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Klinefelter syndrome and success of sperm retrieval

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Learning objectives

By the end of this module the reader should be able to:

- discuss the impact of Klinefelter syndrome (KS) on male fertility
- describe the initial investigations for infertile men with KS
- outline the clinical strategies to manage infertility in KS men.

Introduction

In 1942 Dr Harry Klinefelter, an endocrinologist at the Massachusetts General Hospital in Boston, published a case series of nine men with enlarged breasts, small testes, infertility and decreased hair growth [1]. Initially thought to be an endocrine disorder, it was not until the late 1950s that Jacobs *et al.* demonstrated a chromosomal cause for this presentation [2].

The term 'Klinefelter syndrome' (KS) is used to describe a group of chromosomal disorders in which there is at least one extra X chromosome compared with the normal male karyotype of 46,XY. It is the most common chromosomal abnormality in men, with a prevalence of 2 in 1000 and is the diagnosis in 4% of infertile and 15% of azoospermic men [3].

Unlike many genetic conditions, the syndrome is not inherited. In over 90% of KS individuals there is one extra copy of the X chromosome resulting in a karyotype of 47,XXY. This occurs due to an inability for the paired X chromosomes to separate, termed non-disjunction, in either spermatogenesis or oogenesis, resulting in a reproductive cell with an abnormal number of chromosomes [4]. In spermatogenesis, this is due to X-Y non-disjunction. The remaining 10% of patients with KS carry a 46,XY/47,XXY mosaic change or higher grade X aneuploidies [5].

The physical manifestations of KS are extremely variable and in many cases patients are phenotypically normal. Interestingly those with mosaic KS often demonstrate fewer clinical symptoms than the classic or higher grade X aneuploidies [6]. Other than infertility, 90% of adults with KS suffer from non-obstructive azoospermia and many suffer from hypergonadotropic hypogonadism [7]. These individuals typically present with low to borderline-normal testosterone levels, increased luteinising hormone (LH), follicle-stimulating hormone (FSH) and oestradiol levels, physical changes (decreased facial and body hair, gynecomastia, decreased muscle tone, tall stature, narrow shoulders) and psychosocial changes such as learning difficulties [8]. Individuals with Klinefelter syndrome also have an increased incidence of autoimmune diseases such as diabetes and systemic lupus erythematosus, osteoporosis and tumours, especially extragonadal germ cell tumours and male breast tumours [8]. KS patients also appear to be at a higher risk of cardiovascular disease and thromboembolic events, with a high prevalence of recurrent venous ulcers, venous insufficiency and venous and arterial thromboembolism being noted [9].

Aetiopathogenesis of infertility in Klinefelter syndrome

Klinefelter syndrome can affect fertility in males due to germ cell degeneration in the testes [10]. Some reports suggest that this process starts during infancy, leading to an absence or reduced number of germ cells even before puberty [11]. The reason for this degeneration is not fully understood, but may be due to a negative effect of the extra X chromosome, abnormal gene silencing or an adverse influence of the supporting cell matrix of the germ cell.

When boys with KS enter puberty, where serum testosterone levels typically increase, the depletion of germ cells, the hyalinisation of the tubules, the degeneration of the Sertoli cells and the hyperplasia of the Leydig cells accelerates [12]. This is also associated with a decrease in testicular volume to a prepubertal size of 2-4mL [13, 14]. The degeneration process is associated with a relative Leydig-cell insufficiency reflected by impaired serum testosterone levels and increasing LH levels. The initial adolescent rise in testosterone plateaus at 14 years of age and remains in the low-normal range through puberty [15]. It remains uncertain whether the rise in serum or intratesticular testosterone concentrations in puberty is associated with the accelerated destruction of the seminiferous tubules.

Despite these change, in some areas of the testicle semi-

niferous tubules which bear some resemblance to normal tissue are present and ongoing spermatogenesis has been found, thus offering the possibility of successful testicular sperm extraction (TESE) [15]. As germ-cell degeneration accelerates dramatically at the onset of puberty, it has been suggested that tissue could be retrieved at this age for cryopreservation and future utilisation [16]. Furthermore, as we do not fully understand the reason for this decline, there is an argument that harvesting cells before loss is irreversible may be advantageous and should be done before commencing hormone therapy. There is however a risk of causing potential damage to spermatogonal stem cells. There are also psychological and ethical issues that must be considered when conducting procedures on adolescents.

A number of studies have described the outcome of TESE in pubertal males and the overall average success rate has been reported at 50% - the equivalent of that in adults. Rives et al. evaluated the outcomes of eight KS boys (seven with a 47,XXY karyotype and one with a mosaic form 47,XXY/46,XY) for consideration of semen analysis and testicular biopsy. Patients were seen before the introduction of hormone replacement therapy. Five out of the eight patients were diagnosed during puberty. Bilateral descended and small firm testes were observed in all, with a testicular volume varying from 1.3mL to 3.5mL. Whilst all of the patients had initially agreed to undergo semen analysis and testicular biopsy, only five patients went ahead with the procedure. Histological evaluation of testicular tissue confirmed the presence of spermatozoa in one patient, and germ cells were observed in another. The findings however demonstrated that the percentage of tubules with germ cells was low. The concentration of spermatogonia was reduced in KS patients compared with controls, regardless of age. The study demonstrated that, whilst it was technically possible to obtain semen samples from this patient group after the onset of puberty, the procedure was “not acceptable for all adolescents”. Producing an adequate semen sample was also difficult for many [16].

Aksglæde *et al.* found that only 50% of the Klinefelter boys had germ cells in their testes, indicating a severely impaired fertility potential even in the peripubertal period [12]. Sperm retrieval rates in small studies of the less than 16 years age group are poor and have been reported as ranging from 0-20% [6].

Patient views are absolutely integral to this process and a number of studies evaluating the desire for fertility amongst patients and their parents, have been conducted. Rives *et al.* conducted numerous consultations with patients, interviewing them both alone and with their family. They gauged the patients’ own understanding of their disease, wishes for fertility and other methods to have a family. Seven of the eight patients reported that they had not thought about their future fertility and only began engaging in the process after at least three medical consultations. The adolescents placed less emphasis on their future fertility compared with their parents. Interestingly, the parents were fully convinced of the possibility of fertility preservation even if told that no biochemical or clinical parameters could successfully predict this. The fathers were specifically interested in their sons having a normal sex life [16].

Diagnosis of Klinefelter syndrome

It is estimated that only 25% of men with KS receive a formal diagnosis [17]. Males tend to present at three stages in life: prenatally, at school as part of investigation for developmental delay, or as young men with infertility [18]. The only generally accepted risk factor for KS is older maternal age [3].

In most urological practice, KS will be identified during investigations for male infertility or early hypogonadism. It is thought that infertility affects 97% of all KS patients [19]. From the current European Association of Urology (EAU) guidelines, karyotyping is recommended at sperm concentrations less than 10 million/mL [20, 21]. During the infertility evaluation, fasting hormone levels are conducted, and testicular ultrasound can be performed to evaluate for other pathologies such as obstruction or tumour. Testosterone levels may be normal or low, oestradiol levels normal or elevated, and FSH levels increased. Ultrasound findings in KS men include the presence of small testes, coarse or nodular echotexture, hypervascularisation, and microlithiasis. KS nodules tend to be benign Leydig cell tumours/hyperplasia [22]. Infertility and cryptorchidism, both of which occur with KS, increase the risk of developing testicular cancer, but it is still unclear if this higher risk is directly related to KS.

Management of infertility in Klinefelter syndrome

The production of ejaculated 24XY sperm has been reported in between 0.9% and 7.0% of men with Klinefelter’s mosaicism and in 1.36-25% of men with somatic karyotype 47,XXY [19]. Successful pregnancies have been achieved using ejaculated sperm and assisted reproductive technology (ART) [23].

Surgical sperm retrieval and intracytoplasmic sperm injection (ICSI) have dramatically improved the fertility potential of men with KS [23]. The first reported successful sperm retrieval in men with KS using TESE was undertaken in 1996. The first pregnancies achieved using ICSI of ejaculated and testicular sperm were reported two years later [24]. With the use of microdissection TESE, sperm retrieval rates (around 50%) in patients with KS are considered equivalent to those in men with non-obstructive azoospermia [25].

The management of infertility in KS is multimodal, involving many specialties including geneticists, endocrinologists and urologists. Genetic counselling is essential for new diagnoses. A key aim for many patients is to preserve fertility and maximise their chances of becoming biological fathers. Androgen replacement therapy should be started after fertility issues have been addressed in men with hypogonadism.

Hormone therapy

Spermatogenesis requires a favourable intratesticular hormonal environment and many clinicians advocate the use of testosterone supplementation in adolescents with KS to support age-appropriate pubertal development. Various preparations are available, but gel preparations offer the most physiological pharmacodynamic profile, are safe and efficacious, and avoid the need for painful injections which may not be tolerated well in a paediatric population. Maintenance of steady-state serum testosterone levels may also limit the disruption of spermatogenesis compared with other formulations, such as injections, which are associated with peak and trough variations.

In a study by Ramasamy *et al.* the serum testosterone concentration and the testosterone:oestradiol ratio after preoperative medical therapy was higher in men in whom sperm were found than in men in whom no sperm were found (PZ0.002 and PZ0.05 respectively) [26]. Men with a low baseline testosterone, who responded to the medical therapy with a resultant testosterone of 8.7nmol/L (250ng/dL) had a higher chance of sperm retrieval than men who did not [26].

It has been suggested that medical optimisation prior to micro-TESE or TESE may increase the chance of successful sperm retrieval [27]. Whilst there is no substantial evidence evaluating this in the form of randomised controlled trials, small case series utilising various regimens have been reported. Lower retrieval rates have been reported in a subset of KS adults who previously received exogenous testosterone, although the nature, duration, and reason for therapy in these patients is unknown [24]. Mehta *et al.* performed a retrospective analysis of adolescents with KS, treated with hormone replacement therapy, who underwent surgical sperm retrieval at their institution [28]. An aromatase inhibitor (1mg anastrozole) was administered daily along with the patient's usual testosterone therapy. These were continued until fertility potential has been addressed (sperm cryopreservation or surgical sperm retrieval) for a maximum duration of 24 months. Testicular volume was measured with scrotal ultrasound before the initiation of hormone therapy. Semen evaluation was performed and the presence of sperm in the ejaculate was investigated on two separate occasions for each patient. Four patients underwent unilateral micro-TESE, and six required a bilateral procedure. Sperm were successfully retrieved in 70% and cryopreserved.

At the authors' institution, an early stimulation protocol is commenced after assessing baseline testosterone. Patients receive at least six months (ideally 12-18 months) of stimulation with clomifene citrate or a combination of human chorionic gonadotropin, menotropins and testosterone and the response is closely assessed. Micro-TESE is performed after this and the protocol has improved retrieval rates by 30% since being introduced.

In patients already receiving androgen replacement therapy, it has been suggested to discontinue this treatment for at least 6-9 months prior to micro-TESE [29]. This is still controversial as some authors have not shown a predictive relationship between testosterone and higher retrieval rates [30]. Rohayem *et al.* demonstrated that a combination of serum testosterone greater than 7.5nmol/L and LH less than 17.5U/L resulted in higher retrieval rates in both adolescents and adults [31]. Another large study showed higher testosterone (along with lower FSH and LH) to be positive markers for retrieval in azoospermics in general. However, the predictive value of testosterone in KS is still unclear [29]. The incorporation of non-testosterone hormone therapy (clomiphene, human gonadotrophin, aromatase inhibitors) has been recognised as potentially beneficial in patients with low testosterone prior to retrieval but more work has to be done in this area [19].

Surgical intervention

The micro-TESE technique has proved superior to TESE with respect to minimising the damage to the testicular tissue, and maximising the success rate of sperm retrieval [31]. In this procedure, microsurgical techniques are used to identify individual seminiferous tubes with active spermatogenesis. A recent review of sperm retrieval in men with KS according to method (TESE vs micro-TESE) showed an average sperm retrieval rate of 42% by the use of TESE and 57% by the use of micro-TESE [32]. The sperm retrieved from micro-TESE can then be injected into eggs (ICSI) obtained from the female from in vitro fertilisation (IVF).

The levels of FSH, inhibin B, and the inhibin B/FSH ratio are known predictive factors for fertility in males with normal karyotype, but this does not seem to be the case in KS [33]. Several authors demonstrated that, at a younger age, TESE might improve the fertility outcomes for patients with KS. However, a recent review showed that retrieval rates are much lower in the less than 16 years old age group (0-20%) compared to young adults and late adolescents between 16-30 years old (40-70%). The study pointed out the small numbers in the 76 studies included indicating the need for more work in the area [6].

In addition, one study found a positive predictive value of testicular volume, testosterone levels and response to hCG test for successful TESE [34], but this association was not confirmed in others [32]. Men with the presence of seminiferous tubules that did not have sclerotic changes during the procedure were associated with the most favourable outcomes [35].

In the largest case series of KS patients undergoing micro-TESE (n = 106) and ICSI, pregnancy rates (53%) and live birth rates (42%) were not significantly different from men with non-obstructive azoospermia without KS [35]. Schlegel's group published similar pregnancy and live birth rates of 57% and 45% from 68 men [25].

Pre-implantation genetics

Klinefelter syndrome is not an inherited condition but the risk of a woman having a son with KS may be slightly higher if she is over 35 years of age.

It has been suggested that a method to potentially identify affected embryos is the use of preimplantation genetic diagnosis (PGD) or amniocentesis. There is however limited evidence for the benefit of this technique for the detection of numerical chromosomal abnormalities in embryos to improve implantation rates after IVF. A complicating factor in its use is the need for clinicians to perform early embryo transfer (usually on day three) in order to achieve higher rates of pregnancy [36]. Furthermore, numerous healthy children have been born using ICSI without pre-implantation genetic diagnosis (PGD) and the conception of one 47,XXY fetus has been reported [37].

A comparison of the result of PGD in 113 embryos from 20 couples with KS with 578 embryos from control couples with X-linked disease undergoing PGD for gender determination and found a significantly higher percentage of sex chromosome (13.2 vs 3.1%) and autosome (15.6 vs 5.2%) abnormalities in embryos from KS couples as compared with the X-linked couples [38].

Because of this increase of sex chromosomal and autosomal abnormalities in the embryos of Klinefelter patients, PGD or amniocentesis analysis should be considered, although it is routinely still not offered in many institutions [21].

Other aspects to consider are that ICSI is associated with an increased risk of producing a chromosomal anomaly in offspring [39]. IVF is also associated with an increased risk for *de novo* chromosomal aberrations, especially those involving the sex chromosomes [20].

Summary

The vast majority of males with 47,XXY are usually azoospermic, but the chances of fathering a child by the use of assisted reproductive techniques are increasingly encouraging, with average micro-TESE sperm retrieval rates of 57%. However, reported numbers of live born children of couples with KS is still limited. Spermatozoa may occasionally be found in the ejaculate, and we therefore recommend always performing semen analysis before considering micro-TESE. More work needs to be done to investigate the need and strategies for hormonal optimisation prior to micro-TESE as well as the optimum age for harvest. Finally, follow-up of men with KS is required and androgen replacement therapy should be started after fertility issues have been addressed and when testosterone level is in the range of hypogonadism [20]. Follow-up is especially important in these patients as they display an increased cardiovascular risk profile, and suffer from thromboembolic events as well an increased prevalence of metabolic abnormalities including diabetes mellitus and dyslipidemia [9].

Key learning points

- Klinefelter syndrome is the most common chromosomal abnormality in men, with a prevalence of 2 in 1000, affecting up to 4% of infertile and 15% of azoospermic men.
- Fertility is affected due to germ cell degeneration in the testes. The vast majority of males with 47,XXY are azoospermic, but the chances of fathering a child by the use of ART are becoming increasingly higher, with average micro-TESE sperm retrieval rates of 57% being reported, based on studies of over 741 males with KS.
- More work is required to investigate the need and strategies for hormonal optimisation prior to micro-TESE as well as the optimum age for sperm harvest.
- The follow-up of men with KS is essential and androgen replacement therapy should be started after fertility issues have been addressed.

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