Klinefelter's syndrome

BY JOSIP VUKINA AND PHILIPPA SANGSTER

Klinefelter's syndrome (KF) is the most frequent sex chromosome abnormality with an estimated prevalence of 1 in 500 to 1 in 700 newborn males and 1 in 10 in men with azoospermia. While the majority of cases are an XXY genotype, mosaicisms or other aneuploidies can be detected [1]. It is important to be aware of this syndrome within the urology clinic as it is the most common cause of inherited male hypogonadism, with significant effects on male fertility. Overall, it is seen in 3.1% of infertile males [2]. This article aims to review the etiology and epidemiology of KF as well as the disease's implications on fertility.

Etiology and epidemiology

The origin of the XXY genotype is most commonly from errors related to nondisjunction in paternal meiosis 1 [3]. Approximately 15-20% of KS men are defined as 'mosaics', usually with two cell lines: 47,XXY / 46,XY [4]. Although, this figure may be underestimated due to different chromosomal mosaicism levels in different tissues. In addition, mosaic KS men are more androgenised than true XXY men which may result in under detection of men with mosaic KS [5].

In KF, testis biopsy shows Leydig cell hyperplasia, tubular hyalinisation, thickening of the basement membrane, and Sertoli-only cell phenotype [6]. Improper functioning of Leydig cells causes high estradiol levels and low to normal testosterone.

Unfortunately, KF is severely underdiagnosed with epidemiologic studies demonstrating that only 25% of patients are ever diagnosed and few of these before the onset of puberty [7]. This may be changing rapidly, however, as a recent study demonstrated the ease of KF testing with dried blood spot samples, potentially allowing simple and affordable newborn screening [8].

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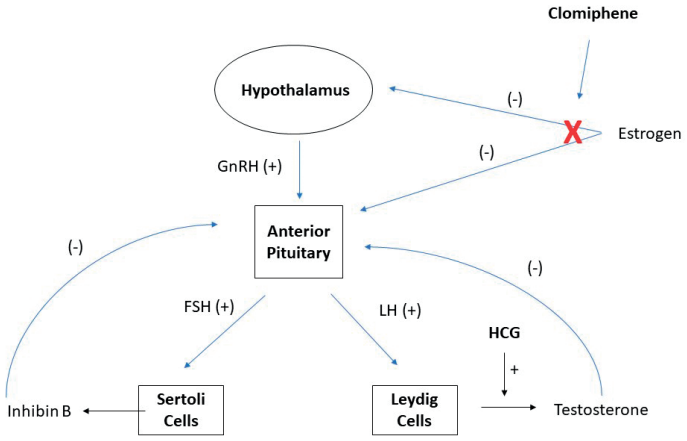

Figure 1: Hypothalamic–pituitary–gonadal (HPG) reproductive hormone axis.

Men are often diagnosed at puberty or in adulthood because of minimal testicle growth or obvious gynaecomastia, however a common presentation will be in the urology clinic with a diagnosis of infertility and azoospermia [9]. In those cases, a full genetic work-up should be completed including karyotype, Y chromosome microdeletion test, and CF carrier status (if any abnormality of vas deferens is detected) [10]; hormones, including testosterone, follicle-stimulating hormone (FSH), and luteinising hormone (LH), should also be checked.

Clinical presentation

Signs and symptoms can differ in different age groups depending on the severity of the phenotype. Clinically, it is worth recognising these differences to aid in diagnosis. In early life, KF patients can present with weak musculature and delayed motor and speech development. While most boys have sufficient testosterone to initiate puberty, most fail to progress properly [11] and are noted to have small firm testes, small penis, and gynaecomastia. Undescended testes are more common in KF although there has been no data showing higher rates of testicular cancer in these patients [12]. In adulthood, patients present with infertility, gynaecomastia, poor libido, decreased body hair, and behavioural problems. Mosaic patients often present with milder phenotypes depending on their degree of mosaicism [1,13].

Sperm extraction – when, how, and will it be successful?

If the man is found to have very low levels of sperm in their ejaculate, there are reports in the literature of successful intracytoplasmic sperm injection (ICSI) using this ejaculated sperm [14,15] in KF men. However, these cases are rare and the majority of patients need surgical sperm retrieval to achieve pregnancy. Even if there is no sperm found with the ejaculate, small numbers of sperm may still be found within the testicle itself. Prior to 1996, when Tournaye et al. first reported successful recovery of sperm by testicular sperm extraction (TESE) in men with KF and azoospermia [16], these patients were considered definitively sterile. One year later, Palermo documented the first pregnancy using TESE / ICSI [17]. Multiple studies have now shown that despite widespread scarring of the seminiferous tubules, there are residual foci of spermatogenesis amenable to surgical sperm retrieval and assisted reproductive methods [13,18].

In traditional TESE, a small incision is made in the tunica albuginea of the testicle, seminiferous tubules are extracted, and the incision is closed. This method does not allow for targeting of any certain areas of the testis based on appearance. Many papers have shown high rates of sperm retrieval (47-69%) using microsurgical testicular sperm extraction (mTESE), which is a more invasive form of sperm retrieval in which the testicle is bivalved and the tubules examined directly with microscopic magnification [18-24]. The technique was first described in a seminal paper

by Schlegel et al. in 1999 which showed a 63% sperm retrieval rate with mTESE as compared to 41% by standard TESE [25] in all cases of azoospermia. In 2009, Ramasamy and Schlegel demonstrated a 68% sperm retrieval rate using mTESE in KF patients [18]. Based on higher sperm retrieval rates, our institution utilises mTESE for all cases of testicular sperm extraction in KF patients. When the testicle is bivalved, it is common to see flat scarred-appearing tubules with small pockets of healthier-appearing tubules that are targeted for retrieval, as seen in Figure 2.

Recent reviews have shown very wide-ranging success rates of sperm retrieval in KF (13-68%) with no convincing predictors of success. Analyses of age, testicle size, FSH, LH, or testosterone levels have not shown any significant association with success of sperm retrieval. Based on previous reports of progressive hyalinisation of seminiferous tubules in post pubertal KF patients, it has been suggested that performing earlier TESE may result in high sperm retrieval rates [26, 27]. However, a more recent meta-analysis from 2017 showed that increasing age was not associated with worsening sperm retrieval rate [28], which is consistent with our institutional series. Notably, numerous studies show comparable sperm retrieval, fertilisation, implantation, and live birth rates between KS and other non-obstructive azoospermia (NOA) patients [21,23,29]. A meta-analysis from 2017 showed that eventually, 16% of KF couples undergoing assisted reproductive techniques will have a child [28].

Hormonal manipulation

If men have been previously diagnosed with KS, they often attend their first infertility consultation receiving supplemental testosterone. This medication will suppress the production of spermatogenesis, thus gonadotrophins and semen analysis results will need to be interpreted accordingly.

While the use of testosterone replacement therapy (TRT) in adolescents with KF has been advocated to allow appropriate pubertal development, hormonal stimulation prior to sperm retrieval in KF is not well studied. There is a paucity of studies on the subject and those that have been done are not randomised and thus, conclusions are limited.

We recommend close collaboration with a reproductive endocrinology specialist to hormonally optimise patients prior to surgical sperm retrieval. Patients should not be given testosterone because of the deleterious effective on spermatogenesis, however, in cases of hypogonadism, testosterone production can be stimulated via the use of recombinant LH and FSH administration. If used, testosterone should be stopped at least six months prior to sperm retrieval to allow for recovery [30,31].

Transmission to offspring?

A significant concern regarding fertility preservation among KF patients is the potential transmission of genetic abnormalities to offspring. While the majority of children born to KF parents are reportedly healthy, there are published occasions of foetuses or embryos diagnosed with 47 XXY karyotype [32-34]. Of additional concern is a 2003 study recommending preimplantation screening due to the increase rate of aneuploidy in KF couples [35]. The literature on this topic is still evolving. We highlight that transmission to offspring is theoretically possible and strongly recommend involvement of a genetic counselor.

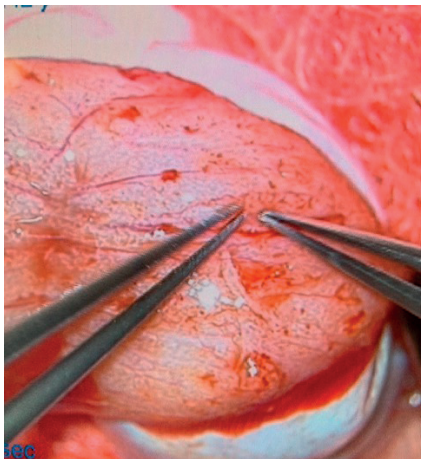

Figure 2: Seminierous tubules under the operative microscope in the testicle of a patient with KF.

Table 1: Varying presentations of KF by age

Infants	Puberty	Adults
Weak musculature	Taller than average stature	Infertility
Delayed motor development	Gynaecomastia	Gynaecomastia
Delayed speech and language	Small penis	Poor libido
Quiet personality	Small, firm testicles	Decreased body hair
Cryptorchidism	Behavioural problems	Small testicles / penis
Slow penile growth		Behavioural problems

Table 2: Clinical investigations (adapted from European Association of Urology Guidelines on Male Infertility [10]).

Recommendations	GR
Obtain standard karyotype analysis in all men with damaged spermatogenesis (spermatozoa <10million/mL) who are seeking fertility treatment by in vitro fertilisation (IVF).	B
Provide genetic counselling in all couples with a genetic abnormality found in clinical or genetic investigation and in patients who carry a (potential) inheritable disease.	A
For all men with Klinefelter's syndrome, provide long-term endocrine follow-up and androgen replacement therapy, if necessary.	A
Do not test for microdeletions in men with obstructive azoospermia (OA) when ICSI is used because spermatogenesis should be normal.	A
Inform men with Yq microdeletion and their partners who wish to proceed with ICSI that microdeletions will be passed to sons, but not to daughters.	A
In men with structural abnormalities of the vas deferens (unilateral or bilateral absence), test the man and his partner for transmembrane conductance regulator (CFTR) gene mutations.	A

Looking into the future – spermatogonial stem cell preservation

Based on the idea that germ cell loss starts with the onset of puberty, some have advocated for the preservation of adolescent or even pre-pubertal testicular tissue with the hope of using spermatogonial stem cells to derive sperm in the future. Many centres around the world, including ours, have begun cryopreservation of pre-pubertal testicular tissue in patients prior to the start of chemotherapy but it has not yet become common practice for KF. Deriving sperm from this tissue has been extensively studied in animal models, however this has not been reproduced in humans or non-human primates [36]. Factors to consider are cost, the ethical challenges of subjecting adolescents to an operation they may not understand, and the still unproven benefit of being able to use this technology in humans. At this point, experts in this field believe that this should be reserved for research purposes but not for the regular clinical use [27].

Conclusion

In summary, urology providers are most likely to encounter KF patients in the setting of infertility, sexual dysfunction, or hypogonadism. We recommend a full hormonal evaluation and referral to a centre with expertise in male infertility. Currently, our unit works closely with reproductive endocrinology specialists to hormonally optimise patients and eventually proceed on to mTESE and, if successful, cryopreservation of sperm. Patients should be referred for extensive genetic counseling and the use of preimplantation genetic diagnosis should be considered. There may be a role for preservation of testicular tissue for future use of spermatogonial stem cells however this therapy is still considered experimental.

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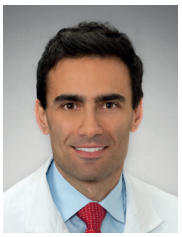
AUTHORS



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