REVIEW

Klinefelter syndrome: phenotype, testicular function and infertility treatment.

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Abstract

Klinefelter syndrome (KS) is a sex chromosomal disorder affecting males, particularly phenotypic manifestations, endocrinology and testicular function. Most KS men exhibit some form of testicular and gonadal dysfunction, and usually present with non-obstructive azoospermia or severe oligozoospermia, severely affecting their reproductive capacity. This review focuses on the molecular mechanisms behind these impairments which are related to the supernumerary X-chromosome universal to KS males. It also describes the varying phenotypes and explores semen quality and infertility in KS men, additionally revealing how factors such as age, genotype and hypogonadism influence these KS manifestations. Lastly, it explores common ART techniques used to overcome infertility in KS males including different sperm retrieval techniques and intra-cytoplasmic sperm injection, as well as the concern surrounding transmission of chromosomal abnormalities to subsequent offspring.

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Introduction

Klinefelter syndrome (KS) refers to the most common spectrum of sex chromosomal aneuploidies in males (Tuttelmann & Gromoll, 2010; Lizarazo et al., 2019). The syndrome is named after Harry Klinefelter, an American endocrinologist who first noticed an unusual phenotypic appearance in some of his male patients. Klinefelter first published the report in 1942 on 9 men whom he considered to have an uncharacterised syndrome, clinically typified by gynaecomastia, small testes, hypogonadism (reduced gonadal function), sparse facial and body hair and azoospermia (Klinefelter et al., 1942). 17 years later, it was discovered that these KS men had a supernumerary X-chromosome(s), portraying a 47, XXY+ genotype instead of the typical male karyotype of 46, XY (Jacobs & Strong, 1959). KS was originally termed an endocrine disorder (Jo et al., 2013), but has since been comprehensively researched and is now found to be the most frequent genetic cause of non-obstructive azoospermia (NOA), occurring as a result of non-disjunction of paternal or maternal sex cells during meiosis I or II (Samplaski et al., 2014). 80-90% of KS cases bear a 47, XXY karyotype, with the remaining 10-20% cases representing males with higher-grade polysomic aneuploidies (48 XXXY or 48, XXYY), varied mosaicism (47, XXY/46, XY) or structurally abnormal X-chromosomes (47, iXq, Y) (Tuttelmann & Gromoll, 2010; Bonomi et al., 2017). The origin of the aberrant X-chromosome, either maternal or paternal, and the associated phenotypes are not entirely understood (Shiraishi & Matsuyama, 2018). However, studies have established a positive association between maternal age and

risk of aneuploidies (such as KS), in subsequent children (Bonomi et al., 2017).

KS is estimated to affect 1 in 600 males in the general male population (Host et al., 2014), making it the most frequently observed male sex chromosomal aneuploidy (Bonomi et al., 2017). It is present in 3-4% of infertile men and 10-12% of azoospermic men (Corona et al., 2017; Vloeberghs et al., 2018), but despite its prevalence, it is estimated only 10% of KS cases are identified in childhood (Akcan et al., 2018). KS is typically diagnosed based on a physical examination, hormonal tests and a karyotype analysis (Dobs & Matsumoto, 2009), although KS males can remain undiagnosed due to the heterogeneity in clinical and genetic presentation. Diagnosis also tends to occur during infertility investigations (Lanfranco et al., 2004; Akcan et al., 2018) and in all KS karyotypes, the primary defect is failure of the Leydig cells to produce sufficient quantities of male steroid hormone testosterone (Schoenwolf et al., 2015). KS is firstly characterised by normal serum levels of testosterone, follicle stimulating hormone (FSH), luteinising hormone (LH) and inhibin B until the onset of puberty where testosterone concentrations plateau and remain low-normal throughout puberty (Wilkstrom & Dunkel, 2008). It is currently thought that an accelerated loss of germ cells occurs during puberty, leading to fibrosis and then hyalinisation of seminiferous tubules, hyperplasia of Leydig cells and ultimately results in small firm testes and NOA or oligozoospermia (Aksglaede et al., 2006; Schoenwolf et al., 2015).

Infertility in KS men has remained an untreatable condition for decades, where semen analyses most often reveal NOA or severe oligozoospermia (Aksglaede et al., 2006; Corona et al., 2017). However, testicular biopsies have shown that many non-mosaic and mosaic KS men have residual foci with preserved spermatogenesis (Aksglaede et al., 2006; Selice et al., 2010). Moreover, the use of modern assisted reproductive technologies (ART) such as conventional testicular sperm extraction (c-TESE) and microsurgical TESE (micro-TESE), have seen high sperm retrieval rates (SRR) and ensuing success rates using intra-cytoplasmic sperm injection (ICSI) (Maiburg et al., 2012; Corona et al., 2017). However, the issue of transmitting chromosomal aneuploidies to subsequent offspring is a concern for KS males (Levron et al., 2000).

Therefore, a contemporary review of the exhibited phenotypes and symptoms, pathophysiology, semen quality and different infertility treatments is warranted in understanding such a prevalent chromosomal aneuploidy. This review has numerous objectives: firstly, it will outline the aetiology of KS, the varying phenotypic presentations, the effect of the supernumerary X-chromosome(s) and the consequential impact on semen quality in KS men. Secondly it will summarise and critically evaluate ART infertility treatment options and their appropriateness.

Aetiology and pathogenesis

Numerical chromosomal aneuploidies in KS arise via non-disjunction during early germ-cell development in meiotic divisions (Lanfranco et al., 2004). The 47, XXY karyotype is acquired through spontaneous non-disjunction which can occur maternally through a mitotic error in the zygote or during stage I or II of maternal germ cell meiosis (Lanfranco et al., 2004). Stage I errors are the most common source of maternal non-disjunction (Lanfranco et al., 2004), but the 47, XXY karyotype can also result from an erroneous stage I of spermatogenesis (Samplaski et al., 2014). Alternatively, mosaicism in KS results from post-fertilisation non-disjunction, which instigates the production of two different cell lines in the body (Paduch et al., 2008). The true prevalence of mosaicisms may be underestimated due to different mosaic expression in different bodily tissues, particularly in the testes (Samplaski et al., 2014). The majority (80-90%) of KS males are of the non-mosaic type, with the remainder presenting mosaicism or a higher-grade aneuploidy (Tuttelmann & Gromoll, 2010; Bonomi et al., 2017). An earlier study by Jacobs et al. (1988) found that in a group of KS men, paternal non-disjunction accounted for 53.2% of cases; 34.4% cases were due to maternal meiotic I errors, 9.3% were maternal meiotic II errors and postzygotic mitotic errors accounted for 3.2% of cases. This study also found that paternal and maternal errors of gametogenesis were equally responsible for causing the syndrome (Jacobs et

al., 1988), which is also still the case today (Bonomi et al., 2017).

Clinical features, phenotypic manifestations and testicular function

Key signs and phenotypic manifestations of KS have been well typified since Klinefelter's initial description of the chromosomal abnormality (Klinefelter et al., 1942). It is important to note that testicular dysfunction, small testes and varying degrees of hypogonadism present in all KS cases (Schoenwolf et al., 2015; Davis et al., 2015). The 'prototypic' KS male has been found to exhibit tall stature, eunuchoid appearance, gynaecomastia, broad hips, sparse body hair, feminine pubic hair distribution and firm testes, all which have been attributed to hypogonadism and/or the supernumerary X (Klinefelter et al., 1942; Amory et al., 2000). In terms of hormonal milieu, low serum testosterone is usually discovered and is coupled with elevated gonadotrophins, NOA or severe oligozoospermia, and fibrosis of the seminiferous tubules (Davis et al., 2015). KS males also exhibit psychosocial and neurobehavioural characteristics such as significant language-based learning disabilities (Geschwind & Dykens, 2004) as well as intellectual and cognitive function disabilities (Verri et al., 2010). However, the classical phenotype and subsequent clinical descriptions of KS, have been characterised on the basis of relatively small numbers of affected men (those seeking medical attention) who most likely exhibit severe clinical features (Bonomi et al., 2017). For example, Okada et al. (1999) investigated Japanese KS men who presented to a fertility clinic and found that of these men, 95% had small testes, only 12.4% exhibited gynaecomastia and only 1/3 of the sample showed feminine pubic hair distribution. The researchers established that the study sample exhibited diverse clinical features in comparison to the classical features of KS as described by Klinefelter, proposing a potential selection bias; those who present for medical attention are more severely affected (Okada et al., 1999). Therefore, our current knowledge about the clinical phenotypes, signs and symptoms of KS is inherently limited.

It is estimated that approximately 64% of KS men remain undiagnosed throughout their life, as there is no standard clinical phenotype that describes KS, and phenotypic variability between males is rather extensive (Bearelly & Oates, 2019). In fact, Kamischke et al. (2003) found that 60% of KS males in their study were not suspected of having KS based on previous clinical examinations. The researchers investigated the clinical and diagnostic features of men with suspected KS and found that no significant differences between KS men and normal karyotypes existed (Kamischke et al., 2003). The high incidence of these mild phenotypes clarifies why a large proportion of males with KS remain undiagnosed, especially as symptoms infrequently exist concomitantly (Bonomi et al., 2017).

KS males have an increased risk of developing additional disorders, or comorbidities, when compared to their eugonadal counterparts (Belling et al., 2017). A number of studies have reported a higher prevalence of metabolic dysfunction commencing in childhood and/or adolescence, as well as increasing reports of type II diabetes mellitus, metabolic syndrome and osteoporosis (Davis et al., 2015), resulting in a two to six-year shorter life expectancy than a healthy 46, XY male (Bojesen et al., 2011b). Many cases of KS remain and will continue to remain undiagnosed, leading to increased patient morbidity, severe complications and difficult clinical management (Bearelly & Oates, 2019).

Many studies have found that less severe or mild clinical phenotypical forms present few symptoms, where the severity of symp

toms are attributed to age, genotype and/or the degree of gonadal dysfunction (Wilkstrom & Dunkel, 2008; Bonomi et al., 2017; Bearelly & Oates, 2019). Although, due to the universality of hypogonadism in KS, it is difficult to determine what manifestations are due to hypogonadism, the aneuploidy itself or a combination of both (Davis et al., 2015). This section of the review therefore outlines these mechanisms reported to influence KS phenotypes.

Effect of age on phenotype

Signs, symptoms and therefore phenotypic presentation depend on male age, where it is argued that KS phenotype worsens with advancing age, especially with the increased incidence of comorbidities and prototypic features (Bonomi et al., 2017). The timing of clinical features is particularly useful in

diagnosing KS as it can distinguish whether signs and symptoms are androgen-dependent or genotypically-related and can also assist in managing KS-related symptoms (Bonomi et al., 2017). The majority of KS gonadal features are clinically evident at or after puberty (Lahlou et al., 2011). However, Lanfranco et al. (2004) found that genital anomalies such as micropenis and undescended testis have been observed in KS infants at birth, but these anomalies are usually rare. Studies have reported that symptoms such as longer legs (Chang et al., 2015) and speech disabilities (Geschwind et al., 2000) can also present in infants.

In adolescent KS males, hypogonadism remains silent until pubertal onset, when testosterone levels are sufficient for secondary sexual characteristic development (Bonomi et al., 2017). This increase in testosterone triggers negative feedback on luteinising hormone (LH) levels which activates aromatase, resulting in increased circulating oestradiol levels. This increase in oestradiol is thought to contribute to gynaecomastia development (Shirashi & Matsuyama, 2018). Furthermore, accompanying normal sexual development and growth, a progressive increase in testicular volume usually occurs, but in KS males, the testes remain small (<4ml) and firm (Kamischke et al., 2003; Lanfranco et al., 2004; Bojesen & Gravholt, 2007). Bonomi et al. (2017) have reported additional features that present during puberty including reduced muscle mass (virilisation), sparse body and facial hair, feminine pubic hair distribution and an impaired oestradiol/testosterone ratio. In KS adults, the degree of virilisation has been found to vary quite significantly but as Bonomi et al. (2017) suggests, there is a tendency for virilisation to decrease and progressively worsen with advancing age. Chang et al. (2015) found that few men were aware of physical symptoms (overt hypogonadism) including poor muscle development, small penis and a lack of or sparse pubic and facial hair, while Host et al. (2014) reported that 65% - 85% of KS males reported overt hypogonadism. KS-associated comorbidities including diabetes mellitus, osteoporosis and metabolic syndrome also frequently emerge during adulthood and increase with advancing age (Wilkstrom & Dunkel, 2008).

Genotype effect on phenotype

A recent plausible explanation for the heterogeneity in phenotype is that the severity of KS clinical presentation is strongly correlated with the severity of the sex-chromosome aneuploidy; phenotypes increasingly deviate from the normal phenotype (46, XY), as the number of X Chromosomes increase (Samplaski et al., 2014; Bonomi et al., 2017). In the majority of KS males (47, XXY), manifestations have been reported as subtle and nonspecific, whereas higher-grade (polysomic) aneuploidies have exhibited a more severe phenotype (Davis et al., 2015). Linden et al. (1995) found that the frequency of speech and language disabilities concomitantly increase with the number of supernumerary X-chromosomes, also estimating a decrease (15-16 points) of intelligence quotient [IQ] per extra X-chromosome. Some studies have also found significant physical differences between mosaic KS and non-mosaic KS. Samplaski et al. (2014) for example, compared mosaic and non-mosaic KS men and found that mosaic KS exhibit less severe clinical manifestations and endocrinological abnormalities compared to their polysomic counterparts. Thus, the supernumerary X is thought to play a role in differential phenotypic expression.

Hypogonadism and phenotype

KS is a common cause of hypogonadism in males in which the hypothalamus and pituitary produce high levels of circulating gonadotrophins, but the gonads do not respond with increased production of sex steroids (Schoenwolf et al., 2015). In KS adolescents, hypogonadism may be so severe that there are minimal signs of pubertal development (Bearelly and Oates, 2019). KS males tend to have altered body composition with reduced virilisation and an increase in body fat, which have been attributed to a combination of genetic factors and the KS hormonal milieu (Host et al., 2014). The degree of androgenisation and/or virilisation is strongly reliant on the level of testicular testosterone production, which tends to be low in KS adult males (Bearelly and Oates, 2019). Tsai et al. (2000) found that in a group of healthy eugonadal men, hypogonadism independently predicted the development of abdominal adiposity. Furthermore, KS men have been found to have a reduced muscle strength in both the biceps and quadriceps muscles, as

well as a lower aerobic capacity associated with reduced testosterone in hypogonadism (Bojesen et al., 2011a). Hypogonadism is also a well-known cause of low bone mineral density (BMD) and osteoporosis in KS men, in which testosterone is fundamental for maintenance of bone mass (Host et al., 2014). In KS there is evidence of a decrease in BMD as demonstrated by earlier studies (Foresta et al., 1983), and in recent studies such as Bojesen et al. (2011a), which found that KS men had decreased BMD in the spine, hip and forearm, and over 40% of the investigated cases had osteoporosis.

Hormonal therapy effect on phenotype

Hormonal therapy, specifically testosterone replacement therapy (TRT), is used to support the normal development of secondary sexual characteristics and virilisation at the time of puberty (Hawksworth et al., 2018). Therefore, it is often initiated in KS boys, particularly if hypergonadotrophic hypogonadism is present (Hawksworth et al., 2018). TRT has been found to be highly effective in improving hypogonadism in KS males and may prevent the development of significant KS-related co-morbidities such as osteoporosis, diabetes and obesity (Hawksworth et al., 2018), but substantial evidence of this effective treatment is lacking since few randomised placebo trials have been published (Host et al., 2014; Bonomi et al., 2017).

However, one non-randomised study investigated TRT in 30 KS men and found it had a positive effect on endurance, strength, concentration and learning ability in over 75% of men (Nielson, Pelsen and Sorensen, 1988). On the contrary, in Bojesen et al. (2006) TRT was not shown to improve body fat distribution or learning ability in KS males. Moreover, excess androgens have been found to negatively affect sperm retrieval rate (SRR) in KS males, as higher levels can suppress already impaired spermatogenesis in these men (Hawksworth et al., 2018). Therefore, it has been suggested that sperm should be recovered prior to the initiation of TRT, to heighten the chances of SRR success (Mehta and Paduch, 2012). Further studies are needed to ascertain the scientific and clinical benefits of TRT in KS men (Host et al., 2014).

Pathophysiology

The origins of KS phenotypic manifestations and other sex-chromosomal aneuploidies remain largely unexplained despite comprehensive research for over 5 decades (Tuttelmann and Gromoll, 2010). While it is established the supernumerary X-chromosome is an aetiology of testicular failure, the molecular mechanisms by which this occurs have not been fully explained (Davis et al., 2015). Recently, the assessment of multiple genetic mechanisms relating to the supernumerary X-chromosome(s) such as parental origin, X-chromosome inactivation (XCI) pattern and androgen receptors (AR), have been found to have a possible impact on KS phenotypes (Tuttelmann and Gromoll, 2010; Gravholt et al., 2018). We explore the influential nature of these genetic mechanisms here.

Parental origin

Parental origin is proposed to have an impact on KS phenotype, emphasising a supposed pathophysiological mechanism (Bonomi et al., 2017; Gravholt et al., 2018). Wilkstrom et al. (2006) investigated the influence of the supernumerary X on testicular degeneration, pubertal development and growth in 14 adolescent KS boys, where 3 subjects were found to inherit the supernumerary X paternally, and 11 maternally. Researchers found that paternal origin of the supernumerary X was associated with longer polymorphic trinucleotide repeat ((CAG)n) of the androgen receptor (AR) and late onset puberty (Wilkstrom et al., 2006). The AR contains (CAG)n, where a length between 9 and 37 repeats is considered normal (Tuttelman & Gromoll, 2010); longer repeats are associated with reduced sensitivity of the AR in humans (Mouritsen et al. 2013). Similarly, Stemkens et al. (2006) found a higher incidence of developmental problems in speech and motor impairment when the supernumerary X-chromosome was paternally inherited. However, many studies have found no association between parental origin and phenotype (Tuttelmann and Gromoll, 2010). Zeger et al. (2008) for example, established the parent of origin of the supernumerary X-chromosome in 40 KS boys (aged 2 to 14), where the supernumerary X was maternally derived in 60% of cases and the remaining 40% of cases were paternally derived. However, no significant differences in physical characteristics such as

penile length and testicular size were observed between the two groups (Zeger et al., 2008). Further studies are needed to clarify these inconsistencies in the available literature.

XCI pattern

X-chromosomes contain over 1000 genes essential for development (unlike the Y-chromosome), however females carry two copies of the X-chromosome resulting in a possible dangerous double dose of X-linked genes (Ahn and Lee, 2008). Females have evolved a mechanism of dosage compensation, termed X-chromosome inactivation in which one of the two X's is transcriptionally silenced (Ahn and Lee, 2008). The inactivated X-chromosome condenses into a Barr body and is maintained in this silent state (Ahn and Lee, 2008). XCI exists in two forms: random or imprinted, where both utilise the same RNAs and silencing enzymes but differ in terms of developmental timing and mechanism of action (Ahn and Lee, 2008). XCI is thought to play a pivotal role in KS phenotype as KS males, like females, are also subject to X-chromosome inactivation, however some genes are thought to escape the XCI process, hence predisposing the KS phenotype (Chung et al., 2006; Tuttelmann and Gromoll, 2010). Chung et al. (2006) found 14 genes in a single KS patient that escaped the XCI process. Only one gene, the growth-related short-stature homeobox-containing (SHOX) gene in the pseudoautosomal region 1 (PAR1) on the short arm (Xp) of chromosome X, has been evidently shown to influence KS phenotypes (Groth et al., 2013). This gene has been found to be associated with the slightly accelerated growth as seen in some KS males (Ottesen et al., 2010), although further studies are needed to enhance our understanding of this particular phenotypic interaction.

AR sensitivities

AR alleles undergo random XCI, which is facilitated by methylation of specific genes on the X-chromosome and where one of the AR alleles is methylated and inactive and the other is active and not methylated (Zitzmann et al., 2004). This random XCI has been described in women with conditions related to increased androgen activity such as polycystic ovarian syndrome (Hickey, Chandy and Norman, 2002). The human AR is therefore of interest concerning phenotypic variation in KS males, as they present at least two AR alleles (as do women) which contain a highly polymorphic trinucleotide repeat (CAG)n (Tuttelmann and Gromoll, 2010). This (CAG)n repeat has been found to be correlated with physiological androgen effects in normal, eugonadal men including prostate size (Giovannucci et al., 1999) and sperm concentration (Von Eckardstein et al., 2001). Zitzmann et al.'s (2004) study of 77 KS men found a positive correlation between (CAG)n length and body height, an inverse relationship with bone density and arm span to body height ratio, and that the presence of long (CAG)n had predictive power for having gynaecomastia and smaller testes. The researchers also noted that shorter (CAG)n in KS men were associated with professions requiring higher education, had an increased likelihood of having a partner and were more likely to present due to fertility problems rather than endocrine-related disorders. Fundamentally, those with long (CAG)n were more likely to encounter problems related to health (especially bone density), professional underachievement and difficulties in finding a partner (Zitzmann et al., 2004). Therefore, phenotypic variation in KS may indeed be influenced by DNA methylation effects and/or the (CAG)n repeat polymorphism of the AR (Tuttelmann and Gromoll, 2010).

Semen quality, spermatogenesis and mechanisms underlying infertility

Darbre (2015) states that semen quality is a measure of the ability of sperm to accomplish fertilisation, including sperm concentration, motility and morphology, and poor-quality semen can ultimately affect and cause infertility- defined as failure to conceive after one year of unprotected sexual intercourse (Hawksworth et al., 2018). KS males have been traditionally described as infertile (Klinefelter et al., 1942), where the majority of KS males most often exhibit NOA (Maiburg et al., 2012; Chihara et al., 2018) or severe oligozoospermia (Hawksworth et al., 2018). Activation of the hypothalamic pituitary gonadal (HPG) axis is attributed to the accelerated testicular demise in puberty, which arises from activation of apoptosis-related genes within the spermatogonial cell line in the process of meiosis (Hawksworth et al., 2018). However, focal spermatogenesis has been found to persist in some seminiferous tubule segments in KS

men (Plotton et al., 2014), in which spermatogonia can escape the wave of apoptosis that occurs during puberty (Hirota et al., 2017). Supernumerary X (karyotype) and azoospermia factor (AZF) deletion, have been found to influence infertility in KS men, which will be discussed thereafter.

Karyotype

Severe spermatogenesis impairment in KS men results in NOA which occurs in 90% of non-mosaic men and in 75% of mosaic men (Majzoub et al., 2016). It has long been thought that non-mosaic XXY cells are meiotically incompetent and there is a traditional belief that KS males who produce sperm are mosaic (Aksglaede et al., 2006; Selice et al., 2010). However, Laron et al. (1982) reported a spontaneous conception from a non-mosaic KS father (Laron et al., 1982). Subsequent studies have also identified sperm in the ejaculate of non-mosaic KS men, including Kitamura et al. (2010) who reported 4 out of 52 men (7.7%) presenting with ejaculated spermatozoa.

It has since been established that mature sperm can be found in non-mosaic testicular biopsies. For example, Foresta et al. (1999) performed fluorescent in situ hybridisation (FISH) on testicular tissue obtained from 10 non-mosaic KS men and found that all men had Sertoli cells and residual spermatogenesis was found in 2 of these men. Schiff et al. (2005) and Ramasamy et al. (2011) have also demonstrated that in approximately 50% of non-mosaic KS males, sperm can be extracted. Fertility in mosaic KS males however, is less severely affected and there is a higher chance of locating sperm in the ejaculate when compared to their non-mosaic counterparts (Aksglaede and Juul, 2013). Moreover, Samplaski et al. (2014) found that mosaic KS men were more androgenised than their non-mosaic counterparts; they had a larger amount of sperm in the ejaculate and the mean testicular volume was almost double that of non-mosaic men. The researchers suggested that this may explain the underestimated prevalence of mosaic KS (Samplaski et al., 2014). The molecular mechanisms behind the preservation of germ cells in mosaic KS males is suggested to be due to the presence of normal 46, XY germ cells, but this hypothesis is contentious (Vialard et al., 2012).

AZF deletion

The AZF locus on the Y chromosome is commonly found to be deleted in infertile males, including subregions AZFa, AZFb and AZFc, which contain several genes involved in different stages of spermiogenesis (Li et al., 2015). It has been suggested that deletions or mutations in these AZF sub-regions may cause spermatogenic disorders such as oligozoospermia and NOA (Li et al., 2015). Many studies have reported Y chromosome microdeletions in KS men, including Mitra et al. (2006) who found that 4 out of 14 KS men showed AZF microdeletions and Li et al. (2015) reported similar results, but also suggested that the types of microdeletions vary between KS males. Similarly, Hadjkacem-Loukil et al. (2009) investigated the genetic association between AZF region polymorphism and KS men and found that 67% of KS men had the microdeletion. KS males therefore may harbour Y microdeletions which may in turn affect fertility (Hadjkacem-Loukil et al., 2009).

Sperm retrieval and infertility treatment

The goal of sperm retrieval in NOA men is to locate the focal area of spermatogenesis, of which there are various methods (Janosek-Albright, Schlegel & Dabaja, 2015). Two key methods of sperm retrieval in KS men with NOA and oligozoospermia have been described, including c-TESE and micro-TESE. The first use of TESE methods in KS males was reported in 1996 by Tournaye et al. using C-TESE, followed by a pregnancy one year later after a C-TESE/ICSI approach (Palermo et al., 1998). Micro-TESE was then developed as a successful, innovative and less-invasive technique by Schlegal in 1999 (Schlegal, 1999). Since these early reports, infertility in KS males has been overcome by utilising aforementioned sperm retrieval methods followed by ICSI, efficaciously allowing KS men to achieve biological paternity, rather than relying on donor sperm as they have traditionally (Elfatah et al., 2014). In fact, it is reported that TESE/ICSI yields similar retrieval rates and pregnancy rates in KS males as in men with NOA and a normal karyotype (Nieschlag et al., 2016) and approximately 16% of KS males who undergo a TESE approach will produce a live birth (Corona et al., 2017). Age at ART treatment has however, been found to influence the success of

SRR in KS males (Ragab et al., 2018). Additionally, although ART techniques can be useful when mature sperm are present, there is a risk of transmitting chromosomal abnormalities to consequential offspring (Levron et al. 2000) as a result of increased incidence of genetically imbalanced sperm (Staessen et al. 2003). Therefore, pre-implantation genetic testing (PGT) of embryos and fluroescent in-situ hybridisation (FISH) analysis of sperm have been suggested as a means of determining the possible risk of transmission to offspring (Levron et al. 2000). This section of the review thus considers these different techniques and their appropriateness.

SRR techniques

Micro-TESE is different to c-TESE in that it involves separating, puncturing and examining spermatic tubules in order to extract sperm (Rogol and Skakkebaek, 2016) whereas c-TESE extracts protruding testicular tissue (Shah, 2011). Chihara et al. (2018), investigated SRR using Micro-TESE in five non-mosaic KS men and reported a SRR of 40%; sperm was extracted in 2 out of 5 cases which researchers considered low but most likely reflected the small sample size. ICSI was subsequently performed and resulted in fertilised oocytes, but only 1 of the 2 men produced a live birth with a normal 46, XY eugonadal karyotype (Chihara et al., 2018). Similarly, Sabbaghian et al. (2014) evaluated micro-TESE in a large group of non-mosaic KS men and achieved a SRR of 28.4% and a live birth rate of 13%, slightly lower than Tanos et al. (2018) who reported a live birth rate of 21%. Madureira et al. (2014) however, reported a similar SRR rate (38.5%) with a smaller sample size and a high birth rate (47.2% [with all newborns exhibiting normal karyotypes]), using c-TESE. Majzoub et al. (2016) evaluated and compared SRR in KS men undergoing c-TESE and micro-TESE and found that the SRR was significantly higher in the latter group, compared with the former (30% vs 0% respectively). On the contrary, the meta-analysis by Corona et al. (2017) found no statistical difference when comparing C-TESE and Micro-TESE (43% vs 45% respectively), which was similar to the findings from Tanos et al. (2018) systematic review, which reported a SRR of 44.4% and 46.3% when using C-TESE and Micro-TESE respectively. Based on the available literature, it is demonstrated that both sperm retrieval methods yield similar SRRs in KS males.

Effect of age on SRR

Age is a practical and clinical predictor of successful SRR in KS males (Ragab et al., 2018) and It has been suggested that performing TESE earlier might result in better outcomes (Franik et al., 2016). This is based on the progressive hyalinisation of the seminiferous tubules observed after puberty (Franik et al., 2016). Supporting this concept, Rohayem et al. (2015) established that males aged 15-25 years had significantly higher chances of successful SRR and reported that the success of micro-TESE gradually declined with age. Similarly, Ragab et al. (2018) demonstrated that sperm retrieval by micro-TESE wasn't significantly different during adolescence compared with early adulthood (15 to 25 years). On the contrary Plotton et al. (2015) compared the SRR between young KS males (15 to 23) and adults (>23) and found that the SRR of both groups were not statistically different. A recent meta-analysis by Corona et al. (2017) also found that SRR in KS was independent of age, establishing that progressive hyalinisation of seminiferous tubules (after puberty) is not ubiquitous and that finding tubules with normal residual activity is possible.

PGT

PGT, formerly known as pre-implantation genetic diagnosis (PGD), involves embryo biopsy to test for monogenic disorders (PGT-M), structural rearrangements (PGT-SR) and/or aneuploidies (PGT-A) in couples undergoing ART. The benefit of PGT in embryos derived from KS patients is contentious due to insufficient evidence suggesting that embryos derived from KS patients demonstrate a higher prevalence of sex-chromosome aneuploidy (Vloeberghs et al. 2018). Nevertheless, it has been established that 47,XXY cells can complete meiosis and produce aneuploid sperm (Bielanska, Tan and Ao, 2000). Tachdjian's (2003) review reported 36 pregnancies using sperm from non-mosaic KS males, resulting in 32 karyotypically normal infants, 2 karyotypically normal pregnancy losses, 1 unkaryotyped infant and 1 KS prenatally diagnosed fetus (of which the parental origin of the supernumerary X was not defined). Similarly, Vloeberghs et al. (2018) found that non-PGT and PGT cycles showed similar implantation (25.6% v 15.8%), clinical

pregnancy (34.5% v 23.3%) and live births rates (24.1% v 20%) per cycle respectively. On the contrary, Staessen et al. (2003) found that only 54% of embryos created using KS male sperm (following biopsy and fluorescent in-situ hybridisation (FISH) analysis), were considered chromosomally normal; 75% embryos obtained with fresh ejaculate, 55.3% obtained with fresh testicular sperm and 45.8% obtained via frozen testicular sperm cells, were considered normal. Additionally, the study found that a significantly higher percentage of abnormal embryos were produced by KS males when compared to normal karyotype controls (Staessen et al. 2003). Thus, based on the available evidence, genetic counselling for KS patients remains difficult (Tachdjian et al. 2003) and a cautious approach is warranted when advising KS patients considering PGT.

Although, authors have suggested that chromosomal analysis of sperm cells might also assist patients considering treatment (Levron et al. 2000), specifically direct analysis of the ejaculated or testicular sperm, which can be carried out using FISH analysis (Staessen et al. 2003). Investigations considering the meiotic products of mosaic KS males have found that a high percentage of ejaculated sperm have normal karyotypes (Guttenbach et al., 1997; Levron et al., 2000). Levron et al. (2000) utilised FISH to analyse sperm produced by mosaic KS males and found that over 90% of sperm cells analysed were chromosomally normal, suggesting that sperm produced by mosaic males is most likely a product of normal germ cell lines. Furthermore, the remaining abnormal cells were found to be aneuploid as a result of non-disjunction errors during meiosis I and II, indicative of errors from 46,XY germ cells rather than 47,XXY germ cell line as this would yield significantly higher aneuploidy rates (Levron et al. 2000). These findings suggest that the risk of transmitting numerical sex chromosome abnormalities are low in mosaic KS males (Levron et al. 2000). Alternatively, in non-mosaic KS males, FISH analysis has shown varying incidences of normal sperm ranging from 50% to 94% (Guttenbach et al., 1997; Staessen et al., 2003). Although, increased incidence of 24,XX and 24,XY hyperhaploid sperm compared with controls have also been found (Foresta et al., 1998), suggesting an increased incidence of genetically imbalanced sperm (Staessen et al. 2003). In view of the potential risks associated with genetically imbalanced sperm cells, Staessen et al. (2003) recommend offering PGT to couples with non-mosaic KS in order to establish possible chromosomal numerical abnormalities and the risk of transmission.

Conclusion

Klinefelter Syndrome represents the most common sex chromosomal aneuploidy in males, occurring in 1 in 600 males (Tuttelmann and Gromoll, 2010). KS genotypically exists as a non-mosaic type, and less frequent observations of varied mosaicism, structurally abnormal X aberrations and higher-grade polysomic X aneuploidies (Bonomi et al., 2017). The abnormal genetic karyotypes arise during non-disjunction in early germ-cell development and can be equally caused by, and during, paternal or maternal non-disjunction (Jacobs et al., 1988).

Phenotypic manifestations of the prototypic KS male have been typified and universally, testicular dysfunction, small testes and varying degrees of hypogonadism are common in all KS cases (Schoenwolf et al., 2015; Davis et al., 2015). Tall stature, eunuchoid appearance, gynaecomastia, broad hips, sparse body hair, feminine pubic hair distribution and firm testes have also traditionally been described (Bonomi et al., 2017), along with impaired psychosocial and neurobehavioural characteristics and cognitive function disability (Geschwind and Dykens, 2004; Verri et al., 2010). Despite these signs and symptoms, a huge proportion of KS men remain undiagnosed as there is considerable variation in KS phenotypes (Bonomi et al., 2017). Male age, the genetic defect itself and/or the degree of hypogonadism, are a few of the pathogenic mechanisms proposed to affect phenotypic appearance in KS males (Bonomi et al., 2017; Bearelly and Oates, 2019). Particular milestones associated with age are useful in diagnosing KS especially around pubertal onset as this is when typical clinical features become evident (Bonomi et al., 2017). Phenotypic variation in KS strongly correlates with genetics, with studies finding that manifestations progressively worsen as the number of X-chromosomes increases (Samplaski et al., 2014; Bearelly and Oates, 2019). Furthermore, hypogonadism is common in KS men, causing reduced virilisation, BMD,

an increase in body fat and triggers osteoporosis (Bojesen et al., 2011a; Host et al., 2014). TRT is a promising therapeutic option for KS males, as it has the potential to overcome certain manifestations associated with hypogonadism and androgen deficiency (Nielson, Pelsen and Sorensen, 1988). The pathophysiology of the KS phenotype remains unexplained, but it is thought that the mechanisms associated with the supernumerary X-chromosome are potential aetiologies (Davis et al., 2015), including parental origin (Wilkstrom et al., 2006), X-chromosome inactivation (Ahn and Lee, 2008) and androgen receptor sensitivities (Zitzmann et al., 2004).

The genetic karyotype (Selice et al., 2010) and the AZF deletion on the male Y chromosome (Li et al., 2015), are some factors reported to influence semen quality and infertility. Although KS males tend to be infertile, recently spermatogenesis has been found to persist in some seminiferous tubule segments in KS men (Plotton et al., 2014). As a result of recent improvements in sperm retrieval techniques (including c-TESE and micro-TESE followed by ICSI), it has been demonstrated that viable sperm can be retrieved from the seminiferous tubules, thus allowing KS males to biologically father offspring (Elfatah et al., 2014). However, age (Ragab et al., 2018) is reported to influence SRR success due to the progressive hyalinisation of the seminiferous tubules post-puberty (Franik et al., 2016). Additionally, PGT and FISH analysis techniques have been suggested for couples using KS male sperm, to determine the potential risk of chromosomal aneuploidy in resultant offspring. However, due to the conflicting literature, caution is warranted in genetic counselling and when advising KS patients considering PGT and FISH.

There are many different postulations relating to KS phenotypical manifestations, semen quality, infertility and infertility treatments. As a result of this, further research is required to fully comprehend the mechanisms and aetiologies underlying KS in order to improve clinical management and diagnosis.

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