role in the transabdominal descent of the testes by acting on the gubernaculum. Mutations in the *INSL3* gene may also be present in testicular dysgenesis syndrome which can include cryptorchidism, hypospadias, testicular cancer and infertility.

Genetic polymorphisms

It is possible that polymorphisms in exon 1 of the androgen receptor (AR) gene [31,32], Y-chromosome haplogroups [33,34], the enzyme 5-methylenetetrahydrofolate reductase

(MTHFR C677T) [35,36], the enzyme human DNA polymerase ϒ (POLG) [37], the *DAZL* (deleted in azoospermia-like) gene [38], the FSH receptor gene [39] and the oestrogen receptor (ER) genes [40] may contribute to male infertility. However, studies have produced conflicting results and have been limited by sample size and heterogeneous phenotypes. Furthermore it is possible that the clinical effects of polymorphisms are influenced by other genetic predispositions or environmental factors.

OBSTRUCTIVE AZOOSPERMIA

Approximately 40% of azoospemia cases arise from obstruction arising anywhere between the rete testis and the ejaculatory ducts [41]. There are a multitude of causes of obstructive azoospermia (OA) including congenital unilateral and bilateral absence of the vas deferens, trauma, infections (including prostatitis), epididymitis previous radiotherapy and surgery. A previous history of infant hernia repair and infant hydrocele surgery substantially increases the risk of OA. Furthermore, epididymitis is becoming a more common cause of OA due to sexually transmitted infections such as chlamydia. Up to 90% of patients with congenital bilateral absence of the vas deferens (CBAVD) have mutations in the cystic-fibrosis transmembrane conductance regulator (*CFTR*) gene located on chromosome 7 [42]. These patients may have intact spermatogenesis which can be retrieved surgically from the testes or epididymis, and later used for treatment with ICSI. Genetic counselling and PGD should be advised due to the increased risk of cystic fibrosis if the female partner also carries a *CFTR* mutation.

HYPOTHALAMO-PITUITARY DISEASE

Secondary hypogonadism – also termed hypogonadotrophic hypogonadism (HH) – is caused by hypothalamic or pituitary disease resulting in low testosterone and low LH and FSH secretion. Pituitary disease accounts for the majority of cases of secondary hypogonadism.

Hypothalamic failure may be due to congenital or acquired GnRH deficiency. Congenital GnRH deficiency (CGD) is a rare disorder characterised by the deficient production, secretion or action of GnRH resulting in low FSH, LH and testosterone with otherwise normal pituitary function [43]. Clinical features of CGD are incomplete or absent puberty and infertility which may or may not occur in association with other developmental abnormalities including cleft lip or palate, dental agenesis, hearing impairment, renal agenesis and skeletal abnormalities. CGD in association with anosmia or hyposmia is termed Kallmann syndrome which results from incomplete embryonic migration of GnRH synthesising neurones from the olfactory placode to the hypothalamus. Alternatively, GnRH neuronal function may be deficient but smell is intact (i.e. normosmic CGD). Over the last two decades, mutations in several genes have been identified to cause either normosmic CGD, Kallman syndrome, or both. These studies are ongoing and reveal an increasingly complex neurohumeral network termed the GnRH pulse generator including kisspeptin, neurokinin B, dynorphin and substance P which regulate the pulsatile release of GnRH from the hypothalamus [44,45]. Over 25 causal genes for CGD have been identified so far and studies suggest at least 20% of cases are oligogenic. Up to 22% of patients with CGD manage to regain sexual function [46]. Hypothalamic GnRH deficiency may also be acquired during obesity, chronic disease, opioid misuse and severe weight loss.

CLINICAL APPROACH TO THE INFERTILE MALE PATIENT

Significant points to discuss when taking the history include shaving, libido, early morning erections, undescended testes and onset of puberty [47]. Past medical history includes mumps, sexually transmitted infections (STI), previous malignancy, any systemic or chronic disease, and erectile or ejaculatory problems. A surgical history of orchidopexy suggests that the patient had undescended testes which may have reduced function. Medication history includes over the counter and recreational drugs and any additional supplements. Adverse lifestyle factors should also be elicited as discussed previously. Clinical examination may reveal secondary sexual characteristics and gynaecomastia [48]. The testes should be palpated to assess volume with a Prader orchidometer or ultrasound. Semen analysis should be performed for all patients following abstinence of a minimum of two and a maximum of 7 days. Current WHO reference ranges [4] represent the 5th centile of the general population: semen volume >1.5 mL; sperm concentration > 15 million per mL; total sperm number >39 million spermatozoa per ejaculate; total motility >40%; vitality, i.e. live spermatozoa >58%; sperm morphology >4%. Sperm autoantibodies may be detected using the mixed antibody reaction (MAR) test or immunobeads with a normal range of <50% binding; however NICE do not currently recommend these tests [49]. Hormonal analysis should include early morning serum testosterone, oestradiol, LH, FSH and prolactin. Sex hormone binding globulin may also be measured to calculate free androgen index. Genetic testing should be performed for all patients with azoospermia and include a karyotype and tests for Y-chromosome microdeletions and *CFTR* mutations. Scrotal ultrasound is not routinely undertaken to assess male infertility unless a specific urological abnormality is found.

TREATMENT OF MALE INFERTILITY

The treatment of male infertility can include medical and surgical management [50,51], assisted reproduction and the use of donor sperm. Prior to embarking upon treatment couples should be advised about their chance of spontaneous conception with expectant management. Couples should also be given information on lifestyle factors, future treatment options and the availability of support groups and counselling services [49].

MEDICAL TREATMENT OF MALE INFERTILITY AND HYPOGONADISM

Testosterone therapy

Testosterone replacement is routinely given for patients with hypogonadism who are not attempting to conceive [50] (as it may inhibit spermatogenesis via negative feedback). Testosterone maintains virilisation and increases libido, sexual function, muscle strength, fat free mass and bone density. Several testosterone preparations are available including injectable depot preparations, cutaneous gels and oral agents. Orally administered testosterone must be conjugated to minimise first-pass metabolism, but topical or injectable testosterone does not require conjugation for bioavailability.

Testosterone therapy stimulates prostatic growth, increases haematocrit and may increase the risk of thrombosis [51–53]. Careful consideration is therefore required when contemplating testosterone therapy for patients with a contraindication such as known

prostate or breast cancer, erythrocytosis and severe cardiovascular disease. Men over 50 should be screened for prostate cancer with measurement of PSA and a digital rectal examination prior to initiating therapy. The haematocrit should also be measured prior to commencing testosterone, again within 6 months and then yearly. Serum testosterone should also be measured within 3 months of initiating treatment or changing the dose and every 6–12 months when a stable level is reached.

It is interesting to consider whether men require testosterone therapy in old age. Unlike women, men do not experience an abrupt reduction in gonadal function (sometimes termed andropause). However, testosterone actions do reduce with increasing age in men. The Baltimore Longitudinal Study of Ageing observed that free and total testosterone levels were reduced in men aged 60–69 years [54]. The Massachusetts Male Aging Study observed that free and bioavailable testosterone reduced at on average 2% annually [55]. The European Male Ageing Study (EMAS) proposed the minimum criteria for the identification of late onset hypogonadism (LOH), which entailed the presence of three sexual symptoms (decreased sexual interest and morning erections and erectile dysfunction) in combination with total serum testosterone below 11 nmol/L and free calculated testosterone below 220 [56]. Using these criteria, the EMAS investigators observed that only 2% of men aged 40–79 years had LOH. Furthermore, they observed that LOH was generally associated with a total serum testosterone below 8 nmol/L. Much debate surrounds the issue of testosterone supplementation in ageing men, but it appears appropriate to consider therapy in patients fulfilling the criteria for LOH. However, the decision to initiate therapy should always involve a discussion with the patient regarding the risks of therapy in the context of their premorbid history.

Induction of spermatogenesis with gonadotrophin injections

Patients with hypothalamic or pituitary dysfunction may benefit from gonadotrophin therapy when conception is desired. Human chorionic gonadotrophin (hCG) is a first line treatment for sperm induction in patients who have undergone pubertal development [57]. hCG mimics the biological activity of LH by stimulating Leydig cells in the testes to produce testosterone. This action results in a high local concentration of testosterone in the testes which may be sufficient to stimulate Sertoli cells and initiate spermatogenesis. hCG may be delivered by subcutaneous or intramuscular injection and the dose should be titrated with the aim of maintaining a normal serum testosterone level. A recent consensus statement on the treatment of congenital hypogonadotrophic hypogonadism [43] recommended that hCG should be the first-line therapy for patients with some pubertal development (testicular volume >4 mL) and no history of undescended testes. If a patient is still azoospermic following 6 months of treatment with hCG then a preparation containing FSH (such as menotrophin) may be used in conjunction with hCG. However, it has been suggested that patients with a testicular volume of <4 mL could benefit from an alternative treatment regime [58,59] commencing with unopposed FSH followed by hCG (or GnRH) to stimulate the proliferation of immature Sertoli and germ cells in male patients who lack the testicular development associated with puberty. Pulsatile GnRH is an alternative treatment for secondary hypogonadism specifically due to hypothalamic dysfunction, but is technically difficult to deliver and is unavailable in most countries including the UK. If spermatogenesis is successfully induced and sufficient numbers of viable sperm are sampled then sperm cryopreservation should be offered which commonly prevents the need to repeat therapy. In a minority of patients, gonadotrophin therapy may fail to induce significant spermatogenesis; such cases may benefit for consideration of surgical sperm retrieval [60,61].

Hormonal therapies to stimulate endogenous gonadotrophin secretion

It is controversial whether any medical therapies successfully increase fertility in men with idiopathic oligo/asthenozoospermia [62–64]. Drugs studied in this context include antioestrogens. For example clomifene and tamoxifen are selective oestrogen receptor modulators which inhibit the negative feedback of oestrogen. One recent meta-analysis [65] reported that clomifene and tamoxifen were associated with a statistically significant increase in pregnancy rates; however another study [66] reported that there is insufficient evidence to indicate that they are an effective empirical treatment. Aromatase inhibitors such as anastrozole have also been used to treat patients with male infertility by blocking the conversion of testosterone to oestrogen. Studies have reported improved semen parameters but again there is insufficient evidence to indicate that aromatase inhibitors are an effective empirical treatment [67,68]. There is insufficient evidence to conclude whether or not drugs boosting endogenous LH and FSH can treat men with idiopathic oligo/asthenozoospermia. More robust evidence is required before advocating the routine use of such therapies to treat men with infertility.

Kisspeptin

Recent studies have highlighted that the hypothalamic neuropeptide called kisspeptin is needed for endogenous GnRH function. Furthermore, inactivating mutations in the kisspeptin signalling pathway cause hypogonadotrophic hypogonadism [69]. Human studies suggest that peripheral, exogenous administration of kisspeptin stimulates gonadotrophin secretion safely and potently [70], thus implicating a potential novel therapeutic application. However, further studies are required before considering kisspeptin as a clinical therapy which has advantages over existing therapeutic approaches.

Surgical management of male infertility

Surgical techniques play a vital role in the management of obstructive infertility, and an emerging role in the management of testicular failure. Percutaneous epididymal sperm aspiration (PESA) or microsurgical epididymal sperm aspiration (MESA) are highly effective techniques for collecting sperm during obstructive azoospermia, since spermatogenesis is often intact in affected patients [60,61]. Surgical sperm retrieval may also be used to obtain sperm from men with non-obstructive azoospermia or severe oligoaesthenospermia, which is insufficient for assisted reproductive technologies (ART) such as ICSI. The technique of testicular sperm extraction (TESE) may have high associated rates of haematoma and testicular devascularisation [71]. Therefore, microdissection TESE (mTESE) was introduced in 1999, which is now considered the gold-standard surgical procedure for surgical sperm retrieval. mTESE allows the microdissection and identification of individual tubules appearing to be engorged with spermatogenesis in situ (**Figure 7.2)** [72]. During mTESE, tubules are dissected by the surgeon and examined intraoperatively for sperm by an assistant. Samples are then sent to an andrological laboratory to cryopreserve sperm for ART. Sperm retrieval rates up to 60% may be observed with mTESE in specialist centres, although results will vary on patient characteristics and surgical experience [73].

Figure 7.2 Intra-operative image from microdissection testicular sperm retrieval (mTESE). A single seminiferous tubule is held using micro-forceps prior to dissection, microscopic examination for sperm, and processing prior to sperm cryopreservation.

CONCLUSION

Male infertility is common and has devastating consequences for patients. Several recent advances have highlighted emerging roles for lifestyle factors and genetics in the pathogenesis of male infertility. However, treatment options for male infertility remain limited, unless gonadotrophin therapy is administered for patients with hypothalamo-pituitary dysfunction. Further advances in therapeutics of male infertility are required to reduce reliance on ART such as ICSI and donor sperm. Testosterone therapy should be considered in patients with hypogonadism associated with sexual symptoms; however, the benefits of therapy should outweigh the risk of potential complications for the individual patient.

Key points for clinical practice

- Investigation of the infertile male should include semen analysis. In 2010 the WHO published revised reference ranges for semen analysis. These values represent the 5th centile of the general population: Semen volume > 1.5 mL; sperm concentration > 15 million per mL, total sperm number > 39 million spermatozoa per ejaculate, total motility > 40%, vitality, i.e. live spermatozoa > 58%, sperm morphology > 4%. Investigations should also include hormonal analysis (including early morning serum testosterone, oestradiol, LH, FSH and prolactin. Sex hormone binding globulin may also be measured to calculate the free androgen index) and genetic testing for all patients with azoospermia (including a karyotpye, Y-chromosome microdeletions and CFTR mutations). Genetic counselling and PGD should be offered if required.
- Patients should be counselled on lifestyle factors including obesity, alcohol intake and smoking.
- Medical management of male infertility may be successful for patients with hypogonadotrophic hypogonadism. hCG is the first line treatment for sperm induction in patients who have undergone pubertal development. There is not yet any evidence-based empirical treatment for idiopathic oligo/asthenozoospermia. Testosterone therapy is not a suitable treatment for patients attempting to conceive.
- Surgical sperm retrieval may be successful for patients with obstructive male infertility and in some cases severe oligo or azoospermia including in the context of Klinefelter's syndrome. Microsurgical testicular sperm extraction (mTESE) is now the gold standard technique and the aim is to facilitate assisted reproduction treatment with ICSI.

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